

**SEED TECHNOLOGICAL RESEARCH ON HYBRID
PIGEON PEA (ICPH-2671)**

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**SEED TECHNOLOGICAL RESEARCH ON HYBRID
PIGEON PEA (ICPH-2671)**

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IN

SEED SCIENCE AND TECHNOLOGY

BY

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CERTIFICATE

This is to certify that the thesis entitled “**SEED TECHNOLOGICAL RESEARCH ON HYBRID PIGEON PEA (ICPH-2671)**” submitted by **Ms. CHANDRAVATHI B.** in partial fulfillment of the requirement for the degree of **DOCTOR OF PHILOSOPHY in SEED SCIENCE AND TECHNOLOGY**, of College of Agriculture, University of Agricultural Sciences, Dharwad, is a record of research work done by her during the period of her study in this university under my guidance and supervision and the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar titles.

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“When performance exceeds expectation, then there is an appreciation”

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ABBREVIATIONS AND ACRONYMS

AFLP	Amplified Fragment Length Polymorphism
CTAB	Cetyl trimethyl ammonium bromide
CcM	Cajanus cajan Microsatellite
DNA	Deoxyribonucleic Acid
dNTPs	deoxynucleotide triphosphates
µl	microlitre
EDTA	Ethylenediaminetetraacetic acid
ICRISAT	International Research Institute for Semi-Arid Tropics
GCV	Genotypic co-efficient of variation
g	gram
kg	Kilo gram
GA	Genetic Advance
GAM	Genetic Advance Mean
H	Heritability in broad sense
M	Molar
mg	milligram
ng	nanogram
PCI	Phenol: chloroform Isoamyl alcohol
p	Pico molar
PCR	Polymerase Chain Reaction
PCV	Phenotypic co-efficient of variation
RNA	Ribonucleic acid
RNAse	Ribonuclease
rpm	Revolution per minute
SSR	Simple Sequence Repeats
<i>Taq</i>	<i>Thermus aquaticus</i>
TBE	Tris/Borate/EDTA
TAE	Tris/Acetate/EDTA
TE	Tris EDTA
Vg	Genotypic variation
Vp	Phenotypic variation

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1. INTRODUCTION

The world population is projected to grow from 7.3 billion (in 2015) to 8.9 billion by 2050 (Anon., 2013). Therefore, increasing food production to provide food and nutritional security is a challenge. Food and nutritional security becomes more important with the certainty of climate change scenario and ever increasing human population. These twin challenges can be addressed to by diversifying the global cropping pattern and by promoting food/grain legume crops, generally called pulses in India. Legumes include 750 genera and 18,000 species. Pulses form an important part of human daily diet especially in several developing and some developed countries and therefore sometimes legumes are considered as poor man's meat.

Pulses in general are nutritionally enriched as they have high protein content relative to staple cereals. In addition to their nutritional content, there are several reasons that strongly 'Protein Calorie Malnutrition (PCM)' is a global concern especially in infants, young children and nursing mothers. The protein content of pulses is substantially higher (20-36 %) compared to major cereals (6.0-15.0 %). The protein content of important grain legumes is pigeon pea (21.7 g /100 g). The fat content of many pulses ranges between 1-6 g/100 g. Pulses are a good source of health promoting fatty acids like linoleic, linolenic, oleic and palmitic acids. Additionally, most of the pulses are also good source of carbohydrates (30-60 %) (Anon., 2013).

Pulses occupy an important place in Indian agriculture within this protein rich group of crops, pigeon pea occupies an important place among rainfed resource poor farmers because, it provides quality food, fuel wood and fodders. Its soil rejuvenation qualities such as release of soil bound phosphorous, fixation of atmospheric nitrogen, recycling of soil nutrients and addition of organic matter and other nutrients make pigeon pea an ideal crop of sustainable agriculture in the tropical and subtropical regions of India.

Most food proteins in the semi-arid tropics are derived from pulses that are generally grown under low input and risk-prone marginal environments with repeatedly low and unstable yields. At present, the protein availability among masses in the developing world is less than one-third of its normal requirements and with growing population and stagnation of yield the protein availability will certainly decline further.

Since food production balance always remains in favour of cereals, this issue assumes more significance from nutrition point of view. Among pulses, pigeon pea occupies an important place in rain fed agriculture.

Pigeonpea (*Cajanus cajan* (L.) Millsp synonymous: *Cajanus indicus* spreng) is an annual often cross-pollinated crop but can be grown as perennial, It belongs to family *Leguminaceae*, $2n= 22$. Annual or biennial or perennial, erect and branching, stem woody and striated, leaves trifoliolate compound, central leaflet longer than lateral ones, stipules small, long lamina hairy with the hairs there are yellow glands which are prominent. Inflorescence terminal or auxiliary racemes carried on long peduncles, flowers are clustered at the top of the peduncle and are yellow or yellow with purple veins or diffuse red flowers are *papilionaceous* bracteates, calyx gamosepalous of five lobes, corolla with keel petals free, stamens diadelphous, ovary superior with few ovules, fruit a pod which is variable in shape, size, constriction, colour texture and pubescence, pods which have deep

constriction are known as beaded, while others are flattish, seeds may be round or lens shaped, colour ranging from silver white, fawn brown or red to purple blotched or speckled seeds are also found, there is much variation in the size of the seeds and the cotyledons are yellow coloured.

The global pulse production area and yield during 2013 was 73 million tonnes (MT), 80.8 million ha and 904 kg ha⁻¹ respectively (Anon., 2015). Pulse production, area and yield during the same period in India was 18.3 million tonnes (25 % of the global production), 28.2 million ha (35 % of global area) and 650 kg ha⁻¹ respectively.

In India pigeon pea was cultivated on 4.65 million ha with a total production of 3.02 million tonnes and yield of 650.0 kg ha⁻¹ during 2013 (Anon., 2015). Expanding pigeon pea hybrid production during the past 5 decades, pigeon pea productivity in India has remained almost stagnant around 700 kg ha⁻¹. In this context, the hybrids can produce more biomass (>50 %) and productivity (>30-40 %) than varieties. ICRISAT and its partners have developed the hybrid technology in pigeon pea. In the past 6 years, several hybrids (ICPH-2671, ICPH-2740 and ICPH-3762) have shown enhanced productivity and adaptability in Maharashtra, Andhra Pradesh, Odisha, Telangana State, Madhya Pradesh, Karnataka and Jharkhand. ICRISAT and partner institutions (Department of Agriculture, State Agriculture Universities, public and private organizations and farmer organizations) can expand commercial hybrids substantially.

In India, the analysis of 2012-13 shows that highest area was under chickpea (8.7 million ha) followed by pigeon pea (3.81 million ha) and lowest was in pea (0.76). Similarly, production was highest in chickpea (8.83 million tonnes) followed by pigeonpea (3.02 million tonnes) (Anon., 2015).

Recent crop improvement efforts of ICRISAT and ICAR led to development of stable cytoplasmic and genetic male sterile system in pigeonpea and hybrids have been developed for different agroclimatic niches. Seed production technology need to be standardized in large scale production of commercial seed specific to different agro climatic zones. There is an immediate need for exploitation of hybrid vigour by breeding heterotic hybrids for different zones in the country to improve productivity and production.

The concept of developing commercial hybrid in pigeonpea was evolved at ICRISAT in 1974, when a source of Genetic Male-Sterility (GMS) was identified.

The level of realized heterosis for seed yield in pigeonpea is comparable to other crops where commercial hybrids have already made a mark in global agriculture. The hybrid breeding program at ICRISAT and ICAR in recent past focused towards developing a more efficient Cytoplasmic-Genetic Male-Sterility (CGMS) system.

Stable male sterility systems were developed from wild relatives *Cajanus cajanifolius* (A₄) and *Cajanus Scaraboides* (A₂) (Saxena *et al.*, 2005). The F₁ hybrid plants derived from this CGMS produce excellent pollen load and pod set. At present, these CMS systems are being used by pigeon pea breeders in India, Myanmar, and China (Saxena, 2009) for genetic diversification of A-lines and to produce commercial hybrids.

The essential reproductive act in the plant world is pollination; the distribution of male reproductive cells in the form of pollen to join with female reproductive cells in the ovary, deep within a flower. Pollination often involves a partnership between a flowering plant and an insect that carries the

pollen on its body and visited the flowering plants, the pollen migrates inside the flower to the ovary where it fertilizes the ovules or egg cells inside. The fertilization process initiates the production of seeds. Insects are the most frequent pollinators in agricultural crops.

Since, the hybrid seed set on the male-sterile plants is chiefly determined by the availability of bee population in the vicinity, pod set is generally poor in the locations with less number of pollinators. Therefore, to harvest good hybrid yields, it is imperative to select suitable seed production sites with good insect pollinator activity. Therefore, for increasing the insect activity for pollination, the insect attractants *viz.*, Bee-Q and other floral scents are sprayed to increase the seed set in crops.

The commercial seed production of pigeon pea hybrids involves large scale seed production of A, B, R - lines and the hybrid combination (A x R). For seed production of A-line, breeder seed of both A and B lines are planted using a female: male row ratio of 4:1. In the production areas where greater bee activity is observed a higher row ratio can be used for getting higher yields. For hybrid seed production (A x R) also, the same ratio can be used. In general, rouging of off-type plants is carried out both at seedling as well as reproductive stages.

Various advances in plant biotechnology and especially genomics together with traditional plant breeding technologies have led to the development of new improved varieties in a number of crop species with greater tolerance/ resistance and higher yield (Varshney *et al.*, 2010). In this context, molecular markers play a very important role as these are used for estimating diversity in germplasm, trait mapping, molecular breeding, genetic purity assessment of hybrid seeds, *etc.* Among a range of molecular markers starting with isozymes, RFLP (restriction fragment length polymorphism), RAPD (random amplified polymorphic DNA), AFLP (amplified fragment length polymorphism), SSR (simple sequence repeat), DArT (diversity array technology), and most recently SNP (single nucleotide polymorphism), that have become available during last two decades (Jones *et al.*, 2009), SSR markers have emerged as the current markers of choice for plant genetics and breeding applications (Gupta *et al.*, 1999). While SNP markers have a promising future in plant breeding applications, and may augment or displace SSR based marker systems, SNP based markers and associated technologies are in their infancy in most crops, including pigeon pea, while SSR marker technologies are better established for wide spread use in molecular breeding. The word 'hybrid' cultivar excites farmers in the expectations of high yields and greater returns. The hybrid breeding technology has demonstrated quantum yield jumps in various cereal (Alexandratos, 2001), (Rai and Rai, 2006) and fruit (Kuznecov, 1966) crops.

The commercial exploitation of hybrids is known to be directly linked to ease with which their hybrid seeds could be produced and delivered economically to farmers. The efficiency of mass pollen transfer from male to female parent through air or insects to affect cross-fertilization plays an important role in commercializing the hybrids in different crops. In most food legumes, the absence of natural cross-fertilization is the major bottleneck in exploiting hybrid vigour at commercial scale because their flower structure forces high level of self pollination (Saxena *et al.*, 1992). Legumes are the major source of protein for the vegetarian population in South Asia. The legume crops are generally grown under low inputs and risk-prone marginal environments, especially in semi-arid tropics. At present, the availability of proteins among poor in the developing world is less than one-third of its normal requirements (Paul *et al.*, 2011), and with growing population (expected to be 8.9

billion by 2050) and continuing low productivity of legumes, the protein availability to the masses is under threat of further decline. As the food production efforts during past in most developing countries favoured cereals, the issue of protein availability assumes even greater significance from nutrition point of view. Globally, pigeonpea (*Cajanus cajan* (L.) Millsp.) is cultivated on 4.79 million ha in 22 countries. Besides India, Myanmar and Nepal are important pigeonpea-growing Asian countries; in the African continent Kenya, Malawi, Uganda, Mozambique and Tanzania produce considerable amounts of pigeon pea (Anon., 2010).

In India, the pigeon pea area has recorded a significant rise from 2.3 million ha in 1950 to 3.53 million ha in 2010; but the crop productivity has remained stagnant at around 700 kg/ha (Anon., 2010). There are a number of factors for low crop productivity, but the lack of high-yielding cultivars has been identified as one of the major constraints underlying the stagnant productivity (Zaveri and Pathak, 1998). The issue of yield plateau in pigeonpea has been a major concern for a long time, and to date, it has remained a challenge. To achieve a breakthrough in pigeonpea productivity, research on breeding a cytoplasmic-nuclear male sterility (CMS) system was initiated at ICRISAT (Reddy and Faris, 1981). In the last decade (2002–2011), significant progress has been made to address various issues related to hybrid breeding and large-scale hybrid seed production technologies.

The hybrid technology in pigeonpea is on the verge of commercialization in India. This study, besides briefly describing the progress in breeding and release of the world's first commercial pigeon pea hybrid, also documents its salient features including productivity in diverse environments. The level of realized heterosis for seed yield in pigeon pea is comparable to other crops where commercial hybrids have already made a mark in global agriculture. The hybrid breeding program at ICRISAT and ICAR in recent past focused towards developing a more efficient Cytoplasmic-Genetic Male-Sterility (CGMS) system.

To overcome the production constraints, an improved hybrids seed production technology based on more efficient Cytoplasmic-Nuclear Male Sterility (CMS) system was planned. Pigeon pea has a considerable natural out-crossing (Saxena *et al.*, 1990). The first report of this event was published by Howard and Khan (1919) but it was treated as a major constraint in breeding and maintenance of pigeon pea varieties. Since most pigeon pea farmers save their seed for subsequent seasons planting and the crop exhibits tremendous variation for most economic traits. This leads to low and inconsistent yields. With the development of CMS lines the pigeon pea breeders have now converted this constraint into opportunity to increase the yield potential of this crop through hybrids. Considering the limitations in large scale hybrid seed production in CMS became imperative. The strategy was to induce CMS by placing pigeon pea genome in wild cytoplasm through hybridization. Very recently, hybrid breeding technology based on the cytoplasmic-nuclear male-sterility (CMS) system has been implemented in the pigeonpea breeding programme at ICRISAT (Saxena *et al.*, 2010) and this technology holds great potential to increase pigeon pea productivity.

Availability of genetically pure seeds of improved cultivars is crucial for realizing their productivity in different agro-ecological niches. The benefit of new hybrids cannot be fully realized until sufficient quantities of genetically pure and healthy seeds are commercially produced and sold at a cost affordable to the farmers. An efficient seed production system that could provide quality seeds at economically viable cost is the back bone of any hybrid breeding technology.

Since, pigeon pea exhibits natural outcrossing, a safe isolation distance is always essential to produce quality seed of parental lines and hybrids. Rouging and strict crop monitoring is a critical aspect of hybrid seed production and rouging should be done at seedling and flowering stages. There is a need to understand the hybrid seed production constraints in a particular area. One of the major constraints for poor harvests could be low population of pollinating insects. This needs to monitor before investing large sums in seed production in a particular area by keeping these constraints in view the present investigation “Seed technological research on hybrid pigeon pea ICPH-2671” was carried out with the following objectives:

- i. Characterization of parental lines through morphological characters
- ii. Maintenance of parental lines (A, B & R line) on seed quality and characterization of A, B, R and F₁ hybrid ICPH-2671 through molecular markers
- iii. Effect of planting ratio and insect attractant on seed yield and quality

2. REVIEW OF LITERATURE

The plant breeders develop and release hybrids for commercial cultivation and the success of hybrids largely depends on the production and supply of quality seeds to the farmers. Among the several factors maintenance of parental lines is a crucial factor in achieving higher hybrid or female parental seed yield. The success of hybrid seed production requires an adequate knowledge on these aspects. Keeping in view of the foregoing points, the review of literature pertaining to pigeon pea classification, floral biology, pollen viability, stigma receptivity, seed development, maintenance breeding, DNA markers, planting ratio, insect attractant, natural out-crossing, CGMS system, morphological characterization of parental lines and hybrid, special features of hybrid ICPH-2671 and hybrid seed production technology, different methods of pollination on seed yield and components, seed quality have been compiled and presented here. The literature available on these aspects in pigeon pea is very meager, hence the review on other related crops is also included.

2.1 Classification

Pigeon pea (*Cajanus cajan* (L.) Millsp) is a drought tolerant legume of the *Fabaceae* family in the order *Fabales*. Other common names are red gram, Congo pea, Gungo pea, Gunga pea, and no-eye pea. It is the only cultivated species in the genus *Cajanus*. Initially, members of this genus were spread between two main genera; *Atylosia* and *Cajanus*. With evidence emerging from morphological, cytological and chemo-taxonomical studies, many *taxa* of *Atylosia*, found to be congeneric with *Cajanus*, were reclassified into *Cajanus* (Van der Maesen, 1981). This genus now comprises 32 species from Asia, Africa and Australia.

2.2 Floral biology and flower morphology

The anthers dehisce in about 80–90 % of the flowers before they open. The length of time the flower remains open depends on environmental conditions such as temperature, intensity of sunlight, and relative humidity. Mahta and Dave (1931) observed that the pigeon pea flowers remained open for 48 hours at Pusa (Bihar) while at Nagpur they remained open only for 6 hours.

Bahadur *et al.* (1981) reported that pigeon pea pollen grains exhibit dimorphism with regard to grain size and exine structure. In general, the pollen grains in the “short” stamens are larger than those in the “long” stamens.

The pigeon pea flowers borne in short racemes are predominantly yellow in color. The peduncles of pigeon pea are 8 cm long. Pedicels are thin 7-15 mm long and covered with hairs. Bracts are 1- 4 mm long and their margins curve inwards to form a boat like structure. The calyx tube is campanulate with numerous glandular hairs with bulbous bases. The tube is dorsally gibbous about 5 mm in length. The corolla is zygomorphic, papilionaceous and generally yellow in color. The petals are imbricate in the bud. The standard petal (vexillum flag) is erect and spreading more or less orbicular, 14-22 mm long, 14-20 mm wide with clawed base. The wing petals are obviate with a straight upper margin, clawed base, asymmetrically bilauriculate, 15-20 mm long and 6-7 mm wide with a callosity. Keel petals are boat-shaped 14-17 mm long, 5-7 mm wide, clawed and dorsally split, and ventrally split near the base. Stamens are 10, diadelphous (9+1), 15-18 mm long, with 4-7 mm free parts, flattening towards the base, tapering towards the top, and geniculate near the base. The

staminal sheath is about 12 mm long. The anthers are ellipsoid, about 1 mm long, dorsifixed and light or dark yellow in color of the 10 stamens, four have short filaments and six, including the odd posterior one, have long filaments. The odd stamen has a groove for the passage of nectar that is secreted from the base of the filaments. The long stamens are antisealous and the short ones antipetalous (Reddy, 1990).

Under different locations the level of out-crossing in this mutant has been reported to vary from zero to a maximum of 2.5 %. Thus, the breeding behavior of an often cross-pollinated crop has been changed in this material to self-pollination. This trait is now being incorporated by breeders to develop high-yielding pure lines. Once a flower bud becomes visible, it takes about two weeks to bloom. In a young pigeon pea bud, the stigma is placed slightly above the level of anthers, and the style is curved at the tip in such a way that the stigmatic surface is directed towards the anthers. The anthers inside flower are arranged around the style in two groups, five above and five below. As the bud grows, the anther filaments elongate and bring the anthers to the level of the stigma. This growth is completed before the anthers start dehiscing in the closed bud, *i.e.* a day before the flower opens (Saxena *et al.*, 1992a).

2.3 Pollen viability

The short period of heat stress can increase bud and flower abortion (Wahid *et al.*, 2007). Though flower drop is due to lack of fertilization, can be influenced by number of factors including reduced gamete viability (Iwahori and Takahashi, 1964) and lesser number of pollen (Abdalla and Verkrek, 1968). Hence, bud drop and failure of pollination can lead to drastic loss of yield in pigeon pea.

Datta and Deb (1970) in pigeon pea studied pollen tube growth in a style pollinated with pollen from the same flower and observed it to be very slow, taking 54 hours to reach the base of the ovary. Those two mechanisms provide a sufficient time gap for foreign pollen to be introduced on to the stigma by insect pollinators before self fertilization takes place.

According to Onim (1981) reported that pigeon pea shed pollen while their flowers are still in the bud stage, and they do not start germinating until the flowers start to wither 24-48 hours after their anthers dehiscence.

Jogendra Singh and Bajpai (2005) reported that communication reports the relative pollen fertility and morphology in inter specific crosses of pigeon pea. *C. cajan* X *C. acutifolius* hybrid showed low pollen fertility in F₁ generation, where high pollen fertility was found in crosses utilizing *C. cajanifolius* and *C. scarabaeoides*. Moderate variation (37.45 µm) was noticed in size of pollen grains among the parents and their hybrids. *C. acutifolius* had the smallest pollen size.

Pollen viability ranged 10-50 % in different stages of floral buds and open flowers at 30 °C-35 °C. Similarly stigmas of the same were receptive with gradual loss. However, the floral units of the same stages lost pollen viability as well as stigma receptivity when the temperature increased beyond 35 °C in wild pigeon pea (Kanak Sahai and Krishna Kumar Rawat (2013).

2.4 Stigma receptivity

In crop plants like pigeon pea where male-sterility system is recently developed, it is necessary to study the environmental effect on stigma receptivity duration. Stigma receptivity is an important factor to have higher success rate of natural out-crossing.

In West Bengal, Reddy (1973) found that the pigeon pea flowers began to open from 06.30 onwards and remained open for 15 to 24 hours and anthesis continued until 1400 with the maximum anthesis taking place between 10.30 and 12.30 hours. Pathak (1970) reported that those flowers which open in the evening usually remain throughout the night and close before noon on the following day. It has also been found that the stigma becomes receptive for pollination 68 hours before anthesis and remains in the same condition for about 20 hours after anthesis (Prasad *et al.* 1977).

Prasad *et al.* (1977) have reported that receptivity of stigmas starts 68 hours prior to anthesis and continues for 20 hours after anthesis. In mature buds, anthers surround the stigma and dehisce a day before the flower opens. Although, this floral trait should favour 100 % self-pollination, partially out crossed and hybrid seed have been raised to plants (El Baradi, 1978).

Luo *et al.* (2009) studied on stigma receptivity in pigeon pea by using two male-sterile lines (ICPA-2039 and ICPA-2043) under field condition. The study revealed that the stigma was receptive 48 hours before flower opening and continued up to 4 days after flower opening. The peak stigma receptivity was on the day of flower opening with 84 and 86 % pod set after hand pollination in the male-sterile lines ICPA 2039 and ICPA-2043, respectively. This floral stage was characterized by the corolla to calyx length ratio of 2.75 ± 0.283 (ICPA-2043). The long time span of stigma receptivity in pigeon pea encourages insect aided natural out crossing.

2.5 Pollination and fertilization

Datta and Deb (1970) reported that when pollinated with the pollen from the same flower, pollen tube growth within the style is very slow, taking 54 hours to reach the base of the ovary.

Reddy and Mishra (1981) reported that the percentage of “selfs” was negligible when flower buds were pollinated with foreign pollen without emasculation. This indicates that the foreign pollen has an advantage in affecting fertilization over the plant's own pollen. Such mechanisms in pigeon pea offer a sufficient time gap for foreign pollen to be introduced onto the stigma and thus favoring out-crossing.

According to Onim (1981), although anthers dehisce during the bud stage the pollen grains do not start germinating until the flower starts to wither, *i.e.* 24–28 hours after dehiscence. Under Hyderabad (17°N) conditions, the fertilization in pigeon pea occurs on the day of pollination and seeds mature within 45–50 days after pollination. In the first three weeks after anthesis, the pod wall grows more rapidly than the young seeds (Narayanan and Sheldrake, 1975).

Bahadur *et al.* (1981) reported that predominantly pigeonpea flowers are self-fertilized with 20-40 % cross fertilization and growth and development of short stamens is faster than those of longer ones. The maturity period of short stamens coincides with that of stigma receptivity. They also postulated that the pollen grains produced by short stamens are predominantly responsible for self-fertilization, whereas those produced by long stamens are utilized in insect-aided out-crossing. Self-pollination occurs in the buds before their petals open while cross-pollination takes place at a later stage when the petals of the flowers unfold and insects visit the flowers to collect nectar.

In general, the pigeon pea flowers are not truly cleistogamous and only part of their life cycle is cleistopetalous. In Lord's (1981) terminology such a condition is known as pre-anthesis cleistogamy. At ICRISAT, however, a case of true cleistogamy has also been reported (Saxena *et al.*,

1992a), where out-crossing is extremely low. This floral variant, where the petals are twisted and inter-locked with each other with an unusual cylindrical appearance, was selected from an inter-specific cross. This trait, controlled by a single recessive gene, ensures a high level of self-pollination in pigeon pea.

2.6 Pod development

In pigeon pea, pods are of various color; green, purple, dark purple, or mixed green and purple. Pods with deep constrictions in shape are beaded, while others are somewhat flat. The seeds per pod range from two to seven, and sometimes up to nine. The seeds are in separate locules and the cross walls develop during the 1st week after fertilization. The pod wall develops more rapidly than the young seeds. Seed development is visible 7 days after pollination. A pod is formed 15-20 days after fertilization. Seeds reach physiological maturity in 30 days and are ready for harvest at lower moisture content in 40 days. There is little or no shattering of mature pods in the field. A week after pollination the pods become visible and complete their development in 15-20 days. The seed attains physiological maturity within 30-35 days and are ready for harvest in 40 days (Rao and Rao, 1974).

In pigeonpea only 10-20 % of flower buds develop into pods with flowering continuing until 75-80 % of the pods mature (Sharma and Green, 1980). At the peak of the flowering, 50 % or more of the flowers may set pods, while towards the end of the flowering period pod set diminishes to zero. An inflorescence may bear two to eight pods. The number in successive inflorescences decreases progressively towards the apical end of the branch. Pods are indehiscent and each contain two to eight seeds (El Baradi, 1978).

The pedicel of the flower contains small vascular bundles surrounded by a ring of fibers. During the first week of anthesis, the endosperm undergoes rapid development. The nuclei take up a parietal position forming a large vacuole in the center of the embryo sac. The embryo sac elongates at the chalazal region and forms a haustorium. The haustorium penetrates into the nucellar tissue. This is instrumental in absorbing food material that is used by the developing embryo. By the end of the second week there are still large amounts of endospermous tissues and within the embryo, distinct cotyledons are seen. Further, developments of the seed involve rapid growth of the cotyledons and almost complete degeneration of the endosperm. In the cotyledons synthesis of starch and protein starts about 17 days after pollination and continues for 14 days (Sehgal *et al.*, 1987). In each raceme 1 to 5 pods may mature, and rarely up to 10.

Reddy *et al.* (2001) reported that in pigeon pea, from the 450 pollinations, only nine mature pods were obtained. Ninety two percent of flowers dropped within four days after pollination and 60 % of the developing young pods either dropped or contained only aborted ovule. From the mature pods seven fully developed and 11 immature shriveled seeds were obtained which produced only five fully developed F₁ plants. Pod setting on the hybrid plants ranged from 17 to 29 % (mean 1.3 %) compared with 52 to 67 % (mean 61.0 %) in pigeon pea and 38 to 47 % (mean 40.4 %) in *C. reticulatus*.

Das and Fakir (2010) concluded that, pigeon pea pod length increased slowly up to 12 days after flowering (DAF) followed by a rapid and linear increase up to 26 DAF. Between 33 and 47 DAF pod length attained a plateau. At 47 DAF, the fresh weight of seed was greater (0.89 g/pod). The

pattern of seed dry weight followed the trend of a sigmoid curve. Changes in dry weight of seed were similar to that of fresh weight. At 47 DAF the longest pod was observed (5.72 cm). Pod width at different DAF followed a trend also similar to that of pod length.

2.7 Seed development

In any crop seeds, the control of seed size involves complex interactions among the zygotic embryo and endosperm, the maternally derived seed coat and the parent plant (Schruff *et al.*, 2006). As the water relations of the developing seed play a fundamental role in seed filling, and seed filling is sensitive to water shortage (Davies *et al.*, 1999).

Chudasama and Thaker (2007) in pigeon pea reported that, based on the growth pattern seed development can be divided in four distinct phases: (i) cell division (0-15 day), (ii) cell elongation (12-36 day in V₁ (black seeded) and 9-36 day in V₂ (brown seeded), (iii) dry matter accumulation (21-42 day in V₁ and 18-39 day in V₂) and (iv) cell maturation (42-54 day in V₁ and 39-54 day in V₂). During embryogenesis zygote divides repeatedly to form an embryo (phase I), these divided cells further enlarge (phase II) together with deposition of storage products (phase III) and the acquisition of desiccation tolerance along with water removal from the maturing seed (phase IV). Since these phases continued for a stipulated time period, a marked overlapping of these phases was observed.

Das and Fakir (2010) in pigeon pea reported that the increase in fresh weight of pericarp was very slow up to 12 Days After Flowering (DAF) which grew rapidly and linearly between 12 and 40 DAF and reached maximum at 40 DAF (0.83 g/pod), followed by a decline afterwards. The pattern of change in dry weight of pericarp was similar to that of fresh weight. There was very little seed fresh weight up to 19 DAF. Fresh weight of seed increased sharply and linearly between 26 and 47 DAF, thereafter it declined. Maximum dry weight (0.29 g/pod) of seed was found indicating physiological mature stage.

In pigeon pea, it takes about 45-50 days from pollination to seed maturity. During this period, both pods and seeds pass through a number of physiological, morphological and chemical changes. Generally, three days after fertilization, the floral petals wither completely and the ovary starts emerging. A young pod of about one centimeter length is visible after about a week. Such pods grow rapidly and achieve their full length in about 25 days. During this period of pod growth, the young seeds (ovules) inside the pods remain intact but do not gain any noticeable size and weight. Soon after achieving the potential pod length, a greater proportion of food reserves of the plant are diverted towards the ovules and rapid increases in their sizes and weights are observed for the next 10-12 days. (Das and Fakir, 2010).

2.8 Natural out-crossing

Unlike other legumes, there is a considerable extent of insect mediated natural out-crossing in pigeon pea. Pathak (1970) was the first to report *Megachile bicolor* and *Apis florea* as cross-pollinating agents in pigeon pea. At ICRISAT's Patancheru campus, although 48 different insect species were found visiting an unsprayed plot of pigeonpea, *Apis dorsata* and *Megachile* spp. were the main pollinators.

The main insect species responsible for natural outcrossing in pigeon pea are bees (*Megachile lanita*) and *Apis florea* (Pathak, 1970), *Apis dorsata* and *Megachile* spp (Williams, 1977).

Prasad *et al.* (1977) reported genetic differences in the natural out crossing among pigeon pea genotypes.

A genotype with cleistogamous flowers that provided a mechanism for almost complete self pollination in pigeon pea was reported. The flower opening in this genotype was delayed and reduced the chances of out-crossing. The development of cleistogamous lines and lines with a high level of natural out-crossing will be helpful in producing pure lines and creating genetic variation in the pigeon pea populations. Further a substantial level of natural out crossing in pigeon pea provides opportunity for exploitation of hybrid vigor (Anon., 1978).

Bahadur *et al.* (1981) reported that, predominantly pigeon pea flowers are self-fertilized with 20-40 % cross fertilization. Growth and development of short stamens is faster than those of longer ones. Self-pollination occurs in the buds before their petals open while cross-pollination takes place at a later stage when the petals of the flowers unfold and insects visit the flowers to collect nectar.

In general, the pigeon pea flowers are not truly cleistogamous and only part of their life cycle is cleistopetalous. In Lord's (1981) terminology such a condition is known as pre-anthesis cleistogamy. Factors affecting natural out-crossing at a given site include flowering habit of a genotype, presence of insect population, temperature, humidity, wind velocity, and wind direction (Bhatia *et al.*, 1981).

Gupta *et al.* (1981a) reported that in pigeon pea, because of frequent natural out-crossing and practical difficulties in maintaining lines by selfing, the existing standard pigeon pea cultivars have become heterogeneous for several important agronomic characters, including disease resistance.

Byth *et al.* (1982) observed in the pigeon pea field, the genotype prabhat exhibited less than 40 % out-crossing while genotype Royes had more than 2 % out-crossing. They attributed the reduced natural crossing in 'Royes' due to the 'wrapped petals,' a modification in its floral morphology. This is a condition where overlapping of the standard petal delays the opening of the flower.

Pigeon pea flowers being cleistogamous favor self pollination. However, natural out crossing of 14 % was reported (Howard *et al.*, 1919). Since then several studies have reported out crossing ranging from 0 % to 70 % (Singh *et al.*, 1990).

A heterosis breeding program in pigeon pea, based on natural out crossing coupled with genetic male sterility have resulted in high-yielding and drought-tolerant hybrids (Saxena *et al.*, 1986b). However, for utilizing natural out crossing for assumed gains in pigeon pea yield, through population breeding and hybrids, it is necessary to determine the level of out crossing in the genotypes at a particular location to develop efficient breeding and seed production strategies (Saxena *et al.*, 1990)

The hybrid seed set on the male-sterile plants is chiefly determined by the availability of bee population in the vicinity. The known prime pollinating vectors in pigeon pea are *Megachile lanata*, *Apis florea* and *Apis mellifera* (Pathak 1970, Brar *et al.*, 1992). Onim (1981) reported that each insect visit lasts for 15–55 days when they trip the un open floral buds, thereby introducing foreign pollen on the stigmatic surface to affect the cross-fertilization. Williams (1977) counted 5,500–1,07,333 pollen grains on the body of a single pollinating insect, of which pigeon pea pollen accounted for 98–100 %

and the pollen basket (*Corbiculae*) of a single pollinating insect approximately 5,000–1,00,000 pigeon pea pollen grains were reported.

In Kenya 24 insect species visited pigeon pea flowers, and each insect visit lasted between 15–55 seconds (Onim, 1981).

In pigeon pea crop, the pollinating insects may visit the male-sterile plants several times and in each visit, a certain proportion of the flowers are pollinated to set the pods while, the un-pollinated flowers should drop. This is followed by the emergence of new flowers on the same plant, and again a proportion of them set pods through open pollination. This cycle continues, and at the end of the season, plenty of crossed pods are observed on each male-sterile plant (Saxena *et al.*, 2005). This phenomenon delays pod maturity on female parent by 3–4 weeks.

The hybrid seed set on the male-sterile pigeon pea plants is chiefly determined by the availability of bee population in the vicinity, pod set is generally poor in the locations with less number of pollinators. Therefore, to harvest good hybrid yields, it is imperative to select suitable seed production sites with good insect pollinator activity (Sameer Kumar *et al.*, 2014).

2.9 Method of pollination

2.9. 1 Selfing method

Selfing is done by covering an individual inflorescence, a branch of a plant, or a whole plot. When a large amount of seed is required, groups of plants are covered with bee-proof cages.

Three types of selfing bags were used for pigeon pea (Gupta *et al.*, 1981b). Glassine bag (13 x 8 cm) to cover an individual inflorescence. A small-muslin-cloth bag (60 x 20 cm) to cover a flowering branch. A large-muslin-cloth bag (135 x 90 cm) to cover an entire plant.

Gupta *et al.* (1981b) reported that, in pigeon pea, number of pods set plant⁻¹ on the selfed plant with non selfed plant (open pollinated) led to the conclusion that selfing done by a large muslin bag gave 225 pods plant⁻¹, against 35 pods plant⁻¹ with a small muslin bag, and 9 pods plant⁻¹ with a glassine bag. The non-selfed plants produced 240 pods plant⁻¹. Therefore, depending on seed requirements, a large- or small-muslin bag is useful for selfing an individual plant.

To produce a large quantity of pigeon pea seed, bee-proof cages were used for handling the segregating generations. These cages were formed with nylon net on a movable angle-iron frame that covers the plot. The objective of a cage was to control the insects. A comparative cost of material indicated that the cost of a cage was four times greater than cloth bags. However, due to reduced labor costs and reusability of the cages, bee-proof cages are preferred over cloth bags for large scale selfing (Gupta *et al.*, 1981b).

In pigeon pea on an average the pod set inside the selfing bags was not very encouraging and it was determined by the extent of foliage growth and availability of sunlight inside the bags. In general the large selfing bags produce of maximum number of selfed pods, the small bags come next and glassine bags the least. In addition, to produce moderate amounts of selfed seeds, varying sizes of bee-proof cages made with nylon mosquito nets were used. Over the years it has been observed that the pod set in these cages is quite high (Gupta *et al.*, 1981b).

Cumaraswamy and Bawa (1989) reported that in pigeon pea, sex allocation theory predicts that resources allocated to androecium should decrease with an increase in selfing. A decrease in

androecium biomass should be accompanied by an increase in the biomass of pistils and a decrease in androecium biomass should be coupled with a decrease in flower size, specifically corolla biomass. Another predicted change in reproductive traits associated with variation in selfing concerns seed to ovule ratios, but does not directly stem from sex allocation theory. It has been postulated that seed to ovule ratios should be positively correlated with the amount of selfing.

2.9.2 Random mating (Mass selection)

In mass selection, seeds are collected from (usually a few dozen to a few hundred) desirable appearing individuals in a population, and the next generation is sown from the stock of mixed seed. This procedure, sometimes referred to as phenotypic selection, is based on how each individual looks. Mass selection has been used widely to improve old “land” varieties, varieties that have been passed down from one generation of farmers to the next over long periods. An alternative approach that has no doubt been practiced for thousands of years is simply to eliminate undesirable types by destroying them in the field. The results are similar whether superior plants are saved or inferior plants are eliminated: seeds of the better plants become the planting stock for the next season.

A modern refinement of mass selection is to harvest the best plants separately and to grow and compare their progenies. The poorer progenies are destroyed and the seeds of the remainder are harvested. It should be noted that selection is now based not solely on the appearance of the parent plants but also on the appearance and performance of their progeny. Progeny selection is usually more effective than phenotypic selection when dealing with quantitative characters of low heritability. It should be noted, however, that progeny testing requires an extra generation; hence gain per cycle of selection must be double that of simple phenotypic selection to achieve the same rate of gain per unit time.

Sanford (1979) reported that a group of biparental crosses of breeding clones and varieties (*Solanum tuberosum* sun sp. *Tuberosum*) from the USDA potato breeding programme, two random mating populations without selection was continued for seven generations, then cycle 1,3,5 and 7 were tested for yield. There was small non significant decrease in tuber weight per hill and a small but non significant increase in tuber number from cycle one to seven.

In the case of open-pollinated species, such as maize, landraces are populations of random mating individuals, approximating the Hardy–Weinberg equilibrium (HWE), with some deviations due to mild selection. Mass selection and recombination of the top-performing portion of the population result in a gradual increase in the frequency of favorable alleles. Successive generations of selection on maize landraces resulted in improved open-pollinated varieties, which were the basis of corn production until the advent of hybrid maize. (Flavio Breseghello, 2013).

2.9.3 Plant to progeny selection

Pure-line selection generally involves three more or less distinct steps:(1) numerous superior appearing plants are selected from a genetically variable population; (2) progenies of the individual plant selections are grown and evaluated by simple observation, frequently over a period of several years; and (3) when selection can no longer be made on the basis of observation alone, extensive trials are undertaken, involving careful measurements to determine whether the remaining selections are superior in yielding ability and other aspects of performance.

Hopkins (1908) developed the progeny selection of ear to row method, extensively used in maize. In its simplest form it consists of 50-100 number of plants are selected on the basis of their

phenotype and are allowed to open pollinate. The seeds from individual plants are harvested separately. A single row of 10-50 plants *i.e.* progeny row is grown from each selected plant.

The progeny rows are evaluated for desirable character and superior are identified. Several phenotypically superior plants are selected from the superior progenies and selected plants are permitted to open pollinate and small progeny rows are again grown from the selected plants and the process of selection is repeated.

Gurumurthy (2005) reported that in maize open pollinated varieties, significant difference in genetic purity percentage was observed due to different pollination methods and variety. It was observed that pollination methods isolated half-sib nursery mating and reciprocal full-sib mating were significantly superior over methods with respect to maintenance of genetic purity. There was no significant effect on germination, field emergence, shoot length, root length, seedling dry weight, seedling vigour index, ear length, ear girth and hundred seed weight.

2.10 Effect of pollination on seed yield and quality parameters

Singh and Rai (1997) reported that, among the open pollinated varieties of Indian rape seed studied, depressing effect of enforced selfing on ovule size was observed to vary between 9.9 % in toria variety D.T.S. to 2.1 % in the yellow seeded variety Sinoy. The mean value of inbreeding depression was 7.0 % in toria, 4.4 % in brown sarson and 2.0 % in yellow sarson. The trend of the depressing effect on the ovule development was also reflected in the similar magnitude in respect of seeds/silique, 1000-seed weight and oil content.

Godawat (1997) found that, Inbreeding effect on seed yield was studied in 125 S_1 lines derived from 25 genetic stock of isabgol. Open pollinated genotypes manifested higher seed yield than their most of the S_1 lines studies. Maximum (45.78 %) and minimum (1.00 %) inbreeding depression was recorded by the S_1 lines of the genotypes FR 161 and ORP 64, respectively. The 81 lines of the genotypes FR 169, ORP 63 and ORP 5₂ exhibited higher seed yield and least inbreeding depression suggested the preponderance of additive gene action and as such these cultivars could be improved by selection.

Susan *et al.* (1999) reported that in crop *Silene douglasii* var. *oraria* (Caryophyllaceae), in comparisons matched by maternal parent, we estimated a selfing rate of 0.38 ± 0.08 from seed viability and 0.26 ± 0.11 from germination. [If the latter is corrected for the loss of products of selfing during seed production. the adjusted rate, S_0 , for seed germination is 0.34.] The selfing rate based on seed number was somewhat higher (0.55 ± 0.03), probably because the equations may overestimate S if open-pollinated capsules fail to set seed or receive less pollen than selfs. Population means based on all progeny (vs. those matched by maternal parents) gave extremely high selfing rates (S): 1.55 (viable seed no. per capsule), 1.26 (percentage viable seed) and 1.14 (percentage germination). In *Amsinckia*, population estimates of selfing (e.g. 160 %) were also extreme, but only when the mean fitness for selfs and natural pollinations exceeded outcrosses.

Hossain *et al.* (2008) reported that, kernel texture is the one of the most important perquisites for success adoption of the Quality Protein Maize (QPM) genotypes by the farmers. The present study was carried out to analyze in detail the effects of pollination mode (controlled- versus open-pollination)

on different kernel attributes (endosperm modification, crown modification and ear appearance) in a set of QPM inbred lines and their experimental crosses. Analysis of ears obtained from different pollination modes indicated significant interaction of the genotypes with the pollination mode, suggesting the importance of the source of pollen and its genetic constitution in conferring the kernel texture. The study also demonstrates that the data derived using controlled-pollination could be more reliable than those using the open-pollination mode for analyzing the effects of complementation of endosperm modifier genes in QPM cross combinations. Ascertaining the kernel modification potential of the QPM genotypes would aid in proper selection of genotypes during QPM breeding.

Pudasaini *et al.* (2014) reported that, the highest germination percent was observed on *Apis cerana* F. pollinated plot seeds (90.50 % germination) followed by *Apis mellifera* L. pollinated plots (87.25 %) and lowest on control plots (42.00 % germination) seeds. Similarly, seed test weight of *Apis cerana* F. pollinated plots (3.22 g/1000 seed) and *Apis mellifera* L. pollinated plots (2.93 gm/1000 seed) were and lowest on control plots (2.26 g/ 1000 seed) recorded. Likewise, oil content was recorded highest on pollinated by *Apis cerana* F. (36.1 %) followed by pollinated by *Apis mellifera* L. (35.4 %) and lowest on control plots (32.8 %). This study clearly indicated pollination increases the seed quality of rapeseed

Yasar Ozyigit *et al.* (2015) reported that pollination is a prerequisite system for reproductive of many plants and it is more important in self-compatible plants. Since, these plants need other flowers pollen for flower fertilization and seed production. In this study, the effects of different pollination methods (open/cross pollination, self-pollination with hand and control) on some features associated with seed production in *Stevia rebaudiana* were investigated. *Stevia* which belongs to the Asteraceae family and is used as sweetener has a self-incompatibility problem. In the experiment, ten plants which were planted in a row were covered with net in the field condition and five of them were selfed with hand pollination and remaining 5 plants were left as it is. Furthermore, five uncovered plants were left to cross-pollination by insects. At the end of the experiment, seed yield per plant, 1000 seed weight, black/filled seed rate, number of day to first germination and germination rate were determined in harvested seeds. According to the results, cross-pollination was more superior in respect to all features in *stevia*. This status shows that insect population (especially bee) must be present in *stevia* fields for successful seed production.

2.11 Cytoplasmic genetic male-sterility (CGMS) system

In pigeon pea, the three - line hybrid system is geared for multiplying A-line seed with the help of B-line and for producing hybrid seed the A-line is pollinated with R-line. In pigeon pea since CMS was not available, plans were made to breed for this trait by placing pigeon pea genome in to the cytoplasm of its wild relative. It was expected that the interaction of such cytoplasm and nuclear genomes would produce male - sterility that would inherited maternally. This endeavour resulted in development of an excellent CMS system that was developed by crossing a pigeon pea line with a wild species, *Cajanus cajanifolius*. It is the most closely related wild species of pigeon pea and is considered as the progenitor of cultivated type that differs only by a single gene (De, 1974). The CMS system derived using this species (Saxena *et al.*, 2005) is considered the best because it has a

number of good maintainers and fertility restorers. The F_1 hybrid plants produce excellent pollen load and pod set. The A- lines with *C. cajanifolius*.

Saxena *et al.* (2005b) reported a stable male-sterile line through wide hybridization. ICPW 29, an accession of *C. cajanifolius* was crossed as female parent with a short-duration cultivar ICP 11501. Subsequently backcrosses were made to develop BC₆F₁ population. They reported that all the 1133 plants in BC₆F₁ generation were complete male sterile. These results were confirmed again in BC₇F₁ generation. The CMS system with cytoplasm from *C. cajanifolius* was denoted as A₄ CMS system. Diversification of this male-sterile line is under progress at ICRISAT.

The CMS-based hybrid pigeon pea technology is now ready for takeoff with all its major components in place. However, considering a vast variation in agro - ecological conditions, fine tuning of the seed production technology is essential to suit local environments. The level of yield superiority observed in hybrids conclusively demonstrated that they have higher yield potential. Now, our major responsibility is to take this new research product to the clients - the farmers. Considering the potential of this technology and excellent prices, it is expected that farmers with both small as well as large holdings will adopt the hybrids. It is also important to keep the hybrid seed costs within the reach of resource poor farmers. In India, both public and private seed sectors are strong and these resources are being used to improve the accessibility of high yielding hybrid seed to farmers (Saxena, 2006a).

Cytoplasmic nuclear male-sterility is the most widely accepted means of producing commercial hybrids in the field crops. The expression of CMS, in part, is controlled by the factors carried only through the female parent, which is never lost or diluted in the succeeding generations of reproduction. Nuclear genes generally influence the expression of this trait, and environmental conditions may also alter its expression in many, but not all, gene-cytoplasm combinations. Cytoplasmic nuclear male-sterility is conditioned by an interaction between nuclear and cytoplasmic factors. The cytoplasmic factor is referred to as 'N' for normal fertile cytoplasm, and 'S' for the sterile cytoplasm (Saxena, 2006b).

The CMS lines are maintained by these cytoplasmic factors. The CMS 'A' line must be homozygous (*msms*) for nuclear genes and the maintainer 'B' line must have a normal (N) cytoplasm and be homozygous (*msms*) for nuclear genes. The F_1 between 'A' and 'B' line is always male-sterile, since the 'N' cytoplasm, which is responsible for fertility in the 'B' line, is not transferred to the F_1 . For producing fertile hybrid seed, the 'A' line in 'S' cytoplasm is crossed with a fertile line with fertility restorer nuclear genes, commonly known as 'Fr' genes. To sum up, the three-line system is geared for multiplying 'A' line with the help of 'B' line and for producing hybrid seed the 'A' line is crossed with 'R' line (Saxena, 2006).

Dalvi *et al.* (2008a) studied the micro sporogenesis in CMS line of pigeon pea with A₄ cytoplasm. They defined that the breakdown of tapetum is the main cause for male-sterility. The F_1 hybrid plants derived from this CMS produce excellent pollen load and pod set. At present, the A₄ CMS system is being used by pigeon pea breeders in India, Myanmar and China (Saxena, 2009) for genetic diversification of A-lines and to produce commercial hybrids.

For commercial development of hybrids the four pre-requisites are; availability of perfect male-sterility system, efficient mass pollen transfer mechanism, hybrid vigor, and the large scale seed production of hybrids for commercialization. The type of male-sterility governs the acceptance of

hybrids by farmers. Genetic male-sterility (GMS) system was not accepted by farmers due to the economics of large scale seed production. The major drawback was rouging of fertile counterpart from the female plot, which was time consuming and labor intensive. Cytoplasmic-genetic male-sterility (CGMS) system usually was a better option for large scale seed production. Hybrid vigor has been utilized in some cereal and vegetable crops. Pigeon pea (*Cajanus cajan*) displays considerable natural out-crossing and now CMS lines are available with different cytoplasmic backgrounds. This mini-review reports the research on development of CMS lines and CMS based hybrids in pigeon pea. Considering the limitations in large scale hybrid seed in production in CMS hybrids, the development of CMS become imperative. The strategy was to induce CMS by placing pigeon pea genome in wild cytoplasm through hybridization. It was believed that the interaction of wild cytoplasm with cultivated nuclear genome would produce male sterility. So far, seven such CMS system have been bred in pigeon pea with varying degrees of these, A₂ and A₄ system derived from crosses involving wild relatives of pigeon pea and cultivated types have shown promise because of their stability under various agro-climatic zones and availability of good maintainers and fertility restorers (Saxena *et.al.*, 2010).

2.12 Isolation distance

Faris (1985) suggested that for quality varietal seed production, two varieties must be separated by at least 100 m, while at distance of 200 m between the varieties essential if the seed is to be used by breeders. In India seed certification standards fixed with regard to isolation distance for pigeon pea inbred varieties are 200 m for breeder seed and 100 m for both foundation and certified seeds (Tunwar and Singh, 1988).

In pigeon pea, the synchronizing the flowering of the parents several hybrids can be produced in the same isolation block where the male parent is used with different male-sterile (female) parents. An isolation of 150-200 m is sufficient for hybrid seed production (Saxena *et al.*, 1986). The isolation block may be ratooned to increase hybrid seed production if both parents are suitable to ratoon and if their flowering nicks.

Sharma (1993) reported that at 100 m contamination due to natural out-crossing was 1.41 % and less than 1 % at the isolation distance of 200 m in pigeon pea.

Saxena (2007) reported that, the commercial seed production of pigeon pea hybrids involves large scale seed production of female (A-) line maintainer (B-) line, restorer (R-) line, and hybrid combination (A x R). For purity maintenance each set of material demands isolation distance of at least 500 m or more.

2.13 Production of hybrid seed

For sustaining the productivity of hybrids, it is important to produce and supply genetically pure hybrid seed to farmers. In general, the purity of hybrid seed is assessed through standard 'grow-out test' using simply inherited morphological traits (Saxena, 2006).

Hybrid seed using male-sterile lines for initial testing can be produced by hand pollination. One trained person can pollinate about 400 buds a day with the efficiency of 30-40 % pods setting seed (Saxena *et al.*, 1986). The production of a large quantity of hybrid seed requires the use of isolation blocks. Four to six rows of the male-sterile parent sown alternately with one row of the

pollinator parent (male) gives good seed-set. The first bud on each plant in the male-sterile rows is examined. All plants with fertile anthers (normally yellow) in the male-sterile rows are rouged out before anthesis to prevent pollination of the sterile plants. Plants with translucent anthers (male sterile) are tagged. The seed is harvested from the male-sterile (tagged) plants. Periodic removal of pods formed on the pollinator (fertile) parent can prolong the availability of pollen.

Hybrid seed production technology pigeon pea with genetic male sterility poses' problems, which involves prompt identification and removal of male fertile sibs, which accounts for 50 % of the population within female rows of a seed production plot. This job is time consuming and laborious involving 40-45 % of the seed production cost. Inefficiency in eliminating the sibs reduces the quality of hybrid seed (Saxena *et.al.*, 1996).

2.14 Female to male planting ratio's

The commercial seed production of pigeon pea hybrids involves large scale seed production of A, B, R lines, and the hybrid combination (A x R). For seed production of A-line, breeder seed of both A- and B- lines are planted using a female: male row ratio of 4:1. In the production areas where greater bee activity is observed a higher row ratio can be used for getting high yields. For hybrid seed production (A x R) also, the same ratio can be used. In general, rouging of off-type plants is carried out both at seedling as well as reproductive stages (Saxena, 2007).

In pigeon pea, for seed increase of A-line, breeder seed of both A- and B- lines are planted at the same time using a female: male row ratio ranging from 4:1 to 8:1. In case of higher insect activity an 8:1 ratio also gives good seed yield, whereas the 4:1 row ratio gives optimum yield at most locations. For hybrid seed production (A x R) also, the row ratios may vary, as above. Timely rouging and strict crop monitoring are critical for seed production. In general, rouging of off-type plants is done at seedling and flowering stages. The available plants descriptors can also be used for effective rouging. Seed production trials conducted for two seasons at ICRISAT have given encouraging results and on average 1135 kg ha⁻¹ seed of A-lines (A x B) was harvested (Saxena, 2007). Similarly for hybrids (A x R), an average of 975 kg ha⁻¹ yield was recorded.

Saxena and Nadarajan (2010) stated that for seed increase of A/B lines, breeder seed of both A and B lines are planted using a row ratio which ranges from 4:1 to 8:1 (female: male), depending upon the insect activity. In case of higher insect activity 8:1 ratio also gives optimum seed yield at most locations. Plant spacing at 150 x50 cm provided the highest mean height (226 cm), biomass (0.25 kg plant⁻¹) pods per plant (359), seed per pod (3.32) and seed yield (98.6 g/plant) while plant spacing of 150 x30 cm give the highest weight of 100- seeds at 14.55 g in pigeon pea (Mula *et al.*, 2010 a).

Mula *et al.* (2010b) conducted an experiment to evaluate agronomic variability for large scale seed production of ICPH 2671 hybrid from a cytoplasmic male-sterile (CMS) line ICPA 2043 viz., direct and interaction effects of row ratio, planting distance and irrigation. The treatments included two planting ratios of 4:1 and 3:1 with male sterile (MS): male fertile (MF) lines; two row spacing (75 cm X 150 cm) two plant to plant spacing (30 cm and 50 cm) the study clearly revealed that individual plants sown at wider spacing have better growth and yield traits than at closer spacing.

Mula *et al.* (2011) reported that growth and yield of female parent ICPA 2043 were significantly affected by row ratio, plant spacing and soil moisture availability. In Alfisols, the spacing of 75 X 30 cm with 3:1 row ratio and irrigation at every 14 days produced A-line seed yield of 1872 kg ha⁻¹. In vertisols, the seed yield of 2357 kg ha⁻¹ was recorded at Patancheru at the spacing of 75 X 30 cm with 3:1 row ratio and irrigation at every 21 days.

2.15 Influence of insect attractants spray on pollinators, seed yield and quality

2.15.1 Pollinators

The role of insects as pollinators was first reported by Kolreuter in 1763. It was estimated that in nature 5 % of flower self-pollinated and 95 % are cross-pollinated, out of which 10 % depends upon wind and 85 % upon the insects. The insects of the family apidae are the most reliable agents for pollination. Among apidae honey bees are particularly important pollinators as they are capable of carrying pollen and nectar with which they feed their immature stages and in the process, the plants visited by them are benefitted. About 100,000 kinds of flowers in this universe are assumed to be dependent on honey bees and other insects (Tewari and Singh, 1983).

Pollination by insects is called entomophily which includes cantherophily by beetles, myophily by flies, mellitophily by bees, psychophily by butterflies and phalanophily by moths. Obtaining adequate pollination in crops that must be pollinated by insects has become a problem in our agricultural economy (Tewari and Singh, 1983).

The importance of the colour, size and shape of flower in the attractants of pollinators has a long been recognized (Dafni and Neal, 1997). Plants pollinated by animals need to attract their pollinators in order to reproduce. This creates a double challenge. First, they must use signals that are detectable by the pollinator, and second, they must provide the pollinators with reward to ensure their repeated visits. Mimicry by plants can reduce the cost of attracting pollinators if no reward is given, but in order to be effective, the plant must change appropriate floral signals to match those of a model plant offering rewards (Macior, 1971 and Rathcke, 1983).

It is often assumed that many flowering plants owe much of their floral polymorphism in colour, shape and odour to the combined effects of attracting different pollinator species and to the spatio-temporal variation in the selection regimes they are subjected to (Galen and Kevan, 1980; Pettersson, 1991; Eckhart, 1992; Ollerton *et al.*, 2006 and Vereecken and Schiestl, 2009). Johnson and Steiner (2000) reviewed pollinator specificity in plant pollination systems and concluded that the degree of specialization evolved via a combination of modified characters, including the advertisement of specific scents and colours, restrictive (canalized) floral morphology, nectar and, in some cases, unusual rewards such as oils, fragrances, resins, *etc.* (Li *et al.*, 2008).

Sanjivan Kumar *et al.* (2000) reported that sunflower crop sprayed with sugar syrup attracted maximum bees (20.74 bees/5flowers/min), followed by Bee-Q (18.10 bees/5flowers/min).significantly more number of *A. dorsata* up to seven days after first and second spray on Niger. Bee-Q @ 12.5 g/l,

cinnamon leaf extract at 5 % and tuberose flower scented water at 10 % were the next best treatments in attracting more number of pollinators.

Guru Prasad (2001) reported that Fruit boost sprayed at 0.5 ml/l of water attracted significantly more number of *A. dorsata* upto seven days after first and second spray on Niger. Bee-Q @ 12.5 g/l, cinnamon leaf extract at 5 % and tuberose flower scented water at 10 % were the next best treatments in attracting more number of pollinators.

Viraktamath and Patil (2002) reported that application of Fruit boost and Bee-Resulted in more attraction of bees on sunflower. Similarly application of Bee-Q, sugar solution, cinnamon leaf extract and molasses enticed more pollinators on mustard flowers

Manjunatha (2003) reported that spraying of Fruit boost and Bee-Q significantly enhanced visitation by *A. dorsata*, *A. mellifera*, *A. cerana* and other pollinators in sunflower. However, the effect of the attractants lasted for three to five days. *A. mellifera* foraged for longer time (0.43-0.51 min) in treated plot.

Chandrashekhar and Sattigi (2009) reported that, the effect of bee pollination on quantitative and qualitative parameters of radish was carried out in rabi-summer season of 2004-05. Crop was raised at Marewada village, nearer to University of Agricultural Sciences, Dharwad. Spraying of bee attractants like cacambe (10 %) and jaggery solution (10 %) were significantly superior in enhancing both quantitative and qualitative parameters of radish seed.

2.15.2 Seed yield parameters

Prasad *et al.* (1989) reported maximum siliqua length in bee pollinated plots of radish. The crop sprayed with cacambe (10 %) and jaggery solution (10 %) produced more number of seeds (13.66 and 13.58 g/1000 seeds) respectively. These treatments were on par with Bee-Q (1.25 %) and sugar solution (10 %) followed by sugar cane juice, crop caged with bees and molasses (10 %). These results indicated that application of attractants could increase the yield due to increase in activity of the pollinators.

Singh and Chamotre (1992) reported that seeds per siliqua was maximum in open pollinated radish. The crop sprayed with cacambe (10 %) recorded higher number of siliqua per plant (500.16 siliqua/plant). This accounted for 27.69 and 55.51 per cent increase over crop without any spray and crop caged without bees, respectively. This treatment was found to be on par with molasses (10 %), Bee-Q (1.25 %), sugarcane juice (10 %), sugar solution and crop caged with bees. The lowest number of siliqua per plant was found in crop without any spray and crop caged without bees.

Bhosle *et al.* (1992) reported that sunflower cv. Morden when sprayed with 2.5 per cent sucrose solution, resulted in greater seed yield than control.

Kalmath (2002) who noticed that the onion yield was increased due to crop sprayed with cacambe (10 %) and jaggery solution (10 %) and Bee-Q (1.25 %) at flowering stage.

Chandrashekar and Sattigi (2009) reported that, in radish crop sprayed with cacambe (10 %) produced higher number of seeds/siliqua (7.40 seeds/siliqua) which accounted for 85.00 per cent and 111.42 per cent increase over crop without any spray and crop caged without bees. This is on par with Bee-Q (1.25 %) and sugar solution (10 %).

Jayaramappa *et al.* (2011) found that spraying of fruit boost @ 0.5 ml/l and Bee-Q @ 12.5 gms/l enhanced yield parameters like number of fruits / plant to 19.00 and 17.00 fruits, when compared to 10.66 fruits / plant in open pollinated plot. Number of fruits was 21.83 and 20.83 fruits / plot, when compared to 15.68 fruits / plot in open pollinated plots. Fruit length was 42.41 and 41.21 cm, when compared to 32.95 cm in open pollinated plots. Fruit weight was 285.00 and 246.33 g, when compared to 229.67 g in open pollinated plots and yield was 154.00 and 152.00 q/ha, when compared to 130.67 q/ha in open pollinated plots of ridge gourd.

Sivaram and Jayaramappa (2013) reported that, in pigeon pea the insect attractant spray (Bee-Q 12.5 g/l) has recorded the significantly the highest number of seeds/pod (5.33 seeds), 100 seed weight (13.45 g) and seed yield per plant (22.68 g) as compared to open pollinated crop without insect attractant spray.

Venkataramgowda Sivaram *et al.* (2013) found that the use of bee attractants, Bee-Q and Fruit Boost™ in the pollination of niger was evaluated. Bee visitations to niger flowers were observed for two weeks and an estimation of seed yield was determined. Different concentrations of Bee-Q and Fruit boost™ were evaluated to understand honeybee visitation patterns on the target crop and pollination efficiency. Results indicate that applications of Bee-Q at 12.5 g/l and Fruit boost at 0.75 ml/l on niger plots significantly increased the number of bee foragers over control plots. In addition, plots sprayed with these bee attractants significantly enhanced the seed set, seed weight of niger. This study suggests that pheromone-based bee attractants applied to niger can increase the marginal percentage of bee visitation and seed yield.

Jayaramappa and Bhargava (2015) studied that the usage of bee attractants, Bee-Q and Fruit Boost™ in the pollination of watermelon. The bee visitations on target crop flowers were made for two weeks followed by estimation of yield parameters. The different concentrations of Bee-Q and Fruit boost™ was evaluated to understand the honeybee visitation pattern of the target crop for improving pollination efficacy. The observations indicate that, Bee-Q at 12.5 g/l and Fruit boost at 0.5 ml/l of watermelon plots meagerly attracted a number of bee foragers than the control plots. In addition, the plots sprayed with bee attractants marginally enhanced of the fruits/plant, weight of fruits. They concluded that the bee attractants increases certain amount of bee visitation in turn, leads to increase in fruits/plant and weight of fruits on watermelon.

Ma *et al.* (2015) reported that the fruit set rate and yield of pear are commonly low due to insufficient pollination, as the species is unattractive to honeybees. To improve honeybee foraging behavior for the pollination of Dangshan pear (*Pyrus bretschneideri* cv. *dangshansuli*), nine methods were used to attract bees. A control treatment of colonies was fed with normal sugar syrup, while six other treatments were fed using sugar syrup mixed with Pear syrup, Gallic acid, Arginine (Arg), Lysine (Lys), Methionine (Met), or 8-Br-cGMP; plates containing Juvenile Hormone analog ZR-512, Brood Pheromone (BP), and Queen Mandibular Gland Pheromone (QMP) were placed inside the hives of another three treatments. Pollination efficacy was compared using the pollen load weight and quantity of foraging bees. The peak time of pear pollen gathering was 10:00–11:00 am regardless of treatment. The pear pollen load weight per day was increased by all nine treatments. Pear pollen load weight per day was 49.11 g in the control. The QMP treatment yielded the heaviest pear pollen load

weight per day (77.56 g), followed by the 8-Br-cGMP (64.45 g) and BP treatments (64.20 g). The percentages of pear pollen weight and quantity in the total pollen per day were both highest in the BP treatment (80.23 %, 87.27 %), followed by those in the QMP (79.32 %, 86.74 %) and Lys treatments (76.25 %, 85.81 %).

2.15.3 Seed quality parameters

The bee pollination not only increased the yield but also improved the quality of the crop. Such improvements have also been reported by some workers. Increase in seedling vigour of safflower due to bee pollination was reported by Lingappa *et al.*, (1999).

Kalmath (2002) who observed highest germination per cent in onion seeds obtained from plot sprayed with cacambe (10 %), jaggery solution (10 %) and Bee-Q (1.25 %).

Chandrashekar and Sattigi (2009) reported that, in radish the seeds from open pollinated crop sprayed with cacambe (10 %) showed significant superiority with higher percentage of germination accounting for 97.13 per cent, shoot length (16.80 cm) and root length (10.83 cm). These resulted in an increase of 14.93 and 18.94 per cent over open pollinated crop without any spray and crop caged without bees, respectively. This treatment was found to be on par with Bee-Q (1.25 %), sugar solution (10 %), sugarcane juice (10 %) and crop caged with bees.

A laboratory experiment was carried out at Seed Quality and Research Laboratory, National Seed Unit, University of Agricultural Sciences, Dharwad during the Rabi-Summer 2010-11. The experiment consisted of eight treatments combination involving two insect attractants, A₁-Jaggery solution @ 2 % and A₂-Commercial attractant (Bee-Q @ 0.175 %). Among the insect attractants sprayed, Bee-Q recorded higher test weight (2.62 g), germination percentage (91.16) and vigour index (1373) sprayed at the time of 50 per cent flowering stage and also showed lower electrical conductivity (0.264 ds/m) in Alfalfa (*Medicago sativa* L.).

Sivaram and Jayaramappa (2013) found that, the insect attractant spray (Bee-Q 12.5 g/l) has recorded the significantly the highest germination percentage (88.00 %), root length (6.28 cm) and shoot length (6.20 cm) as compared to open pollinated crop without insect attractant spray in pigeon pea.

2.16 Morphological characterization

The variety characterization and purity assessment are very important for maintenance, multiplication, seed certification and quality control of released varieties. Hybrid development and its parental lines identification is one of the most important aspects of seed industry and seed trade. The term variety is defined as an assemblage of cultivated plants, which are distinguishable by morphological, physiological, chemical and cytological characters, provided these characters are heritable, stable and distinct. According to International union for the Protection of Plant Varieties, new crop variety has to be distinct from other varieties and uniform in its characteristics which are genetically stable in yield.

Seeds, seedlings and plants of various cultivars are known to exhibit a wide range of morphological distinctness which is helpful in varietal identification and genetic purity testing.

Morphological characters of seeds, seedlings and plants are useful traits for varietal identification and differentiation. These characters are useful for the identification of the varieties by farmers for quality seed production.

The pigeon pea leaflets are trifoliate and lanceolate. Segregation of stipule length was observed as a ratio of 9 long : 7 short with two complementary genes '*Lsta*' and '*Lstb*' (Deokar and D'Cruz 1972). A broadleaflet base was controlled by two dominant genes (*Bdlba* and *Bdlbb*) which were dominant to a narrow leaf base (Kolhe *et al.*, 1972).

Two genes *Gpstd* and *Gpshtd* control the pod color in epistatic manner, 9 purple : 3 green with purple streaks: 4 green with purple shades (Deokar *et al.* 1971a). Four-seeds pod⁻¹ monogenically dominant to three-seeds pod⁻¹ (Rekhi 1966). Singh *et al.* (1980) reported reciprocal difference with poor pod setting as a dominant character. Backcrosses showed a cumulative effect.

D'Cruz *et al.* (1974) reported monogenic inheritance of seed coat color (3 brown : 1 white). D'Cruz *et al.* (1973) also reported complementary genes (9 brown : 7 white) for seed-coat color. The purple-splashed seed coat was incompletely dominant over chocolate seed coat and light-brown seed coat (Ganguli and Srivastava, 1967).

The vein color (*Drv*) of the standard petal was reported as being linked with the seed-coat color (*Plsd*) (Chaudhari and Thombre 1975). The complementary factor of vein color showed a linkage with the basic factor of leaflet shape, with a crossover value of 24 % (Chaudhari and Thombre, 1977).

D'Cruz and Deokar (1970) reported that the spreading branch habit (*Sbr*) forms a linkage group with the purple stem (*Pst*), lanceolate leaflets (*Llt*), and long petiole (*Lpt*); while the maroon-blotched pod (*Gppd*) gene forms a linkage group with the red-brown seed coat (*Brsda*). D'Cruz *et al.*, (1974) reported that stem color (*Pst*) was linked with leaf thickness (*Tnlt*) and leaflet shape (*Llt*) forming a linkage group *Pst*, *Tnlt*, and *Llt*.

Ganguli and Srivastava (1967) reported that in pigeon pea mutual association among length of pod, number of pods, and 100-seed mass that were negatively correlated with grain yield. Total branches plant⁻¹, fruiting branches plant⁻¹, pods plant⁻¹ and leaves plant⁻¹ were significantly correlated with yield and among themselves. These could be used as selection criteria.

Pod number and seed size were reported to be an important component of yield in pigeon pea (Reddy and Faris, 1981). Significant direct effect of pods plant⁻¹ was observed on yield (Pokle and Mahalkar 1976). Hence, Patil *et al.*, (1988) concluded that plant height, pods plant⁻¹, effective branches plant⁻¹ and days to maturity are important attributes for yield improvement in pigeon pea.

Saxena *et al.* (1983) studied that the effect of pod colour on important organo-leptic properties of vegetable pigeon pea. They found that fresh seeds harvested from purple pods had poor structure, flavor and taste as compared to those of green seeds; but after cooking operation such differences disappeared, suggesting that the pod colour does not play any important role in determining the organo-leptic qualities of vegetable pigeon pea.

Yadavendra and Patel (1983) reported that the pods produced on cultivar ICP-7979 were the most preferred because of their good taste, attractive green colour, less stickiness and easy shelling.

Ravi Kumar and Narayanaswamy (1999) reported that morphological characters of ten soybean cultivars. They found that seed coat colour, hilum colour and seed shape can be successfully used for identification of varieties in seed production.

Jain and Khare (2002a) concluded that, among all characters at seed level, seed shape, hilum shape, seed coat colour and lusture on seed are the most stable, uniform and distinguishing characters in green gram.

Khare *et al.* (2002) observed that the genotypes with dark seed coat exhibited greater (64.5 %) survival in comparison with the light-coloured (54.4 %) genotypes. As seed coat colour of ICPH 2671 is dark, the presence of various phenolic and tannins reduced the rate of water uptake in the seeds. They concluded that, the dark-seeded pigeonpea genotypes encountered waterlogged situations much better than those with light-coloured seeds. The greater water logging tolerance in the hybrid may also be related to relatively high initial vigour of the hybrid plants, which experienced less oxygen deprivation during submergence as compared to pure line cultivars.

Godoy *et al.* (2003) reported that 17 inbred lines of pigeon pea morphologically and described their growth cycle and in 2004 Godoy *et al* morphologically described 11 more inbred lines of pigeon pea.

Byre Gowda *et al.* (2003) conducted experiment on BRG-1 A high yielding and bold seeded pigeon pea variety for dhal and vegetable purpose in GKVK, Bangalore and he characterized the variety BRG-1 is a medium duration variety with indeterminate growth habit and many distinguishable features over Hy-3C. The flowers of this variety are pink and pods are green with few black streaks. Time to 50 % flowering ranges from 90 to 95 days and maturity from 175 to 190 days. BRG-1 has bold white, mottled seeds with 100 seed mass of 18.5 to 19.4 g.

Arun kumar *et al.* (2004) reported that 45 cultivars of pearl millet including 14 hybrids and their parental lines were characterized using qualitative morphological characters of seeds (seed colour as green, grey, grey brown and brown), seedling characters and plant morphological characters.

Karivartharaju (2005) found that a list of seed characters viz., fuzz colour, seed size, 100 seed weight, density of fuzz and fibre characters like colour, strength, fineness, uniformity and maturity, ginning percent for characterization of cotton varieties.

Nkouannessi in 2005, characterized 20 cowpea genotypes procured from different countries viz., Cameroon, South Africa, Kenya and characterized them based on seed shape, seed testa texture, hundred seed weight.

Chandrashekar (2005) conducted the experiment on Influence of the age of the seed on plant growth, seed yield and quality and characterization of French bean genotypes in department of seed science and technology, UAS Dharwad. He reported that hypocotyl colour expressed in different French bean genotypes were as purple, light green, light purple and pale green.

Prashanth (2005) reported that, based on seed shape, categorized French bean varieties as cylindrical kidney and oval shape. Seeds also were classed into large, medium and small size. Prominent seed coat colours observed were black, brown and white and some more classes on the

different intensities of these colours are also done. Based on the seed shape RSJ-288 was individually categorized as cylinder, large size with black seed coat colour.

Monica *et al.* (2007) classified nineteen varieties of rice belonging to non basmati groups based on fifty two morphological descriptors at different stages of plant growth. Though the cultivars showed overlapping of descriptor expression in various combination traits, still the identity of all the cultivars in non-basmati as well as basmati group could be established individually and would be helpful for registration/ protection of these varieties.

Nalini Mallikarjun (2007) studied that F_1 hybrids were pollen sterile, but female fertile. Their morphology was intermediate between the two parents. *Cajanus platycarpus* has trailing growth habit and cultivated pigeon pea plant grows upright. F_1 hybrids were obtained by rescuing the aborting hybrid embryos *in vitro*. Morphologically F_1 hybrids had intermediate growth habit with upright main stem and semi-trailing branches.

Manyasa *et al.* (2007) characterized 123 pigeon pea landraces for diversity using 16 qualitative and 14 quantitative traits. Significant polymorphism was recorded in qualitative traits viz., base flower colour, pod colour, flowering pattern, streak pattern seed colour and seed shape.

Provazi *et al.* (2007) identified 11 pigeon pea lines based on morphological characters such as plant height, number of branches, stem diameter, basic flower colour, basic pod colour, seed colour, seed hilum colour and thickness and seed shape.

Meena *et al.* (2008) studied forty five genotypes of Indian mustard were evaluated to assess genetic variability for seedling traits. High genetic variability was found for seed yield, root length, fresh seedling weight, vigour index, seedling dry weight, shoot length and 1000 seed weight.

Bhadru (2008) in pigeon pea determined the genetic parameters of 12 quantitative traits including seed yield in 50 pigeon pea genotypes and observed wide genetic variation for number of pods, seed yield, plant spread, raceme length and plant height.

Saxena (2008) showed that in pigeon pea important economic traits such as seed yield, pods/plant, plant height, seed size and seeds/pod are predominantly controlled by both additive as well as non-additive genetic variances. The level of realized heterosis for seed yield in pigeon pea is comparable to other crops where commercial hybrids have already made a mark in global agriculture (Saxena, 2009).

Saxena *et al.* (2010) concluded that pigeon pea plants produce profuse flowers and pods under normal growing conditions. The number of pods on the plants is also genetically related to their pod size. Pod load on an individual plant is much higher than those of large seeded varieties. Popular vegetable pigeon pea varieties are characterized by their large pods and large seeds. It has been generally observed that in most germplasm, these two traits are linked together and such lines are invariably photo-sensitive, late maturing (>180 days at 17°N) and perennial in nature. These cultivars flower at the onset of short photo-periods and produce fresh vegetable pods for about 40-50 days, allowing a maximum of 2-3 pickings. ICRISAT has a global responsibility for collection, characterization, maintenance and distribution of pigeon pea germplasm and at present a total of 13,632 accessions representing 76 countries are available for use in breeding programmes. Since long pod size is the most important characteristic of vegetable pigeon pea, the accessions with more than 5.5 mean seeds/pod are considered in this group. At present there are 231 accessions in this

group. In this material 50 % flowering ranged from 80 to 229 days. The plant height ranged from 85 to 285 cm, while pod length varied from 3.2 to 11.6 cm.

Sreelakshmi *et al.* (2010) reported that thirty pigeon pea genotypes for genetic divergence and observed considerable variability for days to 50 % flowering, days to maturity, plant height, number of pods per plant and seed yield.

Acharya and Patel (2011) reported that classification of the 45 newly identified stable CGMS (A) and 26 restorer (R) lines of different background of pigeon pea based on seed size and seed colour.

Upadhyaya *et al.* (2011) concluded that the phenotyping composite collection of 1000 pigeon pea accessions for 16 quantitative and 16 qualitative traits resulted in the identification of promising diverse accessions for the four important agronomic traits; early flowering (96 accessions), high number of pods (28), high 100 seed weight (88) and high seed yield per plant (49).

Dixit *et al.* (2011) characterized 29 released Indian cultivars of lentil based on seed size and seed testa colour.

Acharya and Patel (2011) classified 45 newly identified stable CGMS (A) and 26 restorer (R) lines of different genetic background of pigeon pea based on seed size and seed colour.

Girish *et al.* (2012) studied that evaluation of hundred genotypes of cowpea for variability, heritability and genetic advance for eleven quantitative characters. Significant difference among the genotypes for all characters was noticed for all the parameters, except days to flowering and days to maturity. The highest phenotypic and genotypic variances were recorded for plant height (14.93 and 71.03 cm) and seed yield per plant (17.35 and 16.30 g). The lowest phenotypic and genotypic variances were observed for primary branches per plant (1.11 and 0.97) and secondary branches (2.83 and 2.34).

Photo and thermo sensitivity is the major issues in the crop restricting the horizontal expansion to different cropping systems in varied agro ecologies. Traditional cultivars of pigeon pea are of early (120 to 140 days), Medium (140 to 160 days) and Long duration (> 160 days) types which cannot fit in preceding or proceeding crop situations of rainfed and irrigated ecologies. Super early lines mature within 100 days and have yield potential up to 1.0 to 1.5 t/ha (Vales *et al.*, 2012).

As the seed colour of commercial grains of hybrid ICPH 2671 is dark due to specific complementary gene action (Saxena *et al.*, 2012a), its marketing posed some problems in the states of Karnataka, Andhra Pradesh and Maharashtra where brown seeded pigeon pea is preferred. However, in the states of Madhya Pradesh and Jharkhand, the seed colour was not an issue, and hence, this hybrid was released in Madhya Pradesh.

Muniswamy *et al.* (2014) reported that morphological characterization indicated that characters like determinate growth habit can be successfully used to differentiate ICP-7148 and GC-11-39 from rest of the genotypes. Further, oblong leaf shape could separate ICP-7148 and Gulyal (a land race from Gulbarga region of Karnataka) from rest of the entries. Anthocyanin colour on hypocotyl was observed in seven out of 196 genotypes.

Naima *et al.* (2014) found that the 20 land races of cowpea and characterized based on plant morphological characters such as flower colour, seed number per pod, pod purple pigmentation, pod length, number on seeds per pod and days to 50 per cent flowering.

2.17 DNA markers

DNA markers have the potential to enhance the quality of plant breeding and seed technology programme by providing simply inherited, phenotypically neutral identities to complex traits. However, the greatest potential appears to be in marker assisted selection of varieties identification, quantitative and qualitative traits condition complex economic traits. DNA markers also permit plant breeders to correctly map or place the various interacting genes that condition complex agronomic traits.

Molecular marker have been used to identify and characterized QTL associated with several different traits in cereals, pulses and oil seeds including plant height and maturity (Pereira *et al.*, 1994).

DNA markers are simply detected differences in genetic information between two or more individuals, which are phenotypically neutral, polymorphic, abundant and dominant or co-dominant in nature (Tanksley, 1993) and are therefore extensively used in construction of genetic linkage maps. Several types of DNA markers have been used widely (Mohan *et al.*, 1997, Gupta and Varshney, 2000 and Babu *et al.*, 2004) for linkage mapping *viz.*, RFLP's (Botstein *et al.*, 1980).

2.18 Genetic diversity and molecular markers

Genetic diversity is carried out for several purposes including phylogeny, breeding, germplasm conservation and variety identification. Morphological traits and biochemical markers were used before the advent of molecular markers. However, molecular markers, such as RFLPs, AFLPs, RAPDs, SSRs and SNPs are free from environmental influence and can be scored at any stage in plant growth (Gupta *et al.*, 1999). They are now extensively used for genetic mapping, germplasm characterization, and to improve the efficiency of conventional breeding (Rafalski and Tingey, 1993).

2.19 Simple sequence repeats (SSRs)

Powell *et al.* (1996) reported that simple sequence repeat markers are also known as microsatellites or short sequence repeats. They are tandem repeats of between two to six bases occurring throughout all plant genomes. They are abundant across the genome and are developed using primers designed to flank the repeat sequences. SSRs are also highly polymorphic (*i.e.* show up differences between different individuals analysed), easy to use and automate, codominant (*i.e.* can discriminate between individuals that are heterozygous and homozygous) and multi allelic *i.e.* (produce a number of different sized PCR products for a single pair of primers across a range of individuals).

Shuelke. (2000) stated that to allow PCR amplification, primers are designed to flank the SSR loci. The amplification products are separated on silver-stained polyacrylamide gels or by capillary electrophoresis, which incorporates fluorescence detection systems. For separation with fluorescent detection systems the primers (usually the forward primer) are synthesized with a fluorochrome attached to the 5' end. Alternatively, two different forward primers can be used in the PCR step. The first primer, used in the first few PCR cycles, is designed to contain an M13 sequence at the 5' end, in addition to the unique primer sequence.

A tool that will allow the DNA fingerprinting of especially, the most popular cultivated and newly developed and/or released varieties, will allow scientists, seed producers and seed producing organizations as well as bodies that regulate the quality control of seed purity *etc.* to accurately determine if a variety is pure and accurately labeled. The Malawi Seed Alliance, which includes the official seed certification unit in Malawi, has recently expressed a need for such a tool as it will allow

them to better track how well seed purity and identity is being maintained and will allow for improved confidence in testing, maintaining and providing pure, good quality seed to farmers (ICRISAT-Lilongwe, personal communication). For this reason, this study investigated the possibility to identify a small number of polymorphic SSR markers that can provide a DNA fingerprint for the most important cultivated and released varieties of pigeon pea in Malawi, *i.e.* ICP 9145, Mtawajuni, ICPV 87105, ICEAP 00040 and ICEAP 00057 (Soko, 2000 and Jones *et al.*, 2002).

Numerous studies have led to development and subsequent utilization of many SSR markers in pigeonpea diversity analyses. The information on diversity revealed in this study across the cultivated and gene bank pigeon pea genotypes will be important for future breeding programmes, germplasm conservation efforts and seed certification by a DNA fingerprint (Burns *et al.*, 2001; Odeny *et al.*, 2007; Odeny *et al.*, 2009; Saxena *et al.*, 2010b, Saxena *et al.*, 2010c).

Large numbers of SSR markers have been developed and applied in pigeonpea diversity studies (Burns *et al.*, 2001; Odeny *et al.*, 2007; Odeny *et al.*, 2009; Saxena *et al.*, 2010b and Saxena *et al.*, 2010c). Unlike AFLPs, SSRs are co-dominant and abundant across the genome. They are also multi-allelic, amenable to high-throughput applications and detect more polymorphism (Gupta and Varshney 2000; Salgado *et al.*, 2006).

In pigeon pea genotypes SSR markers convincingly brought out differences between the early, medium and late flowering *C. scarabaeoides* accessions of Indian origin, further confirming the congruence with morphological/phenological grouping (Aruna *et al.*, 2004).

Compared to non-coding SSRs, coding SSR (genic-SSR) are less polymorphic since coding region has high level of selection pressure and so evolve slowly. On the other hand, variation in the genic-SSR may alter the function of gene and therefore reflect functional variation (Varshney *et al.*, 2005). Therefore, genic-SSR has great importance for genomic research.

The study clearly demonstrated that all the three marker types could be used for studying diversity among the wild and cultivated pigeon pea. All three marker types yielded highly polymorphic bands. Similar results were observed in wild and cultivated Sorghum (*Sorghum bicolor* L.) species. Where, the levels of polymorphisms ranged from 60 % for AFLPs, 80 % for RFLPs and 100 % for SSRs. SSRs are tandem repeats that are between two and six bases long and occurring abundantly in a genome. Diversity at the SSR loci is due to the variable number of repeat units. This variation is caused by slip-strand mis-pairing, which occurs during DNA synthesis and results in a gain or loss of one or more repeat units (Semagn *et al.*, 2006).

SSR markers are one of the most used molecular markers in genetic analyses of many plants. As they became popular, development of SSRs was confined mostly to important food crops, namely, rice, maize and wheat and the so-called 'orphan crops', like pigeon pea were neglected (Varshney *et al.*, 2009). However there has been an increase in the number of polymorphic SSRs now available due to a recent initiative in pigeon pea genomics (Varshney *et al.*, 2010).

Saxena *et al.* (2010) identified a set of simple sequence repeat (SSR) markers for testing the hybridity of ICPH 2671. They used 148 SSR markers for polymorphism survey on 159 parental lines of hybrids. Of these, a total of 41 markers were found polymorphic

The variation observed in SSRs mostly found to be depends on their occurrence in coding or non-coding region in the genome Singh *et al.* (2010). Furthermore due to their robustness, SSRs are

resourceful in assessing genetic purity and can even distinguish between pigeon pea hybrids (Saxena *et al.*, 2010d; Datta *et al.*, 2010 and Upadhaya *et al.*, 2011).

Bohra *et al.* (2011) used a set of 18 149 SSR markers that could be used in multiplexes for assessing the hybridity of ICPH 2671. From this study a set of 42 SSR diagnostic markers were identified for hybrid ICPH 2671. This assay can now be used by both the public and private seed companies for reliable detection of seed purity within the commercial seed lots of hybrids ICPH 2671 to ensure the supply of high-quality seeds.

SSR are tandemly repeated motifs of 1-6 bases found in all prokaryotic and eukaryotic genomes analyzed to date. They are present in both coding and non-coding regions and are usually characterized by a high degree of length polymorphism (Sonah *et al.*, 2011a).

SSRs are beneficial over other molecular markers as they are more reproducible, highly polymorphic, co-dominant inheritance, abundance and uniform distribution in nuclear as well as organelle genome (Sonah *et al.*, 2011b).

Moreover about 330 of these polymorphic SSRs were used to construct a consensus pigeonpea genetic map and their exact location in the chromosomes is now known. Therefore because of their ease of use and high polymorphism, SSRs were selected for use in this study. (Bhora *et al.*, 2012).

Romi Oinam *et al.* (2015) concluded that, the accuracy in estimation of genetic diversity by using morphological and isozyme markers. In pigeon pea, such tests take more time due to the long duration of the crop and feasibility to raise a single crop in a year, particularly in medium and long duration of pigeon pea hybrids. Therefore, a simple, rapid and cost-effective hybrid seed quality testing approach in pigeon pea based on molecular markers assay is very much needed.

2.20 Hybrid seed purity testing

Supply of adequate quantities of pure hybrid seeds to the farmers and maintenance of parental lines of hybrids are other important challenges. Any trace of impurity in the hybrid seeds can substantially affect the productivity, in addition to the purity of the parental lines which is utmost importance for the success of the hybrid breeding programme. Traditionally, breeders perform Grow Out Test (GOT) on representative sample of the seed lot to assess the purity. GOT depends upon several morphological and floral characteristics for 195 determining the purity of seeds which is time consuming and particularly in pigeon pea it requires a season for assessing the purity and also very much labor intensive in comparison to molecular marker based assessment, which saves time and cost.

To maintain a variety true to type especially in partially out-crossed species, it needs lot of resources in terms of isolation distance, installation of insect proof cages and labor charges for rouging and seed cleaning operations. Considering these facts attention was paid on natural mutant with wrapped flower morphology or cleistogamy (Saxena *et al.*, 1994).

Molecular markers based purity testing of hybrid seeds have provided better options in a number of crops species and are in routine use in many species like rice (Sundaram *et al.*, 2008), maize (Asif *et al.*, 2006), cotton (Ali *et al.*, 2008), safflower (Naresh *et al.*, 2009).

Similarly, in pigeon pea SSR based markers have been developed for purity assessment of hybrids and recently a gene based marker for differentiating CMS lines and maintainer lines derived

from A₄ CMS system developed. In the very first such study, two diagnostic SSR markers were identified for purity assessment in ICPH 2438 (Saxena *et al.*, 2010).

Subsequently, 42 SSR markers for each of the two hybrids (ICPH 2671 and ICPH 2438) have been identified for purity assessment of hybrid seeds (Bohra *et al.*, 2012). Moreover, common markers (CcM0257, CcM1559, CcM1825 and CcM1895) for both hybrids (ICPH 2671 and ICPH 2438) were detected for undertaking multiplex assays. The marker *nad7a_del* derived from *nad7* (gene from mitochondria) differentiated the male sterile line (ICPA 2039) from the fertile line (ICPB 2039). Interestingly, this marker was able to detect as low as 2 % admixtures level of ICPB 2039 specific fragment in DNA of ICPA 2039 (Sinha *et al.*, 2015). Recently seven SSR markers (CCB9, HASSR3, HASSR9, HASSR23, HASSR35, HASSR37 and HASSR43) have also been identified for distinguishing the A- line, B- line and hybrid (Bohra *et al.*, 2015).

2.21 Seed yield and components

The character seed yield has got a complex gene action so, in order to study it properly different factors affecting the yield must be considered and evaluated with regard to their contribution to yield. The information on association, contributes with seed yield and among themselves is of considerable importance in selection programme.

The seed yield per plant was significantly and positively correlated with number of branches per plant, 100-seed weight, days to maturity, number pods per plant and days to 50 per cent flowering at both genotypic and phenotypic levels. Such positive interrelationship between seed yield and these attributes have also been reported in pigeon pea by Bhongale and Raut (1987), Brar (1993), Lobo *et al.* (1996) and Lal (2002).

Sodavadiya *et al.* (2009) reported that the estimates of genotypic correlation coefficients in general were higher than their corresponding phenotypic correlation coefficients indicating strong inherent association among the traits. The seed yield per plant had significant and positive association with days to 50 % flowering, days to maturity, number of branches per plant, number of pods per plant and 100-seed weight at both genotypic as well as phenotypic levels. Characters like 100-seed weight, days to maturity and pod length exerted high direct effects on seed yield. 100-seed weight and days to maturity also contributed indirectly towards seed yield per plant through most of the characters.

Saxena and Nadarajan (2010) reported that, In 2007 rainy season, a few pilot hybrid pigeon pea seed production programmes were organized in the states of Andhra Pradesh, Gujarat, Karnataka and Maharastra. The quantity of hybrid seed produced in isolated ranged a lot. The highest A X R yield of 2267 kg/ha was recorded at Indore.

2.22 Genetic basis of stability

Seelakshmi *et al.* (2010) reported that, thirty Pigeon pea genotypes studied for genetic divergence using Mahalanobis D^2 statistic indicated wider genetic diversity. Considerable variability was observed for days to 50 % flowering, days to maturity, plant height, number of pods per plant and seed yield due to non-linear pooled deviation. The variability was low for number of primary branches per plant and test weight. Among the 7 characters studied, seed yield contributed the most (89.49 %) towards the divergence of genotypes followed by number of pods per plant, days to 50 % flowering and plant height. The genotypes ICPL 98008, ICPHAL 4979-2 and ICP 77303 were identified as desirable and stable for days to maturity. Stability parameters along with per se performance across three *kharif* seasons revealed that ICPL 20036 and ICPL 20058 were stable genotypes for seed yield and were found to be suitable for low input cultivation while, ICPL 13198 was found suitable for input rich cultivation.

2.23 Genetic variability and heritability

The heritability estimates provide a guideline on the efficiency of selection as they refer to the proportion of the phenotypic variance that is due to heritable genetic variance. A greater heritability estimate suggests that the concerned character can be easily selected within a given environment.

They suggested that studies on the estimation of genetic variances should be conducted using cultural practices similar to those followed in commercial cultivation. These estimates are likely to be more close to the reality (Reddy *et al.*, 1981).

Vange and Egbe Moses (2009) conducted a three-year study during the growing season of 2002, 2003 and 2004 at the National Root Crops Research Institute Sub-station, Otobi (07° 10' N, 08° 1' 39"E, 105.1m elevation) located in Southern Guinea Savanna agro-ecological zone of Nigeria. The study was conducted to evaluate 29 new pigeon pea genotypes and a local variety for genetic variability, character association and path coefficients among the yield and yield component traits. The results indicated that the genotypes showed significant variability for all the traits studied. The phenotypic coefficient of variability and genotypic coefficient of variability were relatively high for number of pods per plant, dry pod weight, dry grain yield and number of primary branches. Phenotypic coefficient of variability and genotypic coefficient of variability were generally moderate for days to 50 % flowering, days to maturity and plant height. Heritability (broad sense) was generally high for all the traits with exception of number of seeds per pod (0.58). Relatively high expected genetic advance was obtained for number of branches per plant (58.26 %), number of pods per plants (161.84 %), dry pod weight (68.48 %) and dry grain yield (82.65 %). High heritability and expected genetic advance in these traits indicated the presence of additive gene effects. In this study dry pod weight, pod length and number of pods have been identified as selection criteria for obtaining good parental lines in a pigeon pea breeding program.

The heritability estimates are generally valid for a given population and the environment in which these were estimated. In pigeon pea, a number of reports on heritability estimates for various quantitative traits have been published. Together, these estimates provide general information about the ease of selection for a particular character. For the sake of convenience, these estimates are grouped as high (>75 %), medium (75-50 %) and low (<50 %). There is a large variation in the estimates of heritability for all the important agronomic traits. However, most of the studies suggest that characters such as seed yield, pods plant⁻¹, protein content, *etc.* have relatively low heritability due to the large influence of the environment on their expression. On the contrary, days to flower, days to maturity, plant height and seed size are known to have relatively high heritability estimates (Saxena *et al.*, 2010).

Hamid *et al.* (2011) found that the Principal Component Analysis (PCA) clearly and concisely explained the genetic variability among pigeon pea genotypes. Based on the correlation matrix, the highly related eight plant characters *i.e.* seedling vigor, plant height, days to first flowering, days to 50 % flowering, days to maturity, pods plant⁻¹, pod length and seed yield were analyzed for the principal components (PCs). The PCA revealed that the first four PCs had Eigen values of more than 1 and explained 28.66 %, 18.36 %, 15.87 % and 13.31 % of total variation individually and 76.20 % in combination. The plant traits that separated genotypes along the PC₁ were morphological and phenological characters. The high and positive contributions of PC₂ were pods plant⁻¹ and seed yield and the plant traits that separated genotypes to PC₂ axis were yield attribute and seed yield. The PC₃ was associated mainly with seedling vigor and that of PC₄ with pod length. Genotypic variation and

pattern of diversity of many crop plants were evaluated through PCA by many authors (Meijer *et al.*, 1996 and Bisht *et al.*, 1999).

Visakho Shuny *et al.* (2013) reported that variability and character association were studied in thirty genotypes of pigeon pea for grain yield and its attributing characters. The analysis of variance revealed significant differences among the genotypes for all the characters studied, indicating a high degree of variability in the material. The highest PCV and GCV were recorded for number of pods per plant followed by seed yield per plant and number of cluster per plant indicating the presence of ample variation for these traits in the present material. In the present study high estimates of heritability and genetic advance were obtained for seed yield per plant, days to 50 % flowering, plant height, number of pods per cluster, number of cluster per plant, pod length, 100-seed weight, number of primary branches per plant and days to maturity. Thus, selection of these traits is likely to accumulate more additive genes leading to further improvement of their performance and these traits may be used as selection criteria in pigeon pea breeding program.

Vanisree *et al.* (2013) reported that the genetic analysis and association studies were conducted in 128 germplasm lines of pigeon pea received from NBPGR for yield and yield attributing characters. Higher amount of variation was observed for seed yield per plant followed by number of pods per plant and plant height. High heritability with high genetic advance as percentage of mean were obtained for seed yield and number of pods per plant indicated the presence of additive gene action influencing the inheritance of these characters. Significant positive correlations were observed for 100 seed weight, number of primary branches per plant, number of pods per plant and plant height on seed yield per plant were high and positive. Selection for higher seed weight, days to maturity, primary branches, pods per plant and plant height would be the best criteria for increasing the seed yield per plant in pigeon pea.

2.24 Gene action

Sharma *et al.* (1972) reported in pigeon pea predominance of additive gene action for seed size and the genes controlling small seed size were found to be dominant over the genes responsible for the expression of large seeds.

Saxena *et al.* (1981) observed the predominance of additive gene action for yield and yield components. They demonstrated that in pigeon pea, agronomic considerations in field evaluation trials and inclusion of parents with a moderately different phenology have significant influence on the estimates of genetic variances.

Sidhu and Sandhu (1981) reported the importance of both additive and non-additive gene actions in pigeon pea, while the predominance of non-additive gene action was observed by Dahiya and Brar (1977).

Gupta *et al.* (1981) also confirmed in pigeon pea additive gene action and reported that seed size differences were determined by only 2 or 3 major genes. For days to flower, Pandey (1972), Sharma *et al.* (1973), and Dahiya and Satija (1978) reported additive genetic variance with partial dominance for earliness while Gupta *et al.* (1981) reported predominance of additive gene effects for flowering. Plant height was reported to be controlled by both additive and dominance gene effects

(Sharma 1981). Genes controlling tall stature were dominant over the genes responsible for the expression of short stature.

In pigeon pea both additive and non-additive gene actions have been reported to control seed yield and other important quantitative characters (Saxena and Sharma 1990). However, the critical information on the extent of non-additive effects, particularly dominance and epistasis components, is not very clear and decisive.

Sharma and Dwivedi (1995) suggested that besides over - dominance and dominance, additive and additive x additive inter - allelic and inter - genomic interactions also play an important role in the expression of hybrid vigour. The likelihood of obtaining heterotic cross combinations in pigeon pea is high because it has an inherent capacity to carry a considerable hidden genetic load of recessive genes due to its partial out – crossing nature and with a little effort breeders can make use of it in enhancing yield.

A review of literature in pigeon pea showed that important economic traits such as yield, pods plant⁻¹, plant height, seed size, and seeds pod⁻¹ are controlled by both additive as well as non - additive gene actions (Saxena and Sharma, 1990) and the level for hybrid vigour for yield is comparable with other crops where commercial hybrids have already been successful.

Saxena *et al.* (2012a) reported that the seed colour of pigeon pea hybrid ICPH-2671 is controlled by three dominant genes; the female parent carries two such genes while the third gene is present in the male parent. The purple colour in the seed is produced when one or both the genes present in the female parent interact with the gene present in the male parent.

The 100 seeds of ICPH 2671 weigh between 10.5 and 11.2 g. The fertility restoration in this hybrid is high (95–100 % pollen fertility), stable across environments and controlled by two dominant genes (Dalvi *et al.*, 2008 and Sawargaonkar *et al.*, 2012).

3. MATERIAL AND METHODS

The field experiments were conducted to study the morphological characterization of parental lines of hybrid pigeon pea-ICPH-2671. Maintenance of parental lines (A, B & R line) of hybrid pigeon pea-ICPH-2671 by different pollination methods on seed quality and also to know the effect of different planting ratio's and insect attractant on seed yield and quality of hybrid pigeon pea-ICPH-2671 during *Kharif*-2011 at Seed Farm, University of Agricultural Sciences, Dharwad. Further, the laboratory studies on seed quality parameters were also carried out at Department of Seed Science and Technology, College of Agriculture, Dharwad. Molecular characterization of A, B, R & F₁ hybrid through molecular markers was carried out at Institute of Agricultural Bio-technology, UAS, Dharwad during 2015. The details of the materials used and technologies adopted during the course of these investigations are presented in this chapter.

3.1 General description

3.1.1 Geographical location of the experimental site

Dharwad comes under the Agro climatic zone-8 comprising of Northern Transitional Tract of Karnataka State. It is located between 15° 26' North latitude and 76° 27' East longitude at an altitude of 678 m above the mean sea level.

The field experiments were conducted in 'D' and 'H' block of the seed unit, University of Agricultural Sciences, Dharwad.

3.1.2 Climatic conditions

Meteorological data for the crop year (2011-12) and the average of 25 years as recorded at Meteorological observatory of the Main Agricultural Research station, Dharwad are presented in table 3.1

3.1.3 Soil condition of the experimental site

The experimental site consisted of well drained and porous deep black soil with good fertility.

3.1.4 Previous crop at the experimental site

Chick pea and soybean were grown during the previous season on the site selected for experimentation during *kharif*-2011.

3.1.5 Isolation distance

There was no other pigeon pea crop except experimental crop with in the vicinity of 400 meters from the experimental site.

3.1.6 Seed source

The required seed material of the parental lines of hybrid Pigeon Pea- ICPH-2671 was obtained from the Pigeon Pea breeder, ICRISAT, Patancheru , Hyderabad.

Table 3.1: Monthly meteorological data for the experimental year 2011-12 and the average of 60 years (1950-2010) at Meteorological Observatory, Main Agricultural Research Station, College of Agriculture, University of Agricultural Sciences, Dharwad

Month	Rainfall (mm)		Temperature (°C)				Relative humidity (%)	
			Mean maximum		Mean minimum			
	1950-2010	2011	1950-2010	2011	1950-2010	2011	1950-2010	2011
January	0.062	0	28.2	29.2	14.07	12.5	64.81	59
February	0.547	21.6	32.4	30.8	16.56	14.0	54.41	48
March	15.65	0.80	35.6	35.2	19.71	18.6	64.24	44
April	39.27	77.4	37.6	34.9	20.11	20.2	78.05	57
May	68.39	66.6	35.7	34.7	20.95	21.3	75.78	61
June	108.51	194.0	31.2	27.5	21.68	21.3	86.29	84
July	138.70	131.0	27.7	26.9	20.85	20.6	89.18	85
August	154.45	124.2	27.9	26.2	20.16	20.7	88.60	87
September	135.23	82.8	27.9	28.1	19.96	19.9	86.68	80
October	94.43	219.7	29	29.9	18.65	19.5	79.40	73
November	52.49	4.6	28.4	29.8	15.93	15.8	73.62	55
December	2.83	0.0	27.4	29.6	13.18	13.7	69.24	57
Total	810.55	922.7	-	-	-	-	-	-

3.2 Experiment I: Maintenance of parental lines (A, B & R line) of hybrid Pigeon Pea- ICPH-2671 on seed quality

The field experiment was conducted at NSP/BSP, seed unit farm, UAS, Dharwad during *kharif* -2011. In this study different pollination methods were employed for the maintenance of the parental lines to determine the best method of pollination to obtain the best quality seeds. The details of the materials used and techniques adopted during the course of these investigations are presented below.

3.2.1 Different pollination methods:

P₁: Selfing – 500 plants

P₂: Isolated random mating (Mass selection)-500 plants

P₃: Plant to progeny row selection -250 progenitor seeds.

Assessment of seed quality parameters of parental lines of pigeon pea hybrid ICPH-2671.

3.2.2 Three different parental lines namely

i) Female/seed parent : ICPA-2043

ii) Male /pollen parent : ICPB-2043

iii) Restorer/R-line : ICPR-2671

3.2.3 Description of parental lines

A line-ICPA - 2043 : Female parent is a medium duration wilt and sterility mosaic resistant cytoplasmic nuclear male sterile (*Ms*) line derived from a wild relative of pigeon pea *C.Cajaniifolius* by back crossing method.

B line - ICPB - 2043: Maintainer line of A line ICPA-2043.

R line -ICPR-2671: Male parent is a medium duration wilt and sterility mosaic resistant advanced breeding line (table -3.2)

3.2.4 Isolation distance

There was no other pigeon pea crop except experimental crop with in the vicinity of 400 meters from the experimental site.

3.2.5 Land preparation

The experimental plot was brought to fine tilth by one deep ploughing and three repeated harrowings followed by leveling. The recommended dose of well decomposed FYM was applied to the soil. The required number of experimental plots as per treatment schedule was laid out by small bunds and by making irrigation channels. The seed rows were marked and drawn at a distance of 90cm apart in each plot.

3.2.6 Application of fertilizers

The recommended quantities of fertilizer dose of 25:50:0 Kg/ha NPK was applied completely as basal dose to each experimental plots in the form of urea and diammonium phosphate and incorporated well in to the soil before sowing.

Table 3.2. Distinguisshing morphological characterization of parental lines of hybrid pigeon pea ICPH-2671

Character	ICPA-2043	ICPB-2043	ICPR-2671
Anthocyanin colorations of hypocotyls	Absent	Absent	Present
Days to 50% flowering	120-125	110-115	122-128
Days to pod maturity	150-160	158-165	180-185
Plant height (cm)	170-180	175-185	215-225
Growth habit	Indeterminate	Indeterminate	Indeterminate
Branching pattern	Spreading	Spreading	Spreading
Stem colour	Green	Green	Green
Leaf shape	Lanceolate	Lanceolate	Lanceolate
Flower colour	Light yellow	Light yellow	Yellow with light streaks
Pod form	Cylindrical	Cylindrical	Cylindrical
Pod colour	Green	Green	Green with brown Streaks
No. of seeds/pod	3.8-4.8	3.5-4.2	3.7-4.0
Seed colour	Brown	Brown	Brown
100-seed mass(g)	11.8 - 12.5	11.2 - 11.8	10.7-12.3

3.2.7 Method of sowing

In A x B line seed production, the female parent ICPA-2043 and maintainer line ICPB-2043 of pigeon pea hybrid ICPH-2671 seeds were sown by hand dibbling at 1:1 planting ratio's (female to male rows) and B & R lines are sown in an isolated blocks separately under congenial field conditions of *Kharif* season on 25th June 2011. Two seeds of the respective parents were hand dibbled at two to three centimeter deep in the earlier marked rows at one feet intra row spacing and three feet inter row spacing. The experimental plot was immediately irrigated after sowing to ensure quick germination and faster seedling establishment.

3.2.8 Weeding

Three hand weedings were done at 25, 50 and 80 days after sowing manually. Intercultivation was done to remove the weeds in between the rows during the early period of crop growth in order to keep seed plot free of weeds.

3.2.9 Plant protection

The crop was sprayed with Monocrotophos 36 EC (0.04%) @ 1 ml per litre of water to control the Pod borers (*Helicoverpa armigera* and *Maruca vitrata*), pod fly (*Melanagromyza obtusa*) and blister beetle at flower initiation stage and second and third spray was done at 10- 15 days intervals by using Knapsack sprayer.

The seeds were dressing with Ridomil MZ @ 3 g kg⁻¹ seed before sowing to control the diseases like fusarium wilt, sterility mosaic and phytophthora blight.

3.2.10 Irrigation

Irrigation was provided to the crop depending upon the soil moisture content and prevailing weather conditions during the period of experiment. Four irrigations were given at early growth stage and another at the early podding stage.

3.2.11 Rouging

The pollen shedder and off-types which did not confirm to the specific characters of the parental lines were removed before flower opening and pollination in order to maintain genetic purity. Besides, the diseased and undesirable plants were also removed to prevent contamination.

3.2.12 Harvesting, threshing and cleaning

The pigeon pea hybrid seed crop was harvested when plants started showing the symptoms of drying the leaflets turning to pale colour to brown colour. male rows including boarder rows plants were first harvested by uprooting manually from each plot and subsequently female plants were harvested from the net plot area. In selfing method the bagged plants were harvested separately and mass selected plants were harvest separately and dried completely under shade.

The harvested plants were dried completely under the partial shade for seven days. Subsequently, the seeds were separated out manually by gentle beating the dried plants with the wooden stick. The seeds were again cleaned and dried in the shade until seed moisture content reduced to 8.5 percent.

3.2.13 Maintenance of parental lines by different pollination methods

3.2.13.1 Methods of pollination

3.2.13.2 P₁: Selfing method

3.2.13.3 A line (ICPA-2043)

In an isolated block three thousand plants of 'A' line and 'B' line were raised by interplanting in 1:1 row proportion. Looking to the morphological descriptors in both the parental lines ('A' & 'B') all the off types were rouged and true to type plants were retained. Selected 500 true to type plants from among the 3000 plants in both 'A' and 'B' line were selfed, by bagging (cloth bag) the reproductive branches of 'A' & 'B' line plants. After maturity selfed plants in 'A' line were harvested and threshed individually. Seeds harvested from 500 selfed plants ('A' line) were examined for seed characters and discarded, seeds obtained from selfed plants which were found to be off type, immature and shrivelled and finally ended up with 250 selfed true to type plant seeds. Equal quantity of 50 per cent seed obtained from each selfed plant (250 plants) were mixed and bulked and this balanced mixture was treated as Breeder seed stage I seed. Remaining left over 50 per cent of seed from each selfed plant was retained as progenitor. A part of the balanced mixture was used for assessment of seed quality parameters.

3.2.13.4 B line (ICPB-2043)

In an isolated block three thousand plants of 'B' line were raised. Looking to the morphological descriptors, the off type plants in 'B' line were rouged and true to type plants were retained. Selected 500 true to type plants from among the 3000 plants were tagged and selfed by bagging (cloth bag) the reproductive branches of 'B' line plants. After maturity, selfed plants in 'B' line were harvested and threshed individually. Seeds harvested from 500 selfed plants ('B' line) were examined for seed characters and discarded the, seeds obtained from selfed plants which were found to be off type, immature and shriveled and finally ended up with 250 selfed, true to type plants seeds. Equal quantity of 50 per cent of seed obtained from each selfed plant (250 plants) was mixed and bulked and this was treated as Breeder seed stage I seed. Remaining left over 50 per cent of seeds from each selfed plant was retained as progenitor. A part of the balanced mixture of seed was used for assessment of seed quality parameters.

3.2.13.5 R line (ICPR-2671)

In an isolated block three thousand plants of 'R' line were raised. Similar procedure was followed for 'selfing' of the 'R' line plants as mentioned under 3.2.13.4 and is depicted in Plate 1.

3.2.13.6 P₂: Isolated random mating (Mass selection)

3.2.13.7 A line (ICPA-2043)

In an isolated block three thousand plants of 'A' line and 'B' line were interplanted in 1:1 row proportion. Looking to the morphological descriptors in both the parental lines (A & B) all the off types were rouged and true to type plants were retained. Selected 500 true to type plants from among the 3000 plants in 'A' line allowed for random mating with 'B' line plants. After, maturity 500 true to type plants were harvested separately and seeds from all the selected plants were bulked. Then bulked seed is treated as Breeder stage I seed. Part of the seed sample drawn from the Breeder stage I seed

was used for assessment of seed quality parameters. From among the 500 true to type random mated plants 250 plants were threshed individually and 1/3 of the seed harvested from these individual selected plants was treated as progenitors for raising plant to progeny rows for further studies.

3.2.13.8 B line (ICPB-2043)

In an isolated block three thousand plants of 'B' line were raised. Looking to the morphological descriptors in the parental line ('B') all the off types were rogued and true to type plants were retained. Selected 500 true to type plants from among the 3000 plants in 'B' line allowed for random mating. After, maturity 500 true to type plants were harvested separately and seeds from all the selected plants were bulked. Then bulked seed is treated as Breeder stage I seed. Part of the seed sample drawn from the Breeder stage I seed was used for assessment of seed quality parameters. From among the 500 true to type random mated plants 250 plants were threshed individually and 1/3 of the seed harvested from these individual selected plants was treated as progenitors for raising plant to progeny rows for further studies.

3.2.13.9 R line (ICPR-2671)

In an isolated block three thousand plants of 'R' line were planted. The similar procedure was followed for mass selection of the 'R' line plants as mentioned under 3.2.13.8.

3.2.13.10 P₃: Plant to progeny row selection

Seed sample drawn from balanced mixture of 250 progeny rows of A, B & R lines were used for assessing seed quality parameters.

3.2.14 The different pollination methods used for assessment of seed quality parameters of parental lines of hybrid pigeon pea ICPH-2671

Factor (I): Pollination methods.

P₁: Selfing

P₂: Isolated random mating. (Mass selection)

P₃: Plant to progeny row selection

Factor (II): Genotypes

G₁: 'A' line (ICPA-2043)

G₂: 'B' line (ICPB-2043)

G₃: 'R' line (ICPR-2671)

Design : CRD with factorial concept.

Replication : Four

Treatment combinations:

P ₁ G ₁	P ₁ G ₂	P ₁ G ₃
P ₂ G ₁	P ₂ G ₂	P ₂ G ₃
P ₃ G ₁	P ₃ G ₂	P ₃ G ₃



Plate 3.1: General view of the experimental plot (Expt. -I)

Plate 3.1: General view of the experimental plot (Expt.-I)

3.2.15 Collection of experimental data

3.2.15.1 Sampling procedure

The biometric observations on quality parameters were recorded as per the different pollination methods. The balanced mixture of 'selfed', mass selected seeds and plant to progeny row selected seeds were used for assessing the seed quality parameters.

3.2.16 Observations recorded on pod and seed morphological characters

3.2.16.1 Pod length and Pod breadth

The uniformly developed ten pods from each genotype were collected randomly and used to assess the length and width of pods with the help of graph paper and expressed in centimeter per pod.

3.2.16.2 Pod size

The pod size of five randomly tagged plants from each genotype at the time of pod mature stage was observed and genotypes were grouped into small (<4 cm), medium (4-5 cm) and large (> 5 cm) types.

3.2.16.3 Number of seeds per pod

The number of seeds per pod was counted and average was worked out based on ten pods and was expressed as number of seeds per pod for all the genotypes.

3.2.16.4 Seed colour

Seed colour of the parental lines were observed visually under natural day light condition and were grouped into following colour viz., brown, dark brown and grey.

3.2.16.5 Hundred seed weight

Hundred seeds in eight replications from each genotype were counted and weight was recorded by using electronic balance as per the procedure of ISTA (Anon., 1999). The average weight was recorded in grams. Based on seed weight, genotypes were categorized into small (>7g), medium (7- 9 g), large (> 9-11g) and very large (> 11 g) groups.

3.2.17 Observations recorded on seed quality parameters

3.2.17.1 Germination percentage

The germination test for pigeon pea seeds was conducted in the laboratory using between paper method as per ISTA procedure (Anon., 1996). Hundred seeds of four replicates were placed in between germination papers. The rolled towels were placed in germination chamber at 25 ± 1 °C and 90 ± 1 per cent relative humidity. The germinated seedlings were evaluated on fourth and sixth day of incubation and the cumulative percentage of germination was expressed based on the total number of normal seedlings.

3.2.17.2 Root length

Ten normal seedlings were selected at random from each treatment of the germination test on the sixth day (final count day) and used for measuring root length. The root length was measured from the tip of the primary root to the base of the hypocotyl and the mean root length was expressed in centimeters.

3.2.17.3 Shoot length

Ten normal seedlings used for measuring the root length were used for measuring shoot length also. The shoot length was measured from the base of the primary leaf to the base of the hypocotyl and the mean shoot length was expressed in centimeters.

3.2.17.4 Seedling dry weight

The same ten seedlings in replicates which were used for recording root and shoot length were dried in an oven at 80 ± 1 °C for a period of 24 hours. These dried seedlings were removed and cooled in desiccators for 30 minutes. The dried seedlings weight was recorded and expressed in miligram per 10 seedlings.

3.2.17.5 Seedling vigour index

The seedling vigour index was calculated as per the formula given by Abdul-Baki and Anderson (1973).

$$\text{Seedling vigour index} = [\text{Shoot length (cm)} + \text{root length (cm)}] \times \text{Germination (\%)}]$$

3.2.17.6 Electrical conductivity

Five grams of seeds were drawn from each treatment in three replicates and were treated with HgCl₂ for half a minute and thoroughly washed in distilled water for three times. Then the seeds were soaked in 25 ml distilled water and kept in an incubator maintained at 25 ± 1 °C for 24 hours. The seed leachate was decanted and volume was made up to 25 ml by adding distilled water. The electrical conductivity of the seed leachate was measured in the digital conductivity meter with a cell constant of 0.50 dS per m and expressed in Deci Simons per meter (Presley, 1958).

3.2.17.7 Field emergence

Four hundred seeds were drawn randomly from each treatment and 400 seeds were sown in four replications of 100 seeds each on a well prepared seed bed of 2 x 1 m size at adequate soil moisture conditions. seeds were sown uniformly to a depth of 3-4cm with a spacing of 90cm between the rows and 30 cm between the plants. The number of seedlings considered as emerged at least 3 cm above the soil surface on 12th day after sowing was counted and the average value was expressed as per cent field emergence.

3.3 Experiment II: Effect of planting ratio and insect attractant spray on seed yield and quality

The field experiment was conducted at NSP/BSP, Seed unit, University of Agricultural Sciences, Dharwad during *Kharif*-2011 to know the effect of different planting ratio's and insect attractant spray on seed yield and quality of hybrid pigeon pea ICPH-2671. To get higher F₁ seed yield the best planting ratio with insect attractant spray suited to Dharwad condition. Further, the growth, yield and seed quality parameters were studied.

3.3.1 Experimental details

3.3.2 Treatment details

Factor (I): Planting ratio

P₁: 3:1 → Three rows of A-line and one row of R-line

P₂: 4:1 → Four rows of A-line and one row of R-line

P₃: 5:1 → Five rows of A-line and one row of R-line

P₄: 3:2 → Three rows of A-line and two rows of R-line

P₅: 4:2 → Four rows of A-line and two rows of R-line

P₆: 5:2 → Five rows of A-line and two rows of R-line

Factor (II): Insect attractants

T₁: Without spray

T₂: Spraying with insect attractant Bee-Q @12.5 g/litre

Treatment combinations:

P ₁ T ₁	P ₂ T ₁	P ₃ T ₁	P ₄ T ₁	P ₅ T ₁	P ₆ T ₁
P ₁ T ₂	P ₂ T ₂	P ₃ T ₂	P ₄ T ₂	P ₅ T ₂	P ₆ T ₂

3.3.3 Seed source

The required seed material of the selected parental lines of the pigeonpea was obtained from the pigeonpea breeder, ICRISAT, Patancheru, Hyderabad for conducting the experiments.

3.3.4 Parental lines: A-line: ICPA 2043- Female parent/seed parent

R-line: ICPR-2671-/Male parent/pollen parent

3.3.5 Experimental design and layout

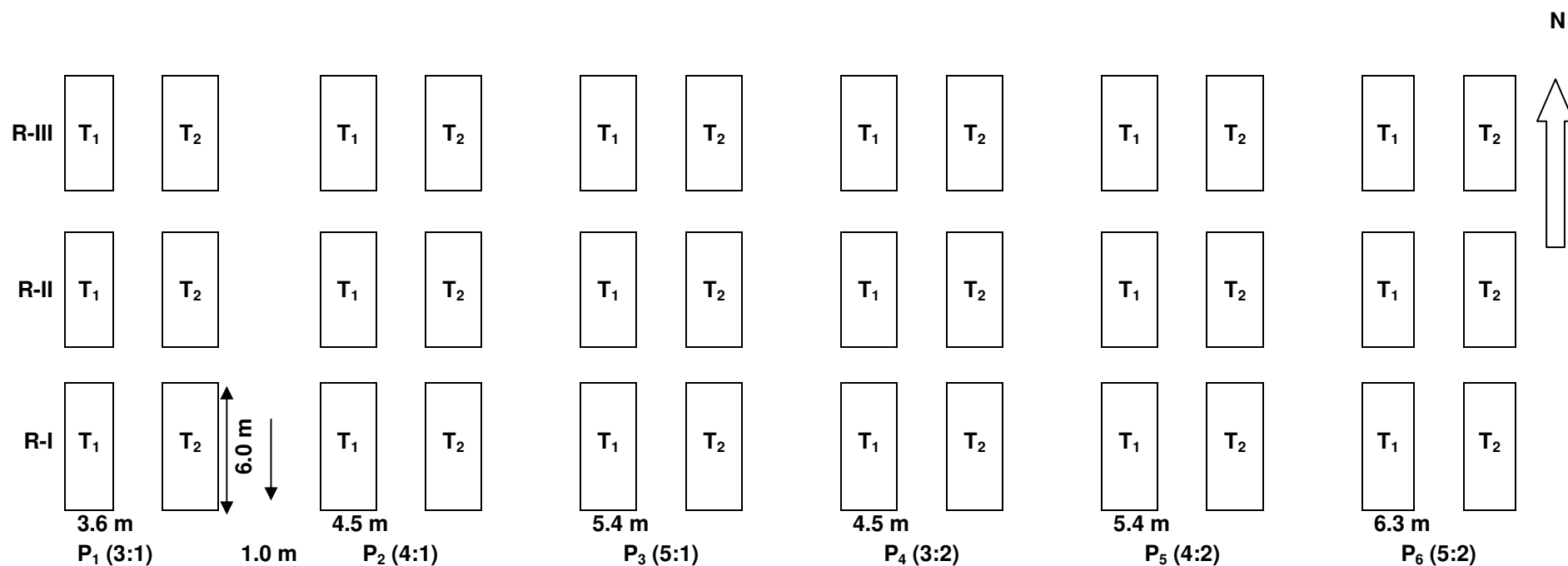
The experiment was laid out in a randomized complete block design (RCBD) with factorial concept with twelve treatment combinations and three replications. The plan of layout of experiment is depicted in fig. 1 and general view of experimental plot is depicted in plate 2.

Replication: Three

Spacing: 3 x1 feet. The distance between plant to plant is 1 feet and row to row is 3 feet.

Plot Size:

Planting ratio	Gross plot size	Net plot size
3:1	3.6 x 6 m ²	2.7 x 6 m ²
4:1	4.5 x 6 m ²	3.6 x 6 m ²
5:1	5.4 x 6 m ²	4.5 x 6 m ²
3:2	4.5 x 6 m ²	3.6 x 6 m ²
4:2	5.4 x 6 m ²	4.5 x 6 m ²
5:2	6.3 x 6 m ²	5.4 x 6 m ²



Factor (I): Planting ratio

P₁: 3:1 → Three rows of A-line and one row of R-line
P₂: 4:1 → Four rows of A-line and one row of R-line
P₃: 5:1 → Five rows of A-line and one row of R-line
P₄: 3:2 → Three rows of A-line and two rows of R-line
P₅: 4:2 → Four rows of A-line and two rows of R-line
P₆: 5:2 → Five rows of A-line and two rows of R-line

Factor (II): Insect attractants

T₁: Without spray
T₂: Spraying with Insect attractant Bee-Q @12.5 g/litre

Fig. 3.1: Plan of layout of experimental plot on effect of planting ratio and insect attractant on seed yield and quality of hybrid pigeon pea (ICPH-2671)



Plate 3.2 : General view of the experimental plot at harvest stage (Expt. -II)

Plate 3.2: General view of the experimental plot at harvest stage (Expt.-II)

3.3.6 Cultural and after care operations

3.3.6.1 Isolation Distance

There was no other pigeon pea crop except experimental crop within the vicinity of 400 meters from the experimental site.

3.3.6.2 Land preparation

The experimental plot was brought to fine tilth by one deep ploughing and three repeated harrowings followed by leveling. The recommended dose of well decomposed FYM was applied to the soil. The required number of experimental plots as per treatment schedule was laid out by small bunds and by making irrigation channels. The seed rows were marked and drawn at a distance of 90 cm apart in each plot.

3.3.6.3 Application of fertilizers

The recommended quantities of fertilizers dose of 25:50:0 Kg/ha NPK per hectare was applied completely as basal dose to each experimental plots in the form of urea and diammonium phosphate and incorporated well in to the soil before the seed sowing.

3.3.6.4 Method of sowing

The female parent ICPS-2043 and male parent ICPS-2671 of pigeon pea hybrid ICPS-2671 seeds were sown by hand dibbling at different planting ratio's (female to male rows) in the congenial field conditions of *Kharif* season on 25th June 2011. The two seeds of the respective parents were hand dibbled at two to three cm deep in the earlier marked rows of at one feet intra row spacing. The experimental plot was immediately irrigated after sowing to ensure quick germination and faster seedling establishment.

3.3.6.5 Weeding

Three hand weedings were done at 25, 50 and 80 days after sowing manually. Intercultivation was done to remove the weeds in between the rows during the early period of crop growth in order to keep seed plot free of weeds. The crop was sprayed with pre-emergence herbicide *Prometryn* @1.5 litre per ha to control the weeds at early growth period of seed crop.

3.3.6.6 Plant protection

The crop was sprayed with Monocrotophos 36 EC (0.04%) @ 1 ml per litre of water to control the Pod borers (*Helicoverpa armigera* and *Maruca vitrata*), pod fly (*Melanagromyza obtusa*) and blister beetle at flower initiation stage and second and third spray was done at 10- 15 days intervals by using Knapsack sprayer.

The seeds were dressed with Ridomil MZ @ 3 g kg⁻¹ seed before sowing to control the diseases like fusarium wilt, sterility mosaic and phytophthora blight.

3.3.6.7 Irrigation

Irrigation was provided to the crop depending upon the soil moisture content and prevailing weather conditions during the period of experiment. Four irrigations were given at early growth stage and another at the early podding stage.

3.3.6.8 Rouging

The pollen shedder and off-types which did not confirm to the specific characters of the parental lines were removed before flower opening and pollination in order to maintain genetic purity. Besides, the diseased and undesirable plants were also removed to prevent contamination.

3.3.6.9 Insect attractant spray

The insect attractant Bee-Q at 12.5g/litre of water was sprayed at every 15 days interval during entire flowering period to increase the honey bee activity for pollination and seed setting.

3.3.6.10 Harvesting, threshing and cleaning

The pigeon pea hybrid seed crop was harvested when plants started showing the symptoms of drying, the leaflets turning to pale colour and pod to brown colour. The male rows including boarder rows plants were first harvested by uprooting manually from each plot and subsequently female plants were harvested from the net plot area excluding five tagged plants used for recording the observations.

Later on the five tagged plants were also harvested separately for recording observations on seed yield and quality parameters. The harvested plants were dried completely under the partial shade for seven days. Subsequently, the seeds were separated out manually by gentle beating the dried plants with the wooden stick. The seeds were again cleaned and dried in the shade until seed moisture content reduced to 8.5 percent.

3.3.7 Collection of experimental data

3.3.7.1 Sampling procedure

The biometric observations on growth, seed yield and quality parameters were recorded as per the treatments. The five competitive plants from net plot area were selected at randomly and tagged with label for recording the observations on plant growth, flowering and reproductive parameters as listed below;

3.3.7.2 Observations on crop growth parameters

3.3.7.2.1 Plant stand at harvest

The number of plants retained at the time of harvest was counted.

3.3.7.2.2 Plant height at harvest stage

The height of five competitive plants randomly selected and tagged were measured at harvest stage from the ground level to the top most tip of the plant by using metric scale and average was worked out and expressed in centimeter for each treatment and replication.

3.3.7.2.3 Number of primary branches/plant

At harvest the number of primary branches per plant was counted on the five earlier tagged plants and their average was expressed as whole number of primary branches per plant for each treatment and under each replication.

3.3.7.2.4 Number of secondary branches/plant

At harvest the number of secondary branches per plant was counted on the five earlier tagged plants and their average was expressed as whole number of primary branches per plant for each treatment and under each replication.

3.3.7.3 Flowering parameters

3.3.7.3.1 Days to flower initiation

The number of days taken for initiation of first flower in each treatment plot was recorded by counting the days taken from the date of sowing to initiation of flowering on the five labeled plants.

3.3.7.3.2 Days to 50 % flowering

The number of days taken for blooming of 50 per cent plants in each plot was recorded by counting the days from the date of sowing.

3.3.7.3.3 Duration of flowering

The number of days that the plant produces flowers in each plot was recorded by counting the days from the date of flower initiation.

3.3.7.4 Pod characters

3.3.7.4.1 Days to pod initiation

The number of days taken to commence first pod in each plot was recorded by counting the actual days taken to initiate podding from the date of sowing.

3.3.7.4.2 Days to pod maturity

The total number of days taken for harvest maturity of the pods in each plot was recorded by counting the actual days from the date of sowing and was expressed as days to maturity for each treatments and replications.

3.3.7.4.3 Number of pods per plant

At harvest the numbers of filled pods present in the five randomly selected and tagged plants were counted individually and average was expressed as total number of pods per plant.

3.3.7.4.4 Number of seeds per pod

After separating out the filled seeds and unfilled seeds from randomly selected ten pods from each treatment were counted manually and average was expressed as number of seeds per pod.

3.3.7.4.5 Pod length and pod breadth

The uniformly developed ten pods from each genotype were collected randomly and used to assess the length and width of pods with the help of graph paper and expressed in centimeter per pod.

3.3.7.4.6 Pod size

The pod size of five randomly tagged plants from each genotype at the time of pod mature stage was observed and genotypes were grouped into small (<4 cm), medium (4-5 cm) and large (> 5 cm) types.

3.3.7.4.7 Pod yield per plant

All matured pods were picked up separately by hand from the earlier five tagged plants at harvest and weight of the pods of individual plants was weighed on analytical balance and their average weight was computed as pod weight per plant in grams.

3.3.7.4.8 Pod yield per plot

The pods harvested from the plants of net plot in each treatment plot was recorded as pod yield per plot in kilo grams.

3.3.7.4.9 Pod yield per ha

The weight of the dried pods from the net plot in each treatment and replication were recorded in kilograms was used for calculating the pod yield/ha.

3.3.7.4.10 Seed yield per plant

After harvest the seeds were separated out manually by beating all the pods harvested from five tagged plants with wooden stick and further, were cleaned and dried. The seed weight from each treatment plot was recorded with analytical balance and average of five plants was expressed as seed yield per plant in grams.

3.3.7.4.11 Seed yield per plot

The seed obtained from each net plot were cleaned, dried and weighed separately. The average was worked out and recorded as seed yield per plot and expressed in kg/plot.

3.3.7.4.12 Seed yield/ha (kg)

Based on seed yield obtained from net plot, the seed yield per hectare was computed and expressed in kilogram per hectare.

3.3.7.4.13 Seed quality parameters

The following laboratory observations were recorded on effect of planting ratios and insect attractants on seed quality.

3.3.7.4.14 Germination per cent

The germination test for pigeon pea seeds was conducted in the laboratory using between paper method as per ISTA procedure (Anon., 1996). Hundred seeds of four replicates were placed in between germination papers. The rolled towels were placed in germination chamber at 25 ± 1 °C and 90 ± 1 per cent relative humidity. The germinated seedlings were evaluated on fourth and sixth day of incubation and the cumulative percentage of germination was expressed based on the total number of normal seedlings.

3.3.7.4.15 Root length

Ten normal seedlings were selected at random from each treatment of the germination test on sixth day (final count day) and used for measuring root length. The root length was measured from the tip of primary root to the base of the hypocotyl and the mean root length was expressed in centimeters.

3.3.7.4.16 Shoot length

Ten normal seedlings used for measuring the root length were used for measuring shoot length also. The shoot length was measured from the base of the primary leaf to the base of the hypocotyl and the mean shoot length was expressed in centimeters.

3.3.7.4.17 Seedling dry weight

The same ten seedlings in replicates which were used for recording root and shoot length were dried in an oven at 80 ± 1 °C for a period of 24 hours. These dried seedlings were removed and cooled in a desiccator for 30 minutes. Then, the dried seedling weight was recorded and expressed in miligram per 10 seedlings.

3.3.7.4.18 Seedling vigour index

The seedling vigour index was calculated as per the formula given by Abdul-Baki and Anderson (1973).

$$\text{Seedling vigour index} = [\text{Shoot length (cm)} + \text{root length (cm)}] \times \text{Germination (\%)}]$$

3.3.7.4.19 Electrical conductivity

Five grams of seeds were drawn from each treatment in three replicates and were treated with HgCl₂ for half a minute and thoroughly washed in distilled water for three times. Then, the seeds were soaked in 25 ml distilled water and kept in an incubator maintained at 25 ± 1 °C for 24 hours. The seed leachate was decanted and volume was made upto 25 ml by adding distilled water. The electrical conductivity of the seed leachate was measured in the digital conductivity meter with a cell constant of 0.50 dS per m and expressed in Deci Simons per meter (Presley, 1958).

3.3.7.4.20 Moisture content

The seed samples were drawn from different treatments to determine the moisture content by adopting the ISTA procedures on oven dry basis following the below formula and expressed in percentage.

$$\text{Moisture content (\%)} = \frac{W_2 - W_3 \times 100}{(W_1 - W_2)} \quad \text{(Dry basis)}$$

W_1 : Weight of the empty aluminium box

W_2 : Weight of aluminium box + crushed seed material (before drying)

W_3 : Weight of box + crushed seed material (after drying).

3.4 Experiment- III: Studies on morphological characterization of parental lines of hybrid pigeon pea ICPH - 2671

3.4.1 Experimental details

To study the Morphological descriptors of the parental lines of pigeon pea ICPH-2671, A, B & R lines were planted at NSP/BSP seed unit, University of Agricultural Sciences, Dharwad during *Kharif* -2011.

The parental lines of A, B & R lines were raised in ten rows of 5 meter length of 3 x 1 feet spacing by dibbling two seed per hill in three replications in an isolated block for recording various plant growth and phenological characters.

Hybrid	Seed parent (A line)	Maintainer line (B line)	Pollen parent (R line)
ICPH-2671	ICPA-2043	ICPB-2043	ICPR-2671

3.4.2 Cultural operations

The cultural operations were carried out as per the procedure mentioned in section 3.3.12.

3.4.2.1 Application of fertilizers

General recommended dose of fertilizer was applied as explained in section 3.3.14.

3.4.2.2 Method of sowing

Sowing of each genotype was done as detailed in section 3.3.15.

3.4.2.3 After sowing

After sowing, irrigation and weeding, plant protection, harvesting of pigeon pea crop was attended as per the operations explained in the experiment –I.

3.4.3 Collection of the experimental data

3.4.3.1 Sampling procedures

Five plants were randomly selected in each parental line and labeled. The observations on plant morphological characterization were recorded on tagged plants at different stages using approved guidelines for the conduct of DUS test according to PPV and FR act (Anon., 2007).

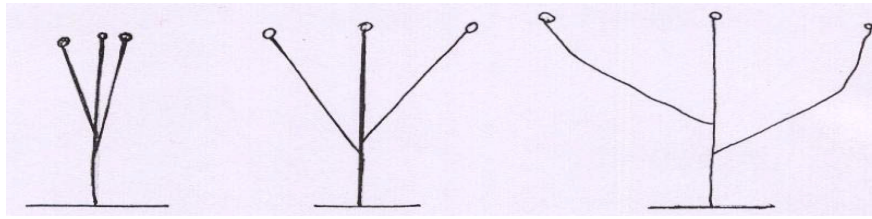
3.4.3.2 Observations recorded as per DUS guidelines

1. Plant: Anthocyanin colouration of hypocotyl

At seedling stage, the hypocotyls colour of the seedling was observed under natural day light condition, after sowing for eight days in the earthen pot and was classified as absent and present in all the test genotypes.

2. Plant: branching pattern

The branching pattern was observed at first insertion of flowering stage. The five randomly tagged plants were grouped into erect ($>30^{\circ}\text{C}$), semi-spreading (30° - 60°C and spreading type ($>60^{\circ}\text{C}$).



Erect

Semi-spreading

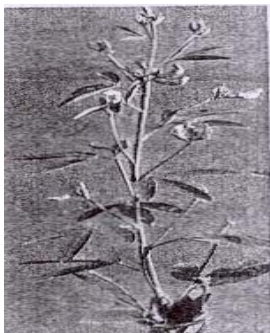
Spreading

3. Time of flowering (50% of the plants with at least one open flower)

The data on which the first flower appeared was noted on five randomly selected plants and actual number of days taken for flowering was calculated from the date of sowing based on 50 percent of plants with at least one flower open. The average was expressed as days to flower initiation and were grouped into very early (<61 days), early (61-90 days), medium (91-130 days), late (131-160 days), very late (>160 days).

4. Plant: growth habit

The growth habit of five tagged plants of each genotypes were recorded at 50 per cent flowering stage and were grouped into a determinate and indeterminate types.



Indeterminate



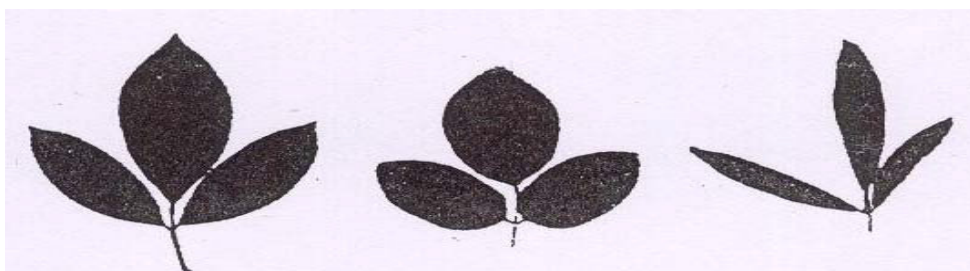
Determinate

5. Stem : Colour

The stem colour of five plants which were tagged randomly in all genotypes were recorded at 50 per cent flowering stage and were grouped into green and purple types.

6. Leaf: shape

The leaf shape of five plants which were tagged randomly in all genotypes were recorded at 50 per cent flowering stage and were grouped into oblong, obovate and narrow oblong types.



Oblong

Obovate

Narrow oblong

7. Leaf : pubescence on lower surface of the leaf

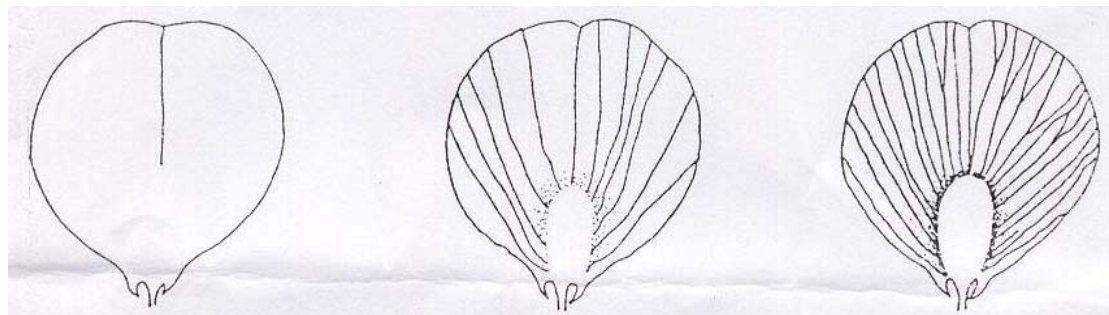
The pubescence on lower surface of the leaf of five labeled plants in each of the genotype were recorded at 50 per cent flowering stage.

8. Flower: colour of base of petal (standard)

The five tagged plants of each genotype were observed at 50 per cent flowering stage and were categorized into light yellow, yellow, orange yellow, purple and red.

9. Flower: pattern of streaks on petal (standard)

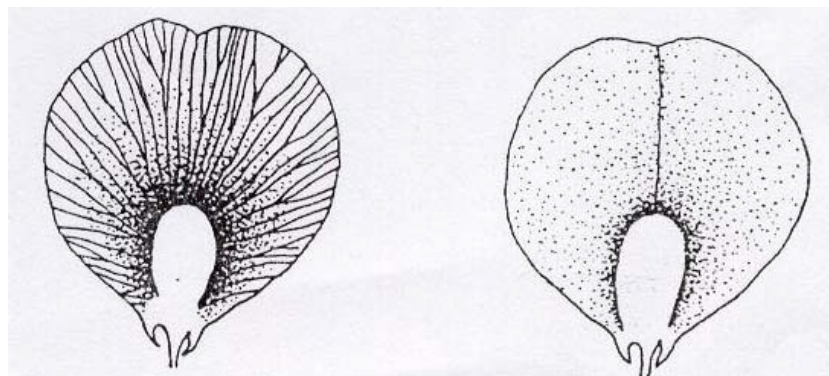
The five tagged plants of each genotype were observed at 50 per cent flowering stage and were categorized into absent, sparse, medium, dense and mosaic types.



Absent

Medium

Dense



Dense

Mosaic

10. Pod: Colour

The Pod colour of five randomly tagged plants from each genotype at the time of premature pod (dough stage) was observed and the genotypes were grouped into green, green with brown streaks, green with purple streaks, purple and dark purple.

11. Pod: Pubescence

The pubescence on the pod was observed at premature pods(dough stage) and was grouped as absent and present in the genotypes.

12.Pod: Waxiness

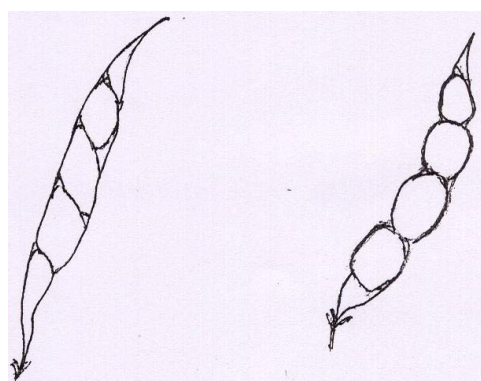
The waxiness on the pod was observed at premature pods(dough stage) and was grouped as absent and present in the genotypes.

13. Pod :Surface stickiness

The surface stickiness on the pod was observed at premature pods (dough stage) and was grouped as absent or present in the genotypes.

14. Pod: Constriction

The pod constriction of five randomly tagged plants from each genotype at the time of pod mature stage was observed and genotypes were grouped into slight and prominent.



Slight

Prominent

15. Pod size

The pod size of five randomly tagged plants from each genotype at the time of pod mature stage was observed and genotypes were grouped into small(<4 cm), medium (4-5 cm) and large (> 5 cm) types.

16. Number of seeds

The number of seeds per pod was counted and average was worked out based on ten pods and was expressed as number of seeds per pod for all the genotypes.

17. Plant :height

The height of five plants were recorded at harvest maturity stage and grouped in to short (< 100 cm), medium (100-150 cm) and tall variety (>150 cm).

18. Seed :colour

Seed colour of the hybrid and its parental lines were observed visually under natural day light condition and were grouped into the following colour groups viz., cream, brown, dark brown, grey and purple based on seed coat colour

19. Seed : colour pattern

The colour pattern of hybrid and its parental lines were observed under natural day light condition and were grouped in to following groups viz., uniform and mottled.

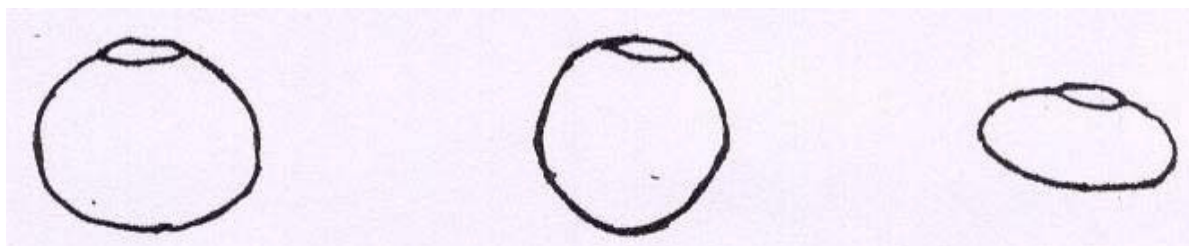


Uniform

Mottled

20. Seed: shape

The seed shape of pigeon pea hybrids were observed under magnifying lens and were grouped into as oval, globular and elongated.



Oval

Globular

Elongated

21. Seed: Test weight (100 seed weight)

Hundred seeds in four replications from each genotype were counted and weight was recorded by using electronic balance as per the procedure of ISTA (Anon.,1999). The average weight was recorded in grams. Based on seed weight, genotypes were categorized into small (>7g), medium (7- 9 g), large (> 9-11g) and very large (> 11 g) groups.

3.5 Experiment-IV: Molecular characterization of pigeon pea hybrid ICPH 2671 and its parental lines by using SSR markers

The seeds of pigeon pea hybrid ICPH-2671 and its parental lines produced during *kharif* season of 2014 were obtained from ICRISAT, Patencharu, Hyderabad. The Initial seed quality and seed morphological characters were studied in the department of Seed Science and Technology, College of Agriculture, Dharwad. The hybrid and parental lines were sown in the earthen pots and studied the Seedling characters and these seedlings were used for the DNA extraction. Further, the laboratory experiment of molecular characterization of hybrid and its parental lines by using SSR markers was carried out in department of Bio-technology, University of Agricultural Sciences, Dharwad during 2015. The methodology adopted for molecular marker analysis is described below.

3.5.1 Pigeon pea hybrid and their parental lines used for the molecular characterization.

Hybrid	Seed parent (A line)	Maintainer line (B line)	Pollen parent (R line)
ICPH-2671	ICPA-2043	ICPB-2043	ICPR-2671

3.5.2 Genomic DNA extraction from pigeon pea seedlings

Total genomic DNA was extracted from young leaves of hybrid and its parental lines by using CTAB method. DNA isolation from leaf was based on procedures outlined by Saghai-Marooof *et al.*, (1984) with slight modification. The reagents and the buffer solutions used for DNA isolation are presented in Appendix I.

3.5.3 Sample collection

Young and healthy and physiologically active leaves of pigeon pea seedlings of A, B, R & F₁ hybrid leaves were collected from 20 days old pigeon pea plants, rinsed with water and surface sterilized with alcohol and used for the extraction of DNA.

3.5.4 DNA extraction protocol

1. Three to four fresh young leaves (not fully expanded and less than a week old) were collected from pigeon pea seedlings are transferred to 2.0 ml eppendorf plastic tubes. For each tube liquid nitrogen was added then these leaves were crush to fine powder by using micro pestle.
2. Then added 800 µl of CTAB extraction buffer to all tubes and again crush the leaves.
3. Incubate in water bath at 65°C for about 20-30 minutes.
4. Centrifuge the tubes at 13,000 rpm for 10 minutes at 4 °C and collect the supernatant in 1.5 ml centrifuge tube using wide bored tip.
5. To the supernatant add equal volume of phenol: chloroform: isoamyl alcohol (PCI) (25:24:1) and mix by inversion for 15 minutes.
6. Centrifuge the tubes at 13,000 rpm for 10 minutes at 4 °C and collect the supernatant in 1.5 ml centrifuge tube.
7. Take supernatant without disturbing the protein layer.
8. To the supernatant add 800 µl of pre-chilled isopropanol to precipitate the DNA.
9. Shake it well for 30 to 40 seconds
10. These samples are incubate it at -20 °C for overnight.
11. Next day Centrifuge the tubes at 13,000 rpm for 10 minutes at 4 °C and remove the supernatant.
12. Wash the pellet 2–3 times with 70 % ethanol and air dry the pellet in room temperature for half an hour.
13. For each tubes add 10 % alcohol to dissolve the pellet and again Centrifuge the tubes at 13,000 rpm for 10 minutes at 4 °C.
14. Dry the pellet removing the supernatant.
15. Add 40 µl of T₁₀ E₁ buffer to dissolve the DNA.
16. Store at -20 °C for further use.

3.5.5 Purification of extracted genomic DNA

1. Equal volume (100 µl) of phenol: chloroform: isoamylalcohol (25:24:1) mixture was added to each tube and the contents were mixed by inverting.
2. The contents were spun at 2500 rpm for 10 min and supernatant was transferred to fresh tubes.
3. Steps 1 and 2 were repeated.
4. To the supernatant above, 10 µl of 3M NaOH (1/10 volume of aqueous layer) and 2.5 volume of chilled absolute ethanol was added, mixed gently and incubated at -20°C for 15 to 20 minutes.
5. The DNA was spooled in 1.5 ml eppendorf tubes using glass hook, washed with 70% alcohol and dried in vacuum.
6. DNA was dissolved in T₁₀E₁ buffer and kept at -20°C till its further use.

3.5.6 Determination of quantity and quality of isolated DNA

The genomic DNA extracted from individual plant samples viz., four DNA samples A-line, B-line, R-line & F₁ hybrid total four DNA samples. The exact amount of DNA was quantified by recording the spectrophotometer (Nanodrop, USA) readings at 260 nm wave length, which allowed the calculation of nucleic acids in the sample.

The DNA quantity was determined by spectrophotometer using a Nanodrop (C) 1000 (Thermo Scientific, USA). Initialization of the instrument was done with nanopure water. The instrument was set blank with the help of 3 µl T₁₀ E₁. The quantity of DNA was measured by loading the 1-2 DNA µl sample on Nano drop spectrophotometer pedestal. The DNA quantity in ng/ml and OD value for each sample was noted. Nucleic acids absorb light at both 260nm and 280nm wavelengths and proteins absorb at 230 nm. The spectrophotometer was programmed to measure absorbance (A) from 220 to 350 nm and display the DNA concentration.

The ratio of absorbance at these wavelengths is an indicator of DNA purity. For pure DNA, the A_{260/280} should be between 1.8 to 2.0 and the A_{260/230} between 1.8 to 2.3 (Sambrook and Russel, 2000). Lower values of the latter ratio indicate the presence of contaminating proteins. All the DNA samples were then diluted to 10ng/µl and used for PCR. The computed OD values were used to dilute the DNA samples to the working concentration of 100 ng/ µl for PCR. The DNA solution were stored in -20°C till further use.

The concentration of DNA in the solution was calculated according to the following formula

$$\text{DNS conc. (10ng/}\mu\text{l)} = \text{OD 260} \times 50 \times \text{dilution factor.}$$

3.5.7 Agarose gel preparation and electrophoresis for visualization of genomic DNA

1) Materials required

Gel electrophoresis apparatus
Gel casting tray and combs
Power supply
Microwave-Oven
UV –trans –illuminator/ Gel Documentation system
Eppendorf pipettes

2) Reagents required

50 X TAE buffer

Tris (Ph 8.3)-242 g

Glacial acetic acid-57.1 ml

EDTA (0.5 M)-100 ml

EtBr-10 mg/ml

Loading dye- Bromophenol blue (6 X)

30% glycerol and 0.25 % Bromophenol blue

Per cent of gel preparation is 8 %

Following procedure was used to accomplish the quality determination

1. Weigh about 0.8 g agarose and add 100 ml of distilled water again add 200 ml of TAE buffer (1 X).
2. Melt the agarose completely into a clear solution in microwave oven at the temperature of 60-65°C for 10-15 min. Then cool the molten agarose to approximately 50°C and add EtBr at concentration of 0.5 µg/ml mix well.
3. The end of the Perspex tray was sealed with spacers and comb was inserted.
4. The prepared Agarose gel (0.8 %) was poured into the tray and air bubbles were removed by using eppendorf pipette.
5. After the Gel was completely set, the gel was placed into electrophoresis tank.
6. Approximately 500 ml of TAE 1X buffer was poured into the electrophoresis tank enough to cover the gel to a depth of 5 mm.
7. Comb was removed carefully.
8. About the 1/10th volume of loading buffer 6X bromophenol blue dye was added to each DNA samples and mixed by gently tapping and spinning 2-3 seconds in a microcentrifuge.
9. DNA samples were loaded into the wells and power supply of about 80V was provided to run the gel.
10. The power supply was switched off when dye front was about 2 cm from positive end and the gel was removed from the gel apparatus (nearly 1 hr is required for run the gel).
11. The gel was viewed and photographed by using gel documentation system (UVI tech england).

3.5.8 Primer dilution

The lyophilized contents of primers may appear as either translucent film and centrifuge the tubes before opening. For primer dilution added 95 µl of biological water to the each primer tubes and shake well and keep it for -20°C. After diluting the primers, these primers are used after two days only. For making working solutions add 95 µl of biological water or distilled water to each primer tubes. After that, add 5 µl of primer of primer total it will become 100 µl.

3.5.9 Polymer chain reaction

The five SSR markers were used for the study. The DNA samples of A, B,R & F₁ hybrid were diluted by using TE buffer for the concentration of 15 ng/μl. The PCR reactions were performed in 20 μl using a gradient thermocycler (Quantum Bio). The annealing temperature is differed for each primer and the reactions were performed based on the temperature of each primer to get specific amplifications. All the reactions were carried out under aseptic conditions, as PCR is very sensitive to contamination and to avoid false amplification. For completion of it will take nearly two hrs. The amplified products were store at -20°C till the electrophoresis was carried out.

3.5.10 PCR reaction mixture

Component	Quantity (μl)
H ₂ O(Biological water)	12.7
PCR Assay buffer (10 X)	2.0
dNTP's (2 mM)	2.0
Primer 1 (forward) (10 p mol/ μl)	1.0
Primer 2 (Reverse) (10 p mol/ μl)	1.0
<i>Taq polymerase</i> (5 U/ μl)	0.3
DNA template (100 ng/ μl)	1.0
Total	20 μl

3.5.11 Touchdown PCR profile used for hybrids

Sl. No.	Step	Temp (°C)	Duration (min)	No. of cycles
1	Initiation of Denaturation	95 °C	5	5
2	Denaturation	94 °C	30 seconds	
3	Annealing	*65 °C	30 seconds	
4	Primer extension	72 °C	30 seconds	
5	Denaturation	94 °C	30 seconds	
6	Annealing	60 °C	30 seconds	35
7	Primer extension	72 °C	30 seconds	

8	Primer extension	72 °C	30 seconds	
9	Final extension	72 °C	10 min	1
	Hold	4 °C		

*Decreased at the rate of 1 ° C per cycle for 5 cycles

3.5.12 Agarose Gel Electrophoresis

The PCR amplified products were fractioned on 4% Agarose horizontal gel electrophoresis. The gel was prepared in 1X TAE buffer. The solution was added with ethidium bromide (5 µl/100 ml). 1 X TAE buffer = 2 ml 100 ml of distilled water and 4% agarose contains 4 g of Agarose in 100 ml of distilled water

Agarose solution containing ethidium bromide was poured into the gel casting platform, after placing the comb in the tray. while pouring the gel sufficient care should be taken to ensure that, there is no air bubbles trapped inside the gel. The comb was removed after the gel solidification. The solidified gel was transferred into the electrophoresis unit containing sufficient buffer so as to cover the wells completely. The amplified PCR products of 1 µl of with 10 µl loading dye was mixed thoroughly by using the micro pipette, then load the 100bp DNA ladder after that samples were carefully load into the wells with the help of eppendorf micro pipette. The electrophoresis gel was run at 100 V for 4 hours at room temperature. After completion of electrophoresis, the gel was carefully removed from the unit and kept it in Gel Documentation system. The banding pattern itself was noted from the digital image of the gels and further these images are used for identifying the banding pattern.

3.5.13 Details of the SSR markers isolated from BES's of pigeon pea used for the molecular analysis (Bohra *et al.*, 2011 and Saxena *et al.*, 2013)

Details of SSR Primer CcM 2626	
Name	CcM 2626 (Forward primer)
Nucleotide sequence	GGATTCTGAAATATTTTGTTACGG
Product size	198 bp
^b SSR motif	(TA) 22
OD	16.1
Yield	505.7 µg
Yield	68.2 n mol
IDO	8020882485-000010

Conc. (volume 1 ml)	505.7 p mol/ µl
Volume for 100 p mol/ µl	682 µl
Mol .weight	7413 g/ mol
Temperature	61.3 °C
Length (no. of nucleotides)	24
GC-content	33.3 %
Scale	25
Purification	DST
Name	CcM 2626 (Reverse primer)
Nucleotide sequence	ACTGCCAATATAAATTACCATGTCT
Product size	198 bp
^b SSR motif	(TA) ²²
OD	14.9
Yield	461.0 µg
Yield	60.7 n mol
IDO	8020882485-000020
Conc. (volume 1 ml)	461.0 p mol/ µl
Volume for 100 p mol/ µl	607 µl
Mol .weight	7584 g/ mol
Temperature	60.4 °C
Length (no. of nucleotides)	25
GC-content	32 %
Scale	25
Purification	DST

3.5.14 Details of SSR Primer CcM 2300

Name	CcM 2300 (Farward primer)
Nucleotide sequence	ACGTCTTCACCGGCATAAAC
Product size	227 bp
^b SSR motif	(A)13n (A)10n (GA)5
OD	11.9
Yield	377.6 µg
Yield	62.4 n mol
IDO	8020882485-000030
Conc. (volume 1 ml)	377.6 p mol/ µl
Volume for 100 p mol/ µl	624 µl
Mol .weight	6046 g/ mol
Temperature	63.8 °C
Length (no. of nucleotides)	20
GC-content	50 %
Scale	25
Purification	DST
Nucleotide sequence	TTTCAACCCTCTCGATTGG
Product size	227 bp
^b SSR motif	(A)13n (A)10n (GA)5
OD	13.8
Yield	469.6 µg
Yield	77.8 n mol
IDO	8020882485-000040

Conc. (volume 1 ml)	469.6 p mol/ µl
Volume for 100 p mol/ µl	778 µl
Mol .weight	6034 g/ mol
Temperature	63.8 °C
Length (no. of nucleotides)	20
GC-content	45 %
Scale	25
Purification	DST

3.5.15 Details of SSR Primer CcM 0724

Name	CcM 0724 (Farward primer)
Nucleotide sequence	AGTTTTCCAATATACCTCAAAAGC
Product size	275 bp
^b SSR motif	(ATT)10
OD	15.0
Yield	476.0 µg
Yield	78.7 n mol
IDO	8020882485-000050

Conc. (volume 1 ml)	476.0 p mol/ µl
Volume for 100 p mol/ µl	787 µl
Mol .weight	6046 g/ mol
Temperature	63.8 °C
Length (no. of nucleotides)	24
GC-content	50 %

Scale	25
Purification	DST
Nucleotide sequence	CAGTGCGGATTTGGATTTTT
Product size	275 bp
^b SSR motif	(ATT) 10
OD	10.8
Yield	367.5 µg
Yield	60.9 n mol
IDO	8020882485-000060
Conc. (volume 1 ml)	367.5 p mol/ µl
Volume for 100 p mol/ µl	609 µl
Mol .weight	6034 g/ mol
Temperature	63.8 °C
Length (no. of nucleotides)	20
GC-content	45 %
Scale	25
Purification	DST

3.5.16 Details of SSR Primer CcM 1707

Name	CcM 1707 (Farward primer)
Nucleotide sequence	ATGCGCGTGAAGGAAAAATA
Product size	162 bp
^b SSR motif	(AT)6
OD	12.9
Yield	374.2 µg
Yield	60.1 n mol

IDO	8020882485-000070
Conc. (volume 1 ml)	374.2 p mol/ µl
Volume for 100 p mol/ µl	601 µl
Mol .weight	6223 g/ mol
Temperature	64.2 °C
Length (no. of nucleotides)	20
GC-content	40 %
Scale	25
Purification	DST
Nucleotide sequence	TTGCTGTTACATTTAGGAGGATAGG
Product size	162 bp
^b SSR motif	(AT)6
OD	15.9
Yield	495.7 µg
Yield	63.8 n mol
IDO	8020882485-000080
Conc. (volume 1 ml)	495.7 p mol/ µl
Volume for 100 p mol/ µl	638 µl
Mol .weight	7767 g/ mol
Temperature	63.1 °C
Length (no. of nucleotides)	25
GC-content	40 %
Scale	25
Purification	DST

3.5.17 Details of SSR Primer CcM 0021

Name	CcM 0021 (Farward primer)
Nucleotide sequence	TGAATGTTTTCCAGGATTTTACA
Product size	280 bp
^b SSR motif	(TTA)10
OD	14.8
Yield	467.2 µg
Yield	63.3 n mol
IDO	8020882485-000090

Conc. (volume 1 ml)	467.2 p mol/ µl
Volume for 100 p mol/ µl	663 µl
Mol .weight	7044 g/ mol
Temperature	62 °C
Length (no. of nucleotides)	23
GC-content	30.4 %
Scale	25
Purification	DST
Nucleotide sequence	GCGCAAATATAAGAGCCCAG
Product size	280 bp
^b SSR motif	(TTA) ₁₀
OD	12.5
Yield	374.4 µg
Yield	60.9 n mol
IDO	8020882485-0000100
Conc. (volume 1 ml)	374.4 p mol/ µl
Volume for 100 p mol/ µl	609 µl
Mol .weight	6144 g/ mol
Temperature	63.4 °C
Length (no. of nucleotides)	20
GC-content	50 %
Scale	25
Purification	DST

3.6 Statistical analysis for the experimental data

The experimental data collected in respect of various parameters on plant growth, flowering periods, seed yield and seed quality parameters were analyzed statistically as described by Gomez and Gomez (1984). The critical difference (CD) values were calculated at 5 per cent ($P=0.05$) for field experiments and 1 per cent ($P=0.01$) for laboratory experiments probability level where 'F' test was found significant for both field and laboratory experiments.

The percentage data were transformed into arcsine transformation wherever, it was applicable prior to the statistical analysis.

3.6.1 Estimation of genetic parameters

Data collected on five individual plants of each genotype in three replication was used to calculate mean, range and genetic parameters as given below. In order to assess and quantify the genotypic variability among the genotypes for important yield contributing characters, the following parameters were estimated

3.6.2 Mean and Range

The mean and range of each character were calculated for each genotype in three replication.

$$\text{Mean (X)} = \frac{\text{Sum of observations of all plants}}{\text{Number of plants}}$$

Range = The minimum and maximum values for each trait.

3.6.3 Analysis of variance (ANOVA)

The analysis of variance (ANOVA) for all the characters was carried out. The Model of analysis of variance is given below;

ANOVA

Sl. No.	Sources of variation	Df	MSS	Expected MSS
1	Replication	r-1	M_1	-
2	Genotypes	g-1	M_2	$\sigma_e^2 + \sigma_g^2$
3	Error	(r-1) (g-1)	M_3	σ_e^2
4	Total	Rg-1	$M_1 + M_2 + M_3$	

3.6.4 Estimation of variance components

Phenotypic and genotypic variances were estimated using the following formula.

$$\text{Genotypic variance } (\sigma_g^2) = \frac{\text{MSS (genotype)} - \text{MSS (error)}}{\text{Number of replications}}$$

$$M_2 - M_3$$

$$r$$

Phenotypic variance (σ_p^2) = σ_g^2 + MSS (error)

$$= \frac{M_2 - M_3}{r} + M_3$$

3.6.5 Co-efficient of variability

Both phenotypic and genotypic co-efficient of variability were computed as per the method suggested by Burton and Devane (1953).

(a.) Genotypic co-efficient of variability (GCV)

$$\text{GCV (\%)} = \frac{\sigma_g}{\bar{X}} \times 100$$

(b.) Phenotypic co-efficient of variability (PCV)

$$\text{PCV(\%)} = \frac{\sigma_p}{\bar{X}} \times 100$$

Where σ_g = Genotypic standard deviation
 σ_p = Phenotypic standard deviation
 $\bar{X}$ = General mean of the character

GCV and PCV values were categorized as low, moderate and high as indicated by Sivasubramanian and Menon (1973).

0-10 % : Low
 10-20 % : Moderate
 20 % and above : High

3.6.7 Heritability (H^2)

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

$$\text{Heritability (H}^2\text{) (\%)} = \frac{\sigma_g^2}{\sigma_p^2} \times 100$$

σ_g^2 = Genotypic variance
 σ_p^2 = Phenotypic variance

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

0-30 % : Low

30-60 % : Moderate

60 % and above : High

3.6.8 Genetic Advance (GA)

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

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

Where, h^2 = Heritability in broad sense

k = Selection differential which is equal to 2.06 at 5% intensity for selection.

σ_p = Phenotypic standard deviation.

3.6.9 Genetic Advance over Mean (GAM)

Genetic advance over mean was estimated using the following formula

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

Where, GA = Genetic advance

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

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

0-10 % : Low

10-20 % : Moderate

20 % and above : High

4. EXPERIMENTAL RESULTS

The experimental results on effect of pollination methods, planting ratio's, insect attractant on maintenance, growth, seed yield and quality parameters in parental lines of pigeon pea hybrid ICPH-2671 and F_1 seed and morphological and molecular characterization studies in parental lines and hybrid pigeon pea ICPH-2671 are presented in this chapter under following different headings.

4.1 Experiment I: Maintenance of parental lines (A, B & R line) of hybrid Pigeon Pea- ICPH-2671 on seed quality

To know the effect of different pollination methods on pod length, pod breadth, size and seed quality parameters viz., germination (%), root length (cm), shoot length (cm), seedling dry weight (m g), seedling vigour index and electrical conductivity (dS/m) and hundred seed weight (g) in parental lines of hybrid Pigeon pea - ICPH-2671 are presented in table -4.3, 4.4, 4.5 and 4.6 and depicted in fig.4.2 to 4.8

4.1.1 Pod length

The data on pod length as influenced by pollination methods in parental lines of hybrid pigeon pea ICPH -2671 and their interaction effects are presented in table -4.1.

The results on pod length revealed non-significant difference due to pollination methods, genotypes and their interactions. However, among pollination methods P_3 : plant to progeny row selection recorded numerically the highest (5.29 cm) pod length and it was lowest (4.79 cm) with P_1 :selfing method.

Among genotypes G_1 : ICPA-2043 recorded numerically the highest (5.20 cm) pod length and it was lowest (4.85 cm) with G_3 : ICPR-2671.

The interaction effects due to pollination methods and genotypes were non-significant. However, the treatment combination G_1P_3 : plant to progeny row selection in ICPA-2043 recorded numerically the highest (5.42 cm) pod length and it was lowest (4.63 cm) with G_3P_1 :selfing in ICPR-2671.

4.1.2 Pod breadth

The results on pod breadth as influenced by pollination methods in parental lines of hybrid pigeon pea ICPH -2671 and their interaction effects are presented in table -4.3

The data on pod breadth revealed non-significant difference due to pollination methods, genotypes and their interactions. Among pollination methods P_3 : plant to progeny row selection recorded numerically the highest (0.85 cm) pod breadth and it was lowest (0.64 cm) with P_1 :selfing method.

Among genotypes G_1 : ICPA-2043 recorded numerically the highest (0.84 cm) pod breadth and it was lowest (0.65 cm) with G_3 : ICPR-2671.

The interaction effect due to pollination methods and genotypes were non-significant. However, the treatment combination G_1P_3 : plant to progeny row selection in ICPA-2043 recorded numerically the highest (0.89 cm) pod breadth and it was lowest (0.51cm) with G_3P_1 : selfing in ICPR-2671.

Table 4.3: Effect of different pollination methods on pod length, pod breadth and pod size of parental lines of hybrid pigeon pea ICPH-2671

Treatments	Pod length (cm)				Pod breadth (cm)				Pod size (cm)			
	P ₁	P ₂	P ₃	Mean	P ₁	P ₂	P ₃	Mean	P ₁	P ₂	P ₃	Mean
G ₁ (ICPA-2043)	4.93	5.24	5.42	5.20	0.76	0.87	0.89	0.84	3.75	4.56	4.82	4.38
G ₂ (ICPB-2043)	4.82	5.18	5.31	5.10	0.64	0.78	0.87	0.76	3.08	4.04	4.62	3.91
G ₃ (ICPR-2671)	4.63	4.77	5.15	4.85	0.51	0.65	0.78	0.65	2.36	3.10	4.02	3.16
Mean	4.79	5.06	5.29	5.05	0.64	0.77	0.85	0.84	3.06	3.90	4.48	3.81
For comparing means of	S.Em ±		CD at 5 %		S.Em ±		CD at 5 %		S.Em ±		CD at 5 %	
Genotypes (G)	0.15		NS		0.12		NS		1.01		NS	
Pollination (P)	0.21		NS		0.15		NS		1.25		NS	
G x P	0.45		NS		0.26		NS		1.35		NS	

NS – Non-significant

P₁ : Selfing method

P₂ : Random mating method

P₃ : Plant to progeny row selection

G₁ : Female parent (ICPA-2043)

G₂ : Maintainer line (ICPB-2043)

G₃ : Male parent (ICPR-2671)

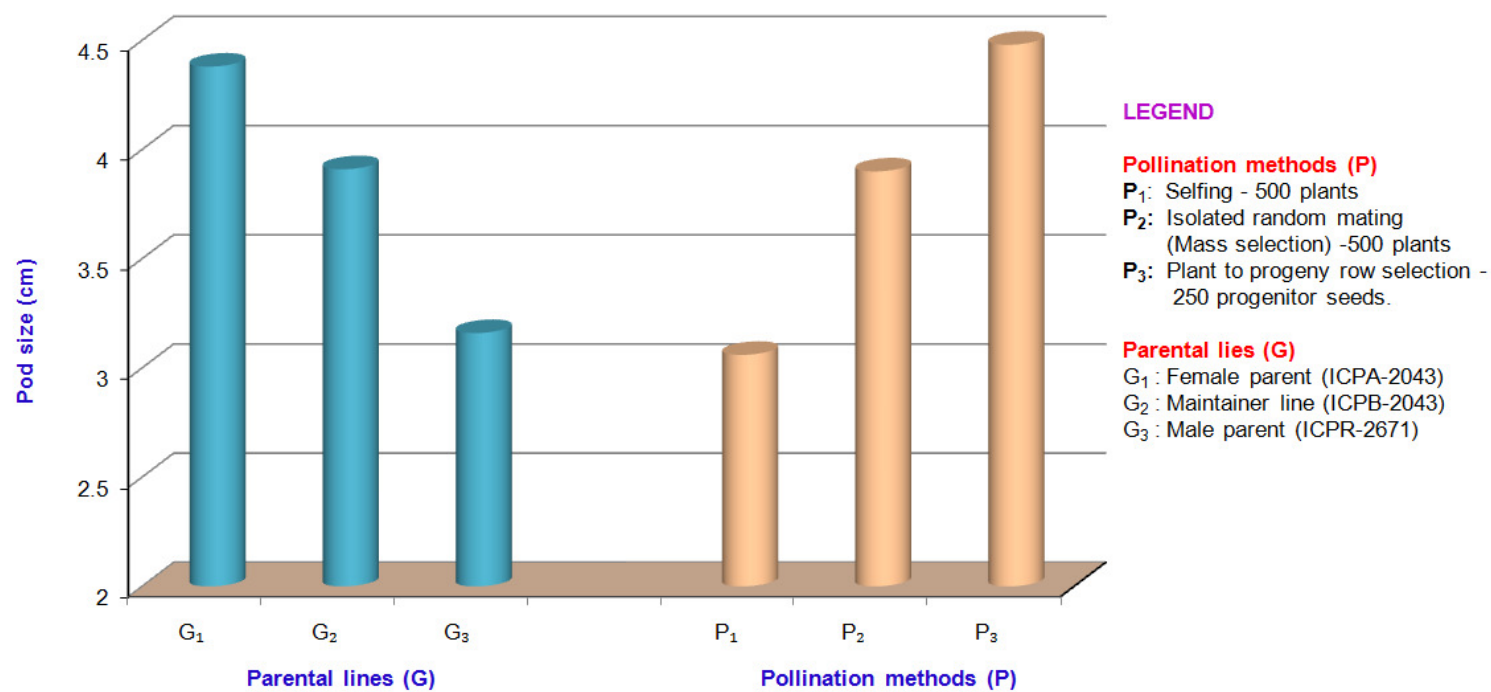


Fig. 4.2 : Effect of different pollination methods on pod size of parental lines of hybrid pigeon pea CPH-2671

Fig. 4.2 : Effect of different pollination methods on pod size of parental lines of hybrid pigeon pea CPH-2671

4.1.3 Pod size

The observation on pod size as influenced by pollination methods in parental lines of hybrid pigeon pea ICPH -2671 and their interaction effects are presented in table- 4.3 and depicted in fig. 4.2

The data on pod size showed non-significant difference due to pollination methods, genotypes and their interaction. However, among pollination methods P_3 :plant to progeny row selection recorded numerically the highest (4.48 cm) pod breadth and it was lowest (3.06 cm) with P_1 : selfing method.

Among genotypes G_1 : ICPA-2043 recorded numerically the highest (4.38 cm) pod size and it was lowest (3.16 cm) with G_3 : ICPR-2671.

The interaction effects due to pollination and genotypes were non-significant. However, the treatment combination G_1P_3 : plant to progeny row selection in ICPA-2043 recorded numerically the highest (4.82 cm) pod size and it was lowest (2.36 cm) with $G_3 P_1$: selfing in ICPR-2671.

4.1.4 Number of seeds per pod

The results on number of seeds per pod as influenced by pollination methods in parental lines of hybrid pigeon pea ICPH -2671 and their interaction effects are presented in table -4.4 and depicted in fig. 4.3

The data on number of seeds per pod showed statistically significant difference due to pollination methods, genotypes and their interactions. However, among pollination methods P_3 : plant to progeny row selection recorded significantly the highest (4.23 seeds) number of seeds per pod and it was lowest (2.38 seeds) with P_1 :selfing method.

Among genotypes G_1 : ICPA-2043 recorded significantly the highest (3.83 seeds) number of seeds per pod and it was lowest (2.93 seeds) with G_3 :ICPR-2671.

The interaction effects due to pollination and genotypes were non-significant. However, the treatment combination G_1P_3 : plant to progeny row selection in ICPA-2043 recorded significantly the highest (4.48 seeds) number of seeds per pod and it was lowest (2.15 seeds) with $G_3 P_1$: selfing in ICPR-2671.

4.1.5 Hundred seed weight

The data on hundred seed weight as influenced by pollination methods in parental lines of hybrid pigeon pea ICPH -2671 and their interaction effects are presented in table-4.4 and depicted in fig. 4.4

The data on hundred seed weight revealed statistically significant difference due to pollination methods, genotypes and their interactions. Among pollination methods P_3 :plant to progeny row selection recorded significantly the highest (12.01 g) hundred seed weight and was lowest (10.94 g) with P_1 :selfing method.

Among genotypes G_1 : ICPA-2043) recorded significantly the highest (11.81g) hundred seed weight and it was lowest (11.27 g) with G_3 :ICPR-2671.

The interaction effects due to pollination and genotypes were found to be significant. Among the treatment combinations G_1P_3 : plant to progeny row selection in ICPA-2043 recorded significantly the highest (12.12 g) hundred seed weight while it was lowest (10.55 g) with $G_3 P_1$: selfing in ICPA-2671.

Table 4.4 : Effect of different pollination methods on number of seeds per pod, hundred seed weight and field emergence of parental lines of hybrid pigeon pea ICPH-2671

Treatments	Number of seeds per pod				Hundred seed weight (g)				Field emergence (%)			
	P ₁	P ₂	P ₃	Mean	P ₁	P ₂	P ₃	Mean	P ₁	P ₂	P ₃	Mean
G ₁ (ICPA-2043)	2.67	4.33	4.48	3.83	11.21	12.10	12.12	11.81	90.67 *(72.25)	91.66 (73.25)	92.33 (73.95)	91.55 (76.56)
G ₂ (ICPB-2043)	2.32	3.67	4.25	3.41	11.07	12.04	12.06	11.72	90.66 (72.24)	91.33 (72.91)	91.66 (73.25)	91.21 (72.79)
G ₃ (ICPR-2671)	2.15	2.67	3.96	2.93	10.55	11.41	11.86	11.27	91.33 (72.91)	91.66 (73.25)	92.66 (74.31)	91.88 (73.48)
Mean	2.38	3.56	4.23	3.39	10.94	11.85	12.01	11.60	90.89 (72.46)	91.55 (73.13)	92.22 (73.84)	91.55 (73.13)
For comparing means of	S.Em ±		CD at 5 %		S.Em ±		CD at 5 %		S.Em ±		CD at 5 %	
Genotypes (G)	0.10		0.30		0.12		0.36		0.56		NS	
Pollination (P)	0.15		0.45		0.14		0.42		0.75		NS	
G x P	0.21		0.65		0.18		0.54		1.12		NS	

NS – Non-significant

* Figures in parentheses indicate arc sine transformed values

P₁ : Selfing method

G₁ : Female parent (ICPA-2043)

P₂ : Random mating method

G₂ : Maintainer line (ICPB-2043)

P₃ : Plant to progeny row selection

G₃ : Male parent (ICPR-2671)

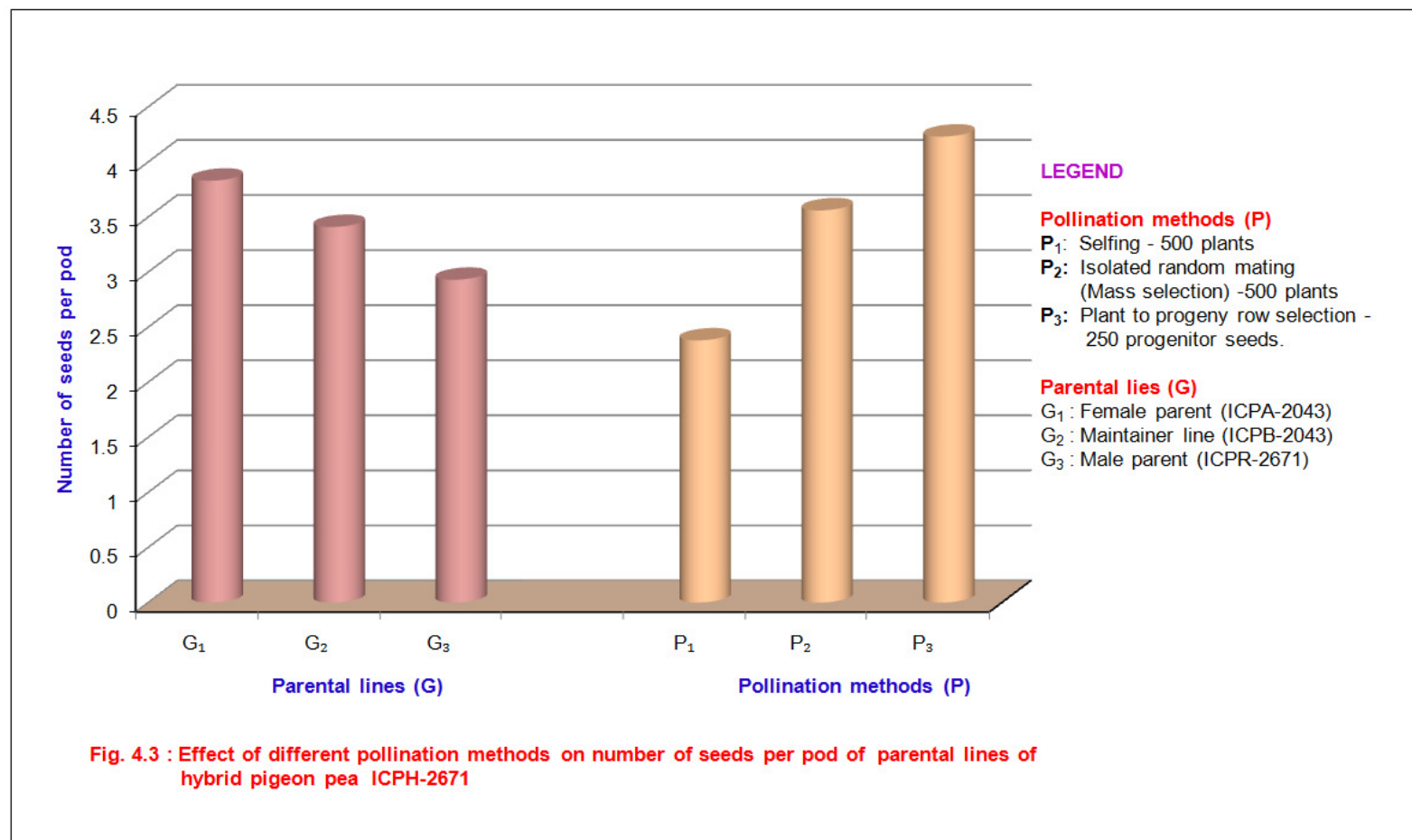


Fig. 4.3 : Effect of different pollination methods on number of seeds per pod of parental lines of hybrid pigeon pea ICPH-2671

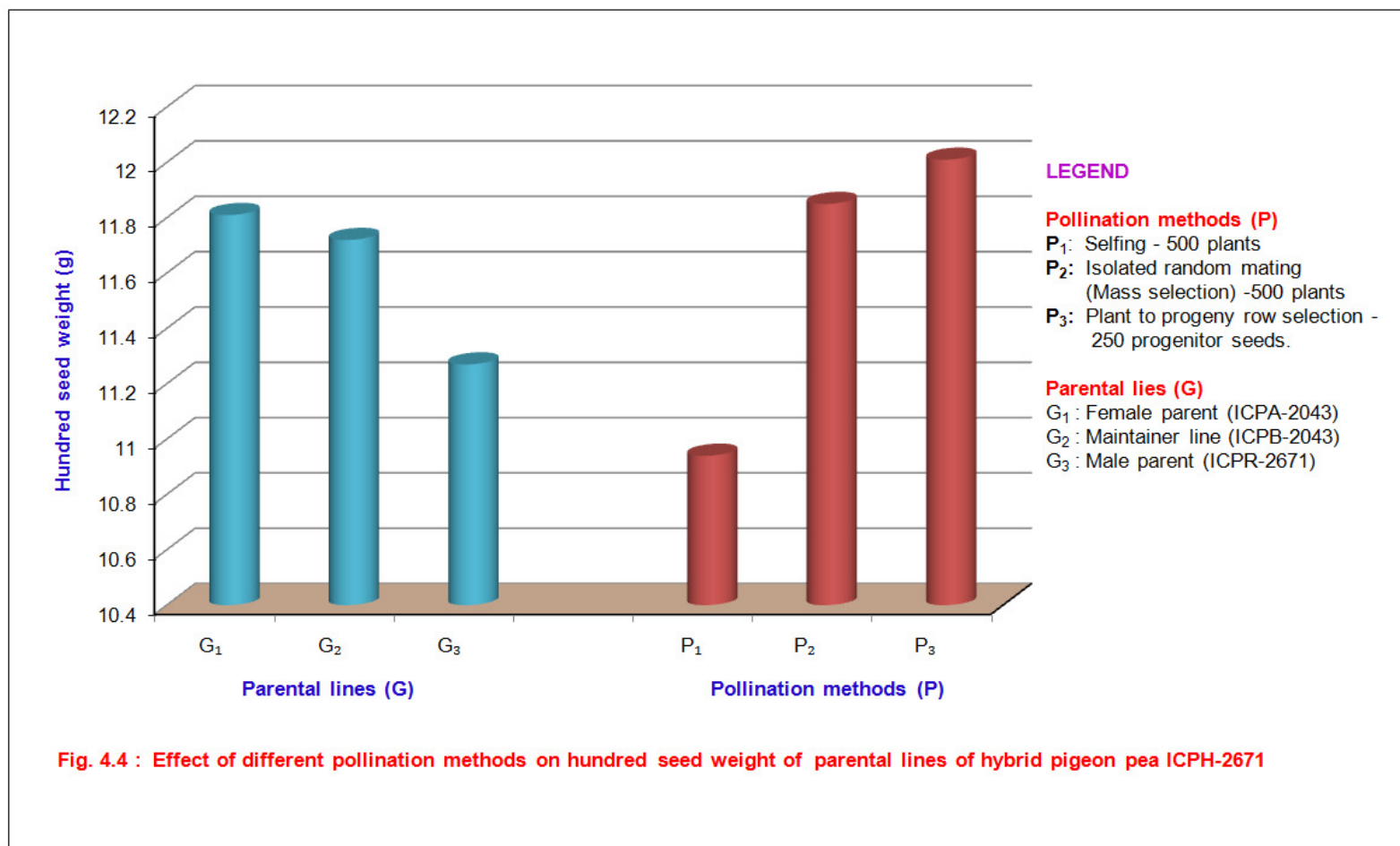


Fig. 4.4 : Effect of different pollination methods on hundred seed weight of parental lines of hybrid pigeon pea ICPH-2671

4.1.6 Field emergence

The data on field emergence percentage as influenced by pollination methods in parental lines of hybrid pigeon pea ICPH -2671 and their interaction effects are presented in table -4.4

The results on field emergence percentage revealed non-significant difference due to pollination methods, genotypes and their interactions. However among pollination methods P_3 :plant to progeny row selection recorded numerically the highest (92.22 %) field emergence and it was lowest (90.89 %) with P_1 :selfing method.

Among genotypes G_3 :ICPR-2671 recorded numerically the highest (91.88 %) field emergence and it was lowest (91.21 %) with G_2 :ICPA-2043.

The interaction effects due to pollination and genotypes were non-significant. However, the treatment combination G_3P_3 :plant to progeny row selection in ICPR-2671 recorded numerically the highest (92.66 %) field emergence and it was lowest (90.67 %).with $G_1 P_1$: selfing in ICPA-2043.

4.1.7 Germination

The data on germination percentage as influenced by pollination methods in parental lines of hybrid pigeon pea ICPH -2671 and their interaction effects are presented in table -4.5 and depicted in fig. 4.5

The results on germination percentage revealed non-significant difference due to pollination methods, genotypes and their interactions. However among pollination methods P_3 : plant to progeny row selection recorded numerically the highest (94.11 %) germination percentage and it was lowest (93.12 %) with P_1 :selfing method.

Among genotypes G_1 : ICPA-2043 recorded numerically the highest (94.23 %) germination percentage and it was lowest (92.99 %) with G_2 : ICPA-2043.

The interaction effects due to pollination and genotypes were non-significant. However, the treatment combination G_1P_3 : plant to progeny row selection in ICPA-2043 recorded numerically the highest (94.67 %) germination and it was lowest (92.33 %) with $G_2 P_1$: selfing in ICPA-2043.

4.1.7 Root length

The data on seedling root length as influenced by pollination methods in parental lines of hybrid pigeon pea ICPH -2671 and their interaction effects are presented in table -4.5 and depicted in fig.4.6

The results on seedling root length revealed significant difference due to pollination methods, genotypes and their interaction effect. While, genotypes G_3 : ICPR-2671 recorded significantly the highest (14.66 cm) seedling root length and it was lowest (12.52 cm) with G_1 : ICPA-2043. Among pollination methods P_3 : plant to progeny row selection recorded significantly the highest root length (14.59 cm) as compared to other pollination methods P_1 : selfing method (12.53) and P_2 : random mating (13.46).

The interaction effects due to pollination methods and genotypes found significant for root length. Among, the treatment combinations G_3P_3 : plant to progeny row selection in ICPR-2671 recorded significantly the highest (16.27 cm) seedling root length and it was lowest (11.73cm) with $G_1 P_1$: selfing in ICPA-2043.

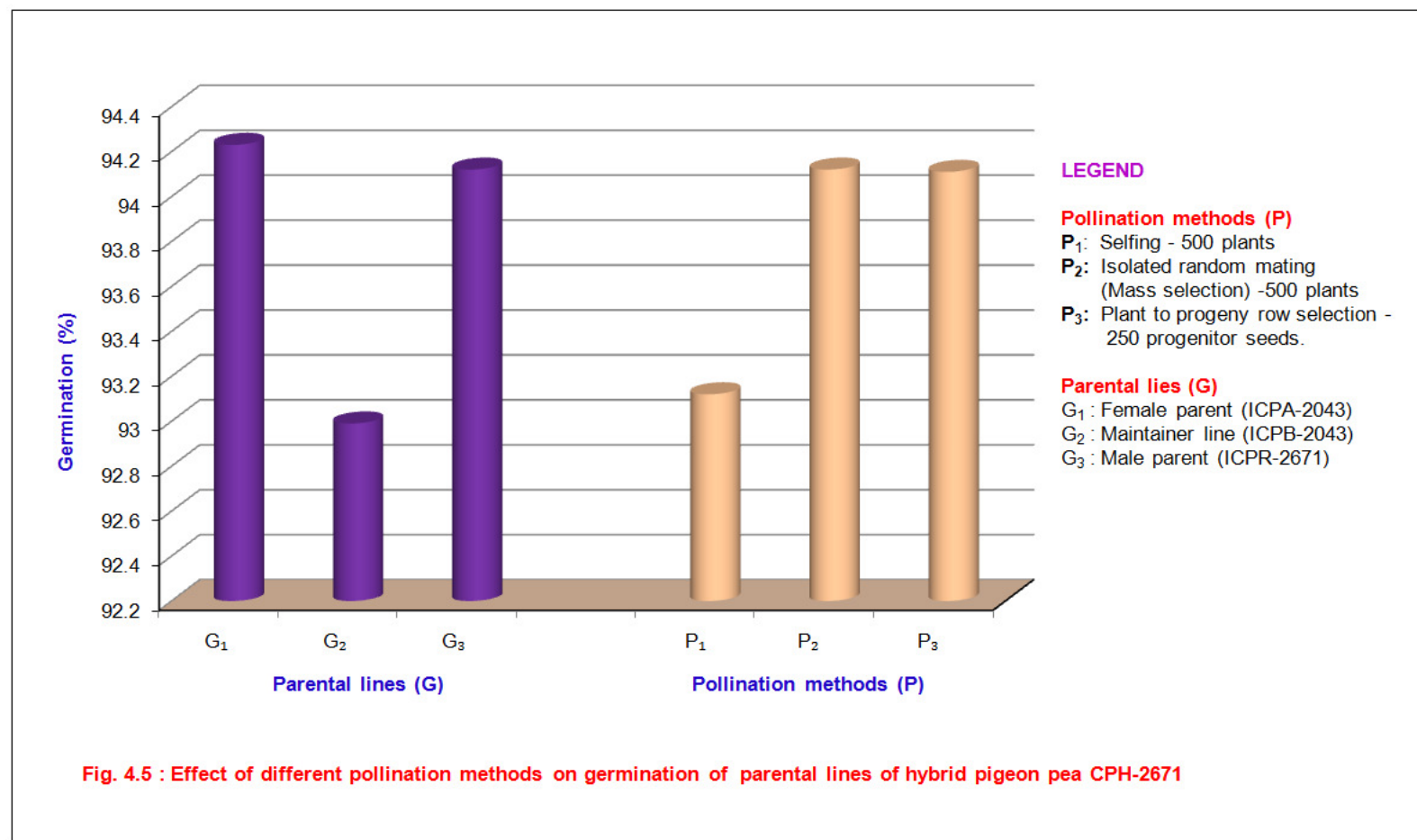


Fig. 4.5 : Effect of different pollination methods on germination of parental lines of hybrid pigeon pea CPH-2671

4.1.8 Shoot length

The observations on seedling shoot length as influenced by pollination methods in parental lines of hybrid pigeon pea ICPH -2671 and their interaction effect are presented in table -4.5 and depicted in fig.4.7

The data on seedling shoot length revealed significant difference due to pollination methods, genotypes and their interaction effects. While, genotypes G_3 : ICPR-2671 recorded significantly the highest (16.57 cm) shoot length and it was lowest (15.23 cm) with G_1 : ICPA-2043. Among pollination methods P_3 : plant to progeny row selection recorded significantly the highest shoot length (16.89 cm) as compared to other pollination methods P_1 : selfing method (13.54) and P_2 : random mating (16.62).

The interaction effects due to pollination methods and genotypes found significant for shoot length. However, the treatment combination G_3P_3 : plant to progeny row selection in ICPR-2671 recorded significantly the highest (17.56 cm) seedling shoot length and it was lowest (12.73cm) with G_1P_1 : selfing in ICPA-2043.

4.1.9 Seedling dry weight

The results on seedling dry weight as influenced by pollination methods in parental lines of hybrid pigeon pea ICPH -2671 and their interaction effects are presented in table -4.6

The data on seedling dry weight revealed non-significant difference due to pollination methods, genotypes and their interactions. Among pollination methods P_3 : plant to progeny row selection recorded numerically the highest (0.97 g) seedling dry weight and it was lowest (0.84 g) with P_1 : selfing method.

Among genotypes G_3 : ICPR-2671 recorded numerically the highest (0.93 g) seedling dry weight and it was lowest (0.89 g) with G_1 : ICPA-2043.

The interaction effects due to pollination and genotypes were non-significant. However, the treatment combination G_3P_3 : plant to progeny row selection in ICPR-2671 recorded numerically the highest (0.98 g) seedling dry weight and it was lowest (0.80 g) with G_1P_1 Selfing in ICPA-2043.

4.1.10 Seedling vigour index

The statistically analyzed data on seedling vigour index as influenced by pollination methods in parental lines of hybrid pigeon pea ICPH -2671 and their interaction effects are presented in table -4.6 and depicted in fig.4.8

The data on seedling vigour index showed statistically significant difference due to pollination methods, genotypes and their interactions effects. However among pollination methods P_3 : plant to progeny row selection recorded significantly the highest (2963) seedling vigour index and it was lowest (2428) with P_1 : selfing method.

Among genotypes G_3 : ICPR-2671 recorded significantly the highest (2941) seedling vigour index and it was lowest (2616) with G_1 : ICPA-2043.

The interaction effects due to pollination and genotypes were non-significant. However, the treatment combination G_3P_3 : Plant to progeny row selection in ICPR-2671 recorded significantly the highest (3191) seedling vigour index and it was lowest (2291) with G_1P_1 : selfing in ICPA-2043.

Table 4.5: Effect of different pollination methods on germination, root length and shoot length of parental lines of hybrid pigeon pea ICPH-2671.

Treatments	Germination (%)				Root length (cm)				Shoot length (cm)			
	P ₁	P ₂	P ₃	Mean	P ₁	P ₂	P ₃	Mean	P ₁	P ₂	P ₃	Mean
G ₁ (ICPA-2043)	93.67 (75.47)	94.37 (76.31)	94.67 (76.69)	94.23 (76.16)	11.73	12.57	13.25	12.52	12.73	16.23	16.75	15.23
G ₂ (ICPB-2043)	92.33 (73.96)	93.33 (75.07)	93.33 (75.07)	92.99 (74.70)	12.70	13.25	14.26	13.40	13.23	16.16	16.36	15.25
G ₃ (ICPR-2671)	93.36 (75.11)	94.67 (76.69)	94.33 (76.26)	94.12 (76.02)	13.16	14.56	16.27	14.66	14.67	17.48	17.56	16.57
Mean	93.12 (74.84)	94.12 (76.02)	94.11 (76.01)	93.78 (75.62)	12.53	13.46	14.59	13.53	13.54	16.62	16.89	15.68
For comparing means of	S.Em ±		CD at 5 %		S.Em ±		CD at 5 %		S.Em ±		CD at 5 %	
Genotypes (G)	0.75		NS		0.24		0.72		0.13		0.36	
Pollination (P)	0.56		NS		0.32		0.96		0.45		1.35	
G x P	0.86		NS		0.92		2.76		1.25		3.75	

NS – Non-significant

* Figures in parentheses indicate arc sine transformed values

P₁ : Selfing method

G₁ : Female parent (ICPA-2043)

P₂ : Random mating method

G₂ : Maintainer line (ICPB-2043)

P₃ : Plant to progeny row selection

G₃ : Male parent (ICPR-2671)

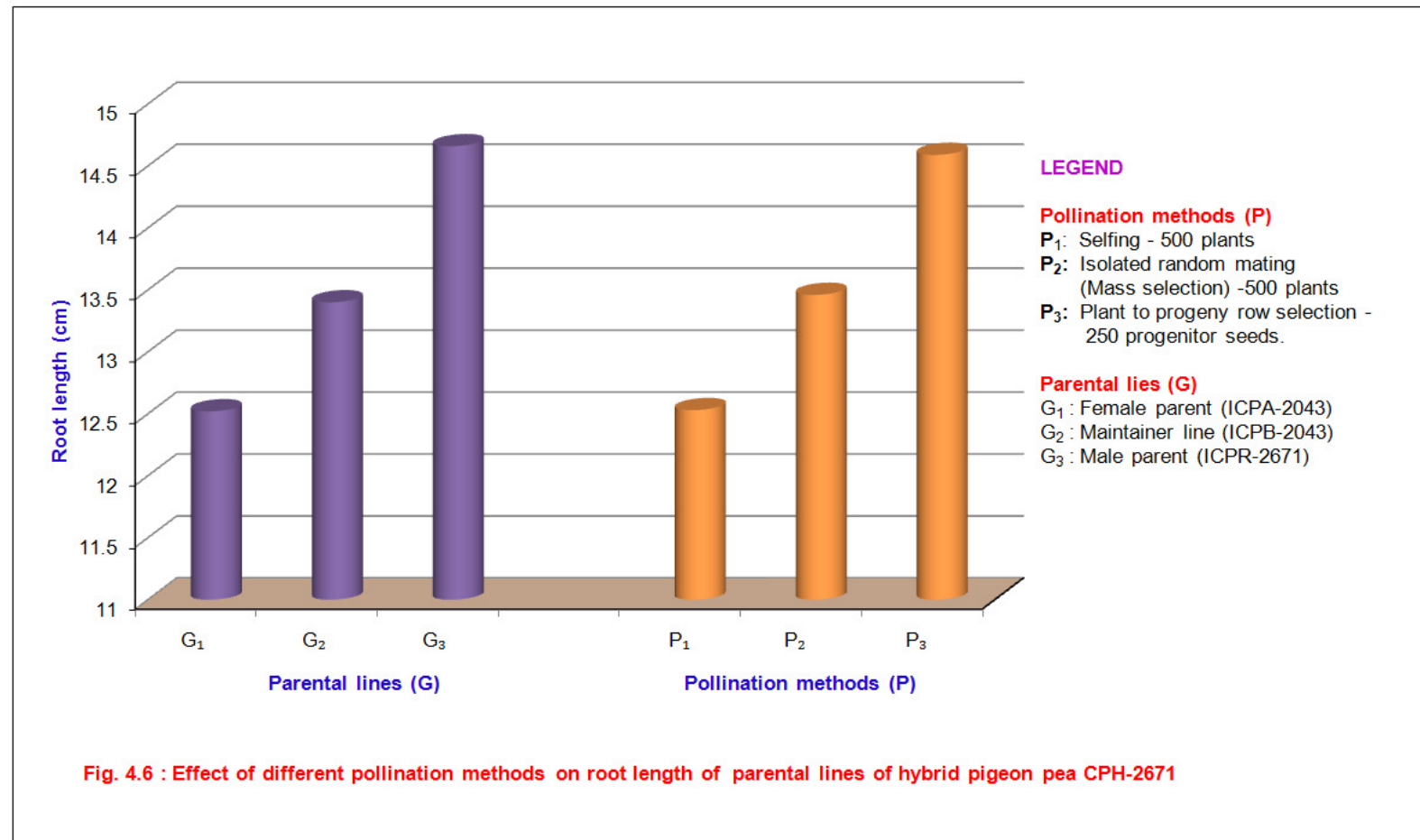


Fig. 4.6 : Effect of different pollination methods on root length of parental lines of hybrid pigeon pea CPH-2671

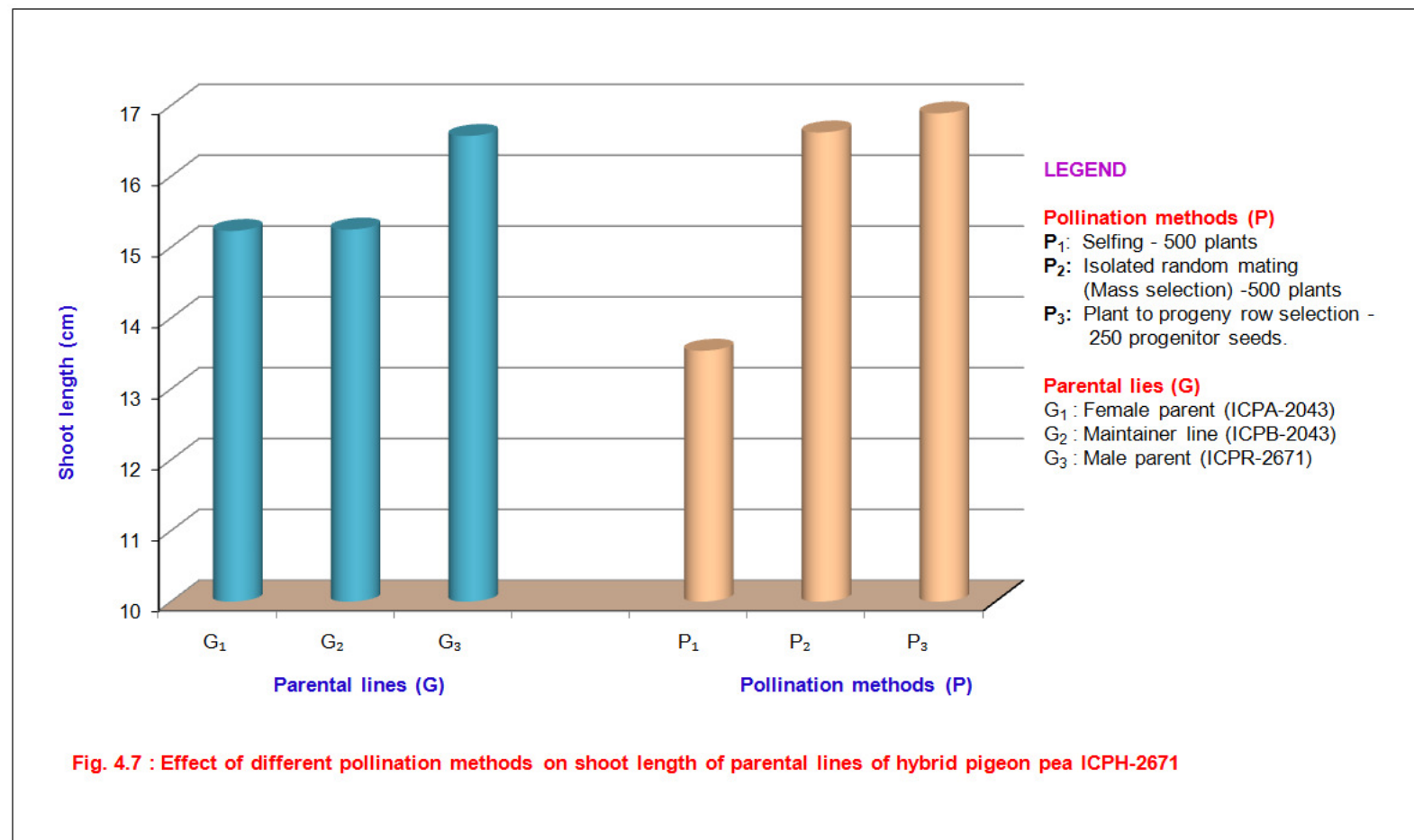


Fig. 4.7 : Effect of different pollination methods on shoot length of parental lines of hybrid pigeon pea ICPH-2671

Table 4.6 : Effect of different pollination methods on seedling dry weight, seedling vigour index and electrical conductivity of parental lines of hybrid pigeonpea ICPH-2671

Treatments	Seedling dry weight (g/10 seedlings)				Seedling vigour index				Electrical conductivity (dS/m)			
	P ₁	P ₂	P ₃	Mean	P ₁	P ₂	P ₃	Mean	P ₁	P ₂	P ₃	Mean
G ₁ (ICPA-2043)	0.80	0.92	0.95	0.89	2291	2718	2840	2616	0.35	0.37	0.28	0.33
G ₂ (ICPB-2043)	0.85	0.94	0.97	0.92	2394	2745	2858	2666	0.42	0.44	0.32	0.39
G ₃ (ICPR-2671)	0.87	0.96	0.98	0.93	2598	3033	3191	2941	0.45	0.46	0.40	0.43
Mean	0.84	0.94	0.97	0.91	2428	2832	2963	2741	0.40	0.42	0.33	0.38
For comparing means of	S.Em ±		CD at 5 %		S.Em ±		CD at 5 %		S.Em ±		CD at 5 %	
Genotypes (G)	0.15		NS		49.47		194.25		0.14		NS	
Pollination (P)	0.25		NS		39.96		119.88		0.12		NS	
G x P	0.35		NS		59.63		178.89		0.16		NS	

NS – Non-significant

P₁ : Selfing method

P₂ : Random mating method

P₃ : Plant to progeny row selection

G₁ : Female parent (ICPA-2043)

G₂ : Maintainer line (ICPB-2043)

G₃ : Male parent (ICPR-2671)

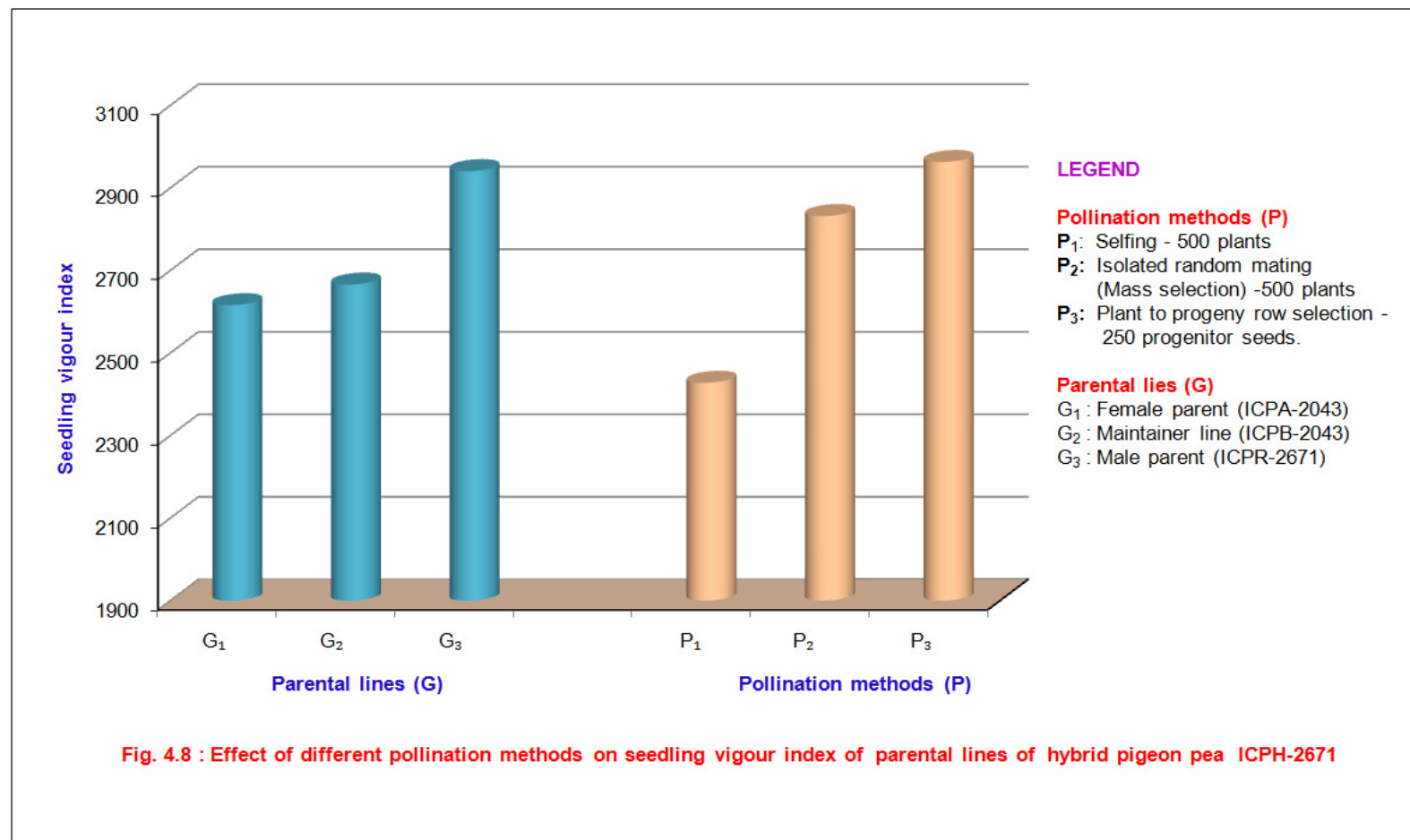


Fig. 4.8 : Effect of different pollination methods on seedling vigour index of parental lines of hybrid pigeon pea ICPH-2671

Fig. 4.8 : Effect of different pollination methods on seedling vigour index of parental lines of hybrid pigeon pea ICPH-2671

4.1.11 Electrical conductivity

The statistically analyzed data on electrical conductivity as influenced by pollination methods in parental lines of hybrid pigeon pea ICPH -2671 and their interaction effects are presented in table - 4.6

The data on electrical conductivity showed non-significant difference due to pollination methods, genotypes and their interactions. However, among pollination methods P_3 : plant to progeny row selection recorded numerically the lowest (0.33dS/m) electrical conductivity and it was highest (0.40 dS/m) with P_1 : selfing method.

Among genotypes G_1 : ICPA-2043 recorded numerically the lowest (0.33 dS/m) electrical conductivity and it was highest (0.43 dS/m) with G_3 :ICPR-2671.

The interaction effects due to pollination and genotypes were non-significant. However, the treatment combination G_1P_3 : plant to progeny row selection in ICPA-2043 recorded numerically the lowest (0.28 dS/m) electrical conductivity and it was highest (0.46 dS/m) with G_3P_2 : random mating in ICPR-2671.

4.1.12 Genetic variability and heritability of growth and yield parameters of parental lines of hybrid pigeon pea ICPH-2671

The estimates of phenotypic and genotypic co-efficient of variation, heritability and genetic advance for all the ten characters of parental lines of hybrid pigeon pea ICPH-2671 are presented in table -4.7

The data on genetic variability parameters with respect to plant height (cm), number of primary branches, number of secondary branches, days to 50 % flowering, days to flower initiation, days to pod initiation, days to pod maturity, number of pods per plant, pod yield per plant and number of seeds per pod showed significant difference. The phenotypic co-efficient of variation (PCV) was higher than genotypic co-efficient of variation (GCV) for all the characters mentioned above.

Number of seeds per pod exhibited high PCV (12.04) and GCV (6.75). Whereas, days to pod maturity also exhibited high heritability (0.98) with genetic advance (14.94).

4.1.13 Influence of pollination methods on genetic variability parameters of parental lines of hybrid pigeon pea ICPH-2671

The estimates of phenotypic and genotypic co-efficient of variation, heritability and genetic advance for seed quality parameters of parental lines of hybrid pigeon pea ICPH-2671 are presented in table -4.8

The data on genetic variability parameters pertaining to seed quality parameters viz., germination, shoot length, root length, seedling dry weight, seedling vigour index, and electrical conductivity showed significant difference. The phenotypic co-efficient of variation (PCV) was higher than genotypic co-efficient of variation (GCV) for all the characters mentioned above.

Number of seeds per pod exhibited high PCV (24.26) and GCV (24.26). Whereas, germination percentage also exhibited high heritability (0.90) with genetic advance (4.15).

Table 4.7 : Genetic variability study of parental lines of hybrid pigeon pea ICPH-2671

Sl. No.	Character	Mean	Max.	Min.	Range	Vp	Vg	PCV	GCV	H ² B	GA	GAM
1	Plant height (cm)	192.17	194.75	188.00	6.75	15.67	12.93	2.06	1.87	0.82	6.73	3.50
2	Number of primary branches	23.13	24.00	22.38	1.63	1.92	0.49	5.99	3.04	0.25	0.74	3.18
3	Number of secondary branches	30.00	31.25	29.00	2.25	1.67	1.26	4.30	3.74	0.75	2.01	6.71
4	Days to 50% flowering	117.08	123.25	112.50	9.75	49.25	25.85	5.99	4.34	0.52	7.59	6.48
5	Days to flower initiation	99.67	106.50	94.75	11.75	42.75	36.49	6.56	6.06	0.85	11.50	11.53
6	Days to pod initiation	133.38	136.75	130.63	6.13	11.00	9.48	2.49	2.31	0.86	5.89	4.42
7	Days to pod maturity	171.88	180.13	166.25	13.88	53.83	53.22	4.27	4.24	0.98	14.94	8.69
8	Number of pods per plant	287.17	273.00	307.75	34.75	444.67	316.79	7.34	6.20	0.71	30.95	10.78
9	Pod yield per plant	58.61	62.30	55.65	6.66	12.36	11.34	6.00	5.75	0.91	6.65	11.34
10	Number of seeds per pod	3.79	4.13	3.63	0.50	0.21	0.07	12.04	6.75	0.31	0.30	7.79

Vp = Phenotypic variation Vg= Genotypic variation, PCV= Phenotypic co-efficient of variation

GCV= Genotypic co-efficient of variation, H²B(%)= Heritability in broad sense

GA= Genetic advance , GAM= Genetic advance mean

Table 4.8: Influence of pollination methods on genetic variability parameters of parental lines of hybrid pigeon pea ICPH-2671

Sl.No.	Character	Mean	Max.	Min.	Range	Vg	Vp	GCV	PCV	H ² B	GA	GAM
1	Pod length (cm)	4.79	5.10	4.59	0.52	0.07	0.09	5.70	6.15	0.77	0.52	10.89
2	Pod breadth (cm)	0.75	0.84	0.65	0.19	0.01	0.01	12.85	13.62	0.89	0.19	24.97
3	Pod size (cm)	5.05	5.20	4.85	0.35	0.03	0.04	3.46	3.74	0.85	0.33	6.58
4	Number of seeds per pod	3.54	4.38	2.63	1.75	0.74	1.00	24.26	24.26	0.23	1.52	42.93
5	100 seed weight	11.68	11.91	11.31	0.60	0.10	0.12	2.75	2.92	0.88	0.63	5.35
6	Germination (%)	92.46	94.88	90.88	4.00	4.46	4.92	2.29	2.40	0.90	4.15	4.49
7	Shoot length (cm)	13.65	15.00	12.79	2.21	1.19	2.82	8.00	12.30	0.42	1.46	10.72
8	Root length (cm)	12.11	12.38	11.80	0.57	0.07	0.20	2.15	3.69	0.33	0.31	2.57
9	Seedling dry weight (g)	0.90	0.80	0.74	0.16	0.01	0.01	8.40	11.00	0.81	0.16	20.49
10	Field emergence	93.08	94.25	91.25	3.00	2.43	3.67	1.67	2.06	0.66	2.61	2.81
11	Electrical conductivity (ds/m)	0.48	0.58	0.38	0.20	0.01	0.02	28.45	19.13	0.45	0.13	26.50

Vp = Phenotypic variation Vg= Genotypic variation, PCV= Phenotypic co-efficient of variation

GCV= Genotypic co-efficient of variation, H²B= Heritability in broad sense

GA= Genetic advance , GAM= Genetic advance mean

4.2 Experiment-II: Effect of different planting ratio's and insect attractants on growth, seed yield and quality of hybrid pigeon pea ICPH-2671

4.2.1 Effect of different planting ratio's and insect attractant on growth parameters

The observations on growth parameters viz., plant stand, plant height (cm) at harvest, number of primary branches and secondary branches per plant at harvest, days to flower initiation, days to 50 % flowering, duration of the flowering, days to pod initiation and days to physiological maturity in parental lines of pigeon pea hybrid ICPH-2671 as influenced by effect of insect attractant spray, planting ratio's and their interaction effects are presented here under.

4.2.2 Growth parameters

The observations on growth parameters viz., plant stand, plant height (cm) and number of primary and secondary branches per plant at harvest both male and female parental lines as influenced by insect attractant spray, planting ratio's and their interaction effects are presented in tables 4.9, 4.10, 4.11 and 4.12

4.2.2.1 Plant stand

Female/seed parent (ICPA-2043 x ICPR-2671)

The data on plant stand due to insect attractant spray, planting ratio's and their interaction effect is presented in table -4.9

The data on plant stand due to insect attractant spray and their interaction effect did not show any significant difference and significant difference was noticed due to planting ratio's in female parental line.

Among different planting ratio's P_6 : 5:2 and P_3 :5:1 (female to male planting ratio's) recorded the significantly higher (96.50 and 95.17 plants) plant stand respectively as compared to other planting ratio's.

However, irrespective of planting ratio's in female parental line T_2 (insect attractant spray) recorded the numerically highest (76.11 plants) plant stand as compared to T_1 : control without spray (76.00 plants).

Male/pollen parent (ICPR-2671)

The data on plant stand due to insect attractant spray and their interaction effects did not show any significant difference and significant difference was noticed due to planting ratio's in male parental line (table -4.9)

Among different planting ratio's P_6 : 5:2, P_5 :4:2 and P_4 :3:2 (female to male planting ratios) recorded the significantly higher (38.83, 38.67 and 38.28 plants) plant stand respectively as compared to other planting ratio's.

However, irrespective of planting ratio's in male parental line the treatment T_2 :insect attractant spray has recorded the numerically highest (29.11 plants) plant stand as compared to T_1 :control without spray (28.57 plants).

4.2.2.3 Plant height at harvest

Female/seed parent (ICPA-2043 x ICPR-2671)

The data on plant height (cm) at harvest due to planting ratio's, insect attractant spray and their interaction effect is presented in table -4.10

The experimental results did not show any significant difference on plant height (cm) at harvest due to insect attractant spray, planting ratio's and their interaction effects.

However, it was numerically higher (183.22 cm) plant height T_2 : with insect attractant spray and lower (182.00 cm) plant height without insect attractant spray (T_1 : control without spray) in female parental lines.

Male/pollen parent (ICPR-2671)

The data on plant height (cm) did not differed significantly due to insect attractant spray, planting ratio's and their interaction effects in male parental line (table -10).

However, the numerically higher plant height (203.50 cm) was recorded with (T_2) insect attractant spray compared to without insect attractant spray (T_1 :203.17 cm).

4.2.2.4. Number of primary branches per plant at harvest

Female/seed parent (ICPA-2043 x ICPR-2671)

Number of primary branches per plant at harvest in female parental line as influenced by insect attractant spray, planting ratio's and their interaction effect did not show any significant difference as presented in table -4.11

Irrespective of planting ratio's, the insect attractant spray (T_2) recorded the numerically higher (23.06) number of primary branches per plant as compared to without insect attractant spray (T_1 :22.61).

Male parent (ICPR-2671)

The data on number of primary branches per plant at harvest did not show significantly difference due to insect attractant spray, planting ratio's and their interaction effect in male parental line (table -4.11).

However, there was numerical difference with respect to T_2 which recorded the more number of primary branches per plant (22.44) as compared to without insect attractant spray (T_1 :22.28).

4.2.2.5 Number of secondary branches per plant at harvest.

Female/seed parent (ICPA-2043 x ICPR-2671)

The data on number of secondary branches per plant at harvest as influenced by insect attractant spray, different planting ratio's and their interaction effect did not show any significant difference in female parental line of hybrid pigeon pea ICPH-2671 (table -4.12).

However, the insect attractant spray (T_2) recorded numerically more number of secondary branches per plant (30.06) as compared to without insect attractant spray (T_1 :29.83).

Male/pollen parent (ICPR -2671)

Male parental line of hybrid pigeon pea ICPH-2671 did not show any significant difference with respect to number of secondary branches per plant at harvest due to insect attractant spray, planting ratio and their interaction effect (table -12).

In male parental lines, numerically more number of secondary branches per plant (24.61) and less (23.56) with respect to insect attractant spray and without insect attractant spray respectively.

Table 4.9: Effect of different planting ratio's and insect attractant spray on plant stand in pigeon pea hybrid ICPH-2671 seed production

Treatments	Plant stand *A -line (ICPA-2043)			Plant stand R-line (ICPR-2671)		
	T ₁	T ₂	Mean	T ₁	T ₂	Mean
P ₁ = (3:1 Planting ratio)	55.67	56.00	55.83	18.55	20.00	19.28
P ₂ = (4:1 Planting ratio)	76.00	76.00	76.00	18.67	19.00	18.83
P ₃ = (5:1 Planting ratio)	95.33	95.00	95.17	19.33	19.00	19.17
P ₄ = (3:2 Planting ratio)	56.00	56.00	56.00	38.22	38.33	38.28
P ₅ = (4:2 Planting ratio)	77.00	76.67	76.83	38.33	39.00	38.67
P ₆ = (5:2 Planting ratio)	96.00	97.00	96.50	38.33	39.33	38.83
Mean	76.00	76.11	76.06	28.57	29.11	28.84
For comparing means of	S.Em. ±		C.D. at 5 %	S.Em. ±		C.D. at 5 %
Insect attractant (T)	0.14		NS	0.23		NS
Planting ratio (P)	0.25		0.73	0.40		1.18
P × T	0.35		NS	0.57		NS

NS – Non-significant

* A-line (F₁ hybrid) = A x R line seed production

T₁ : Without spray

T₂ : Insect attractant spray

Table 4.10: Effect of different planting ratio's and insect attractant spray on plant height in pigeon pea hybrid ICPH-2671 seed production

Treatments	Plant height (cm) *A -line (ICPA-2043)			Plant height (cm) R-line (ICPR-2671)		
	T ₁	T ₂	Mean	T ₁	T ₂	Mean
P ₁ = (3:1 Planting ratio)	181.33	180.00	180.67	203.00	204.67	203.83
P ₂ = (4:1 Planting ratio)	180.00	180.67	180.33	202.67	203.00	202.83
P ₃ = (5:1 Planting ratio)	180.67	183.33	182.00	202.00	204.33	203.17
P ₄ = (3:2 Planting ratio)	182.33	184.67	183.50	205.33	203.00	204.17
P ₅ = (4:2 Planting ratio)	183.67	182.33	183.50	203.67	203.00	203.33
P ₆ = (5:2 Planting ratio)	184.67	185.67	184.67	202.33	203.00	202.67
Mean	182.00	183.22	184.50	203.17	203.50	203.33
For comparing means of	S.Em. ±		C.D. at 5 %	S.Em. ±		C.D. at 5 %
Insect attractant (T)	0.86		NS	0.54		NS
Planting ratio (P)	2.50		NS	0.93		NS
P × T	3.08		NS	1.32		NS

NS – Non-significant

* A-line (F₁ hybrid) = A x R line seed production

T₁ : Without spray

T₂ : Insect attractant spray

Table 4.11: Effect of different planting ratio's and insect attractant spray on number of primary branches in pigeon pea hybrid ICPH-2671 seed production

Treatments	Number of primary branches per plant *A -line (ICPA-2043)			Number of primary branches per plant R-line (ICPR-2671)		
	T ₁	T ₂	Mean	T ₁	T ₂	Mean
P ₁ = (3:1 Planting ratio)	22.67	23.00	22.83	23.33	21.67	22.50
P ₂ = (4:1 Planting ratio)	24.67	22.33	23.50	21.33	21.00	21.17
P ₃ = (5:1 Planting ratio)	23.33	21.33	22.33	22.33	24.00	23.17
P ₄ = (3:2 Planting ratio)	21.00	24.00	22.50	23.33	22.00	22.67
P ₅ = (4:2 Planting ratio)	21.00	25.33	23.17	22.33	24.00	23.17
P ₆ = (5:2 Planting ratio)	23.00	22.33	22.67	21.00	22.00	21.50
Mean	22.61	23.06	22.67	22.28	22.44	22.69
For comparing means of	S.Em. ±		C.D. at 5 %	S.Em. ±		C.D. at 5 %
Insect attractant (T)	0.68		NS	0.50		NS
Planting ratio (P)	1.18		NS	0.87		NS
P × T	1.67		NS	1.23		NS

NS – Non-significant

* A-line (F₁ hybrid) = A x R line seed production

T₁ : Without spray

T₂ : Insect attractant spray

4.2.2.6 Flowering parameters

The results on flower and reproductive components viz., days to flower initiation, days to 50 per cent flowering and duration of flowering in both female and male parental lines as influenced by different planting ratio's, insect attractants spray and their interaction effects are presented in tables – 4.13, 4.14 & 4.15.

4.2.6.1 Days to flower initiation

Female/seed parent (ICPA-2043 x ICPR-2671)

The results on number of days to flower initiation due to the influence of planting ratio's, insect attractant spray and their interaction effects did not show any significant difference in female parental line as presented in table - 4.13

Insect attractant spray (T_2) took numerically less number of days (94.72) days for flower initiation as compared to without insect attractant spray (T_1 :95.56 days).

4.2.6.2 Male/pollen parent (ICPR -2671)

The data on days to flower initiation due to planting ratio's, spraying of insect attractant and their interaction effects did not show significant difference in male parental line (table -4.13).

The insect attractant spray (T_2) took numerically less (106.44 days) number of days to flower initiation as compared to without insect attractant spray (T_1 :106.06 days) in male parental line.

4.2.6.3 Days to 50 per cent flowering

Female/seed parent (ICPA-2043 x ICPR-2671)

Days to 50 per cent flowering differed significantly due to insect attractant spray and did not show any significant difference due to planting ratio's and their interaction effect in female parental line as presented in table -4.14.

The statistical difference was noticed on days to 50 per cent flowering due to insect attractant spray. Insect attractant spray (T_2) took significantly less (109.89 days) number of days to 50 per cent flowering as compared to (T_1 :116.89).

Male/pollen parent (ICPR -2671)

The data on days to 50 per cent flowering due to insect attractant spray differed significantly and non-significant difference was noticed due to planting ratio's and their interaction effects (table - 4.14).

However, In male parental line took significantly less (122.61days) number of days to 50 per cent flowering due to insect attractant spray (T_2) as compared to without insect attractant spray (T_1 :124.89 days).

4.2.6.4 Duration of flowering

Female/seed parent (ICPA-2043 x ICPR-2671)

The statistical data on duration of flowering due to insect attractant spray, planting ratio's and their interaction effects did not show any significant difference in female parental line as presented in table -4.15.

Table 4.12: Effect of different planting ratio's and insect attractant spray on number of secondary branches in pigeon pea hybrid ICPH-2671 seed production.

Treatments	Number of secondary branches per plant *A -line (ICPA-2043)			Number of secondary branches per plant R-line (ICPR-2671)		
	T ₁	T ₂	Mean	T ₁	T ₂	Mean
P ₁ = (3:1 Planting ratio)	30.00	30.00	30.00	24.00	25.67	24.83
P ₂ = (4:1 Planting ratio)	28.33	31.00	29.67	23.67	25.33	24.50
P ₃ = (5:1 Planting ratio)	29.67	31.00	30.33	23.33	24.67	24.00
P ₄ = (3:2 Planting ratio)	30.67	30.33	30.50	22.67	24.33	23.50
P ₅ = (4:2 Planting ratio)	30.67	29.00	29.83	23.67	23.67	23.67
P ₆ = (5:2 Planting ratio)	29.67	29.00	29.33	24.00	24.00	24.00
Mean	29.83	30.06	29.94	23.56	24.61	24.09
For comparing means of	S.Em. ±		C.D. at 5 %	S.Em. ±		C.D. at 5 %
Insect attractant (T)	0.37		NS	0.49		NS
Planting ratio (P)	0.64		NS	0.85		NS
P × T	0.96		NS	1.20		NS

NS – Non-significant

* A-line (F₁ hybrid) = A x R line seed production

T₁ : Without spray

T₂ : Insect attractant spray

Table 4.13: Effect of different planting ratio's and insect attractant spray on days to flower initiation in pigeon pea hybrid ICPH-2671 seed production

Treatments	Days to flower initiation *A -line (ICPA-2043)			Days to flower initiation R-line (ICPR-2671)		
	T ₁	T ₂	Mean	T ₁	T ₂	Mean
P ₁ = (3:1 Planting ratio)	94.33	95.67	95.00	103.33	105.67	104.50
P ₂ = (4:1 Planting ratio)	94.33	95.33	94.83	108.00	105.67	106.83
P ₃ = (5:1 Planting ratio)	94.33	95.00	94.67	106.33	107.33	106.83
P ₄ = (3:2 Planting ratio)	94.33	96.33	95.33	106.67	106.33	106.50
P ₅ = (4:2 Planting ratio)	95.00	96.67	95.83	106.33	106.00	106.17
P ₆ = (5:2 Planting ratio)	96.00	94.33	95.17	105.67	107.67	106.67
Mean	94.72	95.56	95.14	106.06	106.44	106.25
For comparing means of	S.Em. ±		C.D. at 5 %	S.Em. ±		C.D. at 5 %
Insect attractant (T)	0.40		NS	0.84		NS
Planting ratio (P)	0.89		NS	1.45		NS
P × T	1.98		NS	2.05		NS

NS – Non-significant

* A-line (F₁ hybrid) = A x R line seed production

T₁ : Without spray

T₂ : Insect attractant spray

Table 4.14: Effect of different planting ratio's and insect attractant spray on days 50 per cent flowering in pigeon pea hybrid ICPH-2671 seed production

Treatments	Days to 50 per cent flowering * A -line (ICPA-2043)			Days to 50 per cent flowering R-line (ICPR-2671)		
	T ₁	T ₂	Mean	T ₁	T ₂	Mean
P ₁ = (3:1 Planting ratio)	117.33	109.00	113.17	124.00	122.33	123.17
P ₂ = (4:1 Planting ratio)	117.33	110.00	113.67	124.00	123.00	123.50
P ₃ = (5:1 Planting ratio)	117.67	110.33	114.00	126.00	123.00	124.50
P ₄ = (3:2 Planting ratio)	116.00	107.00	111.50	125.33	123.00	124.17
P ₅ = (4:2 Planting ratio)	116.33	110.33	113.33	127.00	122.67	124.83
P ₆ = (5:2 Planting ratio)	116.67	112.67	114.67	123.00	121.67	122.33
Mean	116.89	109.89	113.39	124.89	122.61	123.75
For comparing means of	S.Em. ±		C.D. at 5 %	S.Em. ±		C.D. at 5 %
Insect attractant (T)	0.49		1.44	0.53		1.54
Planting ratio (P)	1.85		NS	0.91		NS
P × T	4.20		NS	3.87		NS

NS – Non-significant

* A-line (F₁ hybrid) = A x R line seed production

T₁ : Without spray

T₂ : Insect attractant spray

Table 4.15: Effect of different planting ratio's and insect attractant spray on duration of the flowering in pigeon pea hybrid ICPH-2671 seed production

Treatments	Duration of the flowering (days) *A -line (ICPA-2043)			Duration of the flowering (days) R-line (ICPR-2671)		
	T ₁	T ₂	Mean	T ₁	T ₂	Mean
P ₁ = (3:1 Planting ratio)	84.67	85.33	85.00	75.00	76.00	75.50
P ₂ = (4:1 Planting ratio)	85.00	85.67	85.33	74.00	75.33	74.67
P ₃ = (5:1 Planting ratio)	85.00	86.00	85.50	75.00	77.33	76.17
P ₄ = (3:2 Planting ratio)	86.33	84.67	85.50	75.33	75.00	75.17
P ₅ = (4:2 Planting ratio)	85.67	85.67	85.67	76.00	76.33	76.17
P ₆ = (5:2 Planting ratio)	84.67	86.67	85.67	76.67	77.00	76.83
Mean	85.22	85.67	85.44	75.33	76.17	75.75
For comparing means of	S.Em. ±		C.D. at 5 %	S.Em. ±		C.D. at 5 %
Insect attractant (T)	0.32		NS	0.32		NS
Planting ratio (P)	0.55		NS	0.56		NS
P × T	0.78		NS	1.79		NS

NS – Non-significant

* A-line (F₁ hybrid) = A x R line seed production

T₁ : Without spray

T₂ : Insect attractant spray

However, the insect attractant spray (T_2 : with insect attractant spray) took numerically higher (85.67 days) duration of flowering as compared to without insect attractant spray (T_1 :85.22 days) in female parental line.

Male/pollen parent (ICPR-2671)

The results on duration of flowering due to insect attractant spray, planting ratio's and their interaction effects did not show any significant difference (table -15).

However, numerical difference was noticed on duration of flowering in male parental lines of hybrid pigeon pea ICPH-2671 due to insect attractant spray. It was numerically highest (76.17 days) duration of flowering due to insect attractant spray (T_2) and lowest (75.33 days) duration of flowering without insect attractant spray (T_1) in male parental line.

4.2.7 Seed yield components

The results on days to pod initiation, days to pod maturity and number of pods per plant in both female and male parental lines as influenced by planting ratio's and insect attractant spray and their interaction effects are presented in tables 4.16 to 4.25

4.2.7.1 Days to pod initiation

Female/seed parent (ICPA-2043 x ICPR-2671)

The data on days to pod initiation due to insect attractant spray, planting ratio's and their interaction effect are presented in table -4.16

The results on days to pod initiation revealed significant difference due to insect attractant spray and did not show any significant difference due to planting ratio's and their interaction effects.

However, insect attractant spray (T_2) took significantly less number of days to pod initiation (129.11 days) as compared to treatment without insect attractant spray (T_1 :133.50 days).

Male/pollen parent (ICPR -2671)

The results on days to pod initiation due to insect attractant spray showed significant difference and non- significant difference was noticed due to planting ratio's and their interaction effects in male parental line (table -4.16)

The statistical analysis on days to pod initiation due to insect attractant spray differed significantly. The T_2 : insect attractant spray took significantly less (134.22 days) number of days to pod initiation as compared to treatment without insect attractant spray (T_1 :137.44 days).

4.2.7.2 Days to pod maturity

Female/seed parent (ICPA-2043 x ICPR-2671)

The data on days to pod maturity due to insect attractant spray, planting ratio's and their interaction effects revealed significant difference in female parental line as presented in table -4.17 and depicted in fig.4.9

In female parental line, the crop sprayed with insect attractant (T_2) recorded significantly less number of days (163.00 days) to pod maturity as compared to without insect attractant spray (T_1 :167.11 days).

Among the different planting ratios the P_6 : 5:2, P_5 : 4:2 and P_4 :3:2 (female to male planting ratios) recorded the significantly less number of days (162.67, 163.33 & 164.83 days respectively) to pod maturity as compared to P_1 :3:1 (166.17 days) P_2 :4:1 (167.17 days) and P_3 :5:1 (166.17 days respectively).

Table 4.16: Effect of different planting ratio's and insect attractant spray on days pod initiation in pigeon pea hybrid ICPH-2671 seed production

Treatments	Days to pod initiation *A -line (ICPA-2043)			Days to pod initiation R-line (ICPR-2671)		
	T ₁	T ₂	Mean	T ₁	T ₂	Mean
P ₁ = (3:1 Planting ratio)	135.00	128.33	131.67	137.67	132.33	135.00
P ₂ = (4:1 Planting ratio)	134.67	129.33	132.00	138.33	135.67	137.00
P ₃ = (5:1 Planting ratio)	133.33	129.33	131.33	137.67	136.33	137.00
P ₄ = (3:2 Planting ratio)	132.33	130.00	131.17	138.33	137.33	137.83
P ₅ = (4:2 Planting ratio)	133.00	127.67	130.33	136.00	131.00	133.50
P ₆ = (5:2 Planting ratio)	132.67	130.00	131.33	136.67	132.67	134.67
Mean	133.50	129.11	131.30	137.44	134.22	135.83
For comparing means of	S.Em. ±		C.D. at 5 %	S.Em. ±		C.D. at 5 %
Insect attractant (T)	0.32		0.94	0.65		1.90
Planting ratio (P)	0.75		NS	1.26		NS
P × T	4.78		NS	3.59		NS

NS – Non-significant

* A-line (F₁ hybrid) = A x R line seed production

T₁ : Without spray

T₂ : Insect attractant spray

The interaction effect due to planting ratio's (P) and insect attractant spray (T) showed significant difference with respect to days to pod maturity. The treatment combination P_6T_2 :(5:2): female to male planting ratio + insect attractant spray and P_5T_2 :(4:2):female to male planting ratio + insect attractant spray has recorded significantly less (160.33 days) and (160.67 days) number of days to pod maturity as compared to P_3T_1 (168.33 days), P_2T_1 (168.00 days) P_1T_1 (168.00 days), and P_4T_1 (167.33 days) planting ratio's.

Male/pollen parent (ICPR -2671)

In male parental line exhibited significant difference on days to pod maturity due to insect attractant spray and non significant difference was noticed due to planting ratio's and their interaction effects as presented in table -4.17 and depicted in fig.4.9

The treatment insect attractant spray (T_2) took significantly less number (180.94 days) of days to pod maturity as compared to treatment without spraying of insect attractant (T_1 :183.00 days).

4.2.7.3 Number of pods per plant

Female/seed parent (ICPA-2043 x ICPR-2671)

The data on number of pods per plant exhibited significant difference due to insect attractant spray, planting ratio's and their interaction effect in female parental line as presented in table -4.18 and depicted in fig. 4.10

The treatment T_2 : insect attractant spray has recorded significantly highest number pods per plant (350.83) as compared to without insect attractant spray (T_1 :315.50 pods).

Among, different planting ratio's P_6 :5:2 female to male planting ratio has recorded significantly higher (336.00) number of pods per plant as compared to P_1 :3:1(330.33 pods), P_2 :4:1 (330.17) and P_3 :5:1 (333.50) planting ratio's respectively.

The interaction effect due to planting ratio's (P) and insect attractant spray (T) showed significant difference with respect to number of pod per plant. Among, the treatment combinations P_6T_2 :5:2 (female to male planting ratio's with insect attractant spray) and P_5T_2 :4:2 (female to male planting ratio's with insect attractant spray) recorded significantly the higher (354.00) and (351.67) number of pods per plant as compared to all other treatment combinations in female parental line.

Male/pollen parent (ICPR-2671)

Number of pods per plant exhibited significant difference due to insect attractant spray and non-significant difference was noticed due to planting ratio's and their interaction effects in male parental line as presented in table -4.18 and depicted in fig. 4.10. However, the treatment T_2 has recorded significantly the higher number of pods per plant (300.11 pods/plant) as compared to T_1 : without insect attractant spray (263.78 pods/plant).

4.2.7.4 Pod yield per plant

Female/seed parent (ICPA-2043 x ICPR-2671)

The data on pod yield (g) per plant due to insect attractant spray, planting ratio's and their interaction effects are presented in table -4.19 and depicted in fig.4.11

The results on pod yield (g) per plant exhibited significant difference due to insect attractant spray, planting ratio's and their interaction effects in female parent. The treatment T_2 : insect attractant spray has recorded highest pod yield (63.61 g) per plant as compared to without insect attractant spray (T_1 :56.18 g/plant).

Table 4.17: Effect of different planting ratio's and insect attractant spray on days to pod maturity in pigeon pea hybrid ICPH-2671 seed production

Treatments	Days to pod maturity *A -line (ICPA-2043)			Days to pod maturity R-line (ICPR-2671)		
	T ₁	T ₂	Mean	T ₁	T ₂	Mean
P ₁ = (3:1 Planting ratio)	168.00	164.33	166.17	181.67	181.33	181.50
P ₂ = (4:1 Planting ratio)	168.00	166.33	167.17	182.00	180.00	181.00
P ₃ = (5:1 Planting ratio)	168.33	164.00	166.17	183.33	180.33	181.83
P ₄ = (3:2 Planting ratio)	167.33	162.33	164.83	183.33	182.33	182.83
P ₅ = (4:2 Planting ratio)	166.00	160.67	163.33	182.67	181.00	181.83
P ₆ = (5:2 Planting ratio)	165.00	160.33	162.67	185.00	180.67	182.83
Mean	167.11	163.00	165.05	183.00	180.94	181.97
For comparing means of	S.Em. ±		C.D. at 5 %	S.Em. ±		C.D. at 5 %
Insect attractant (T)	0.46		1.35	0.44		1.29
Planting ratio (P)	0.80		2.40	0.76		NS
P × T	1.13		3.39	2.24		NS

NS – Non-significant

* A-line (F₁ hybrid) = A x R line seed production

T₁ : Without spray

T₂ : Insect attractant spray

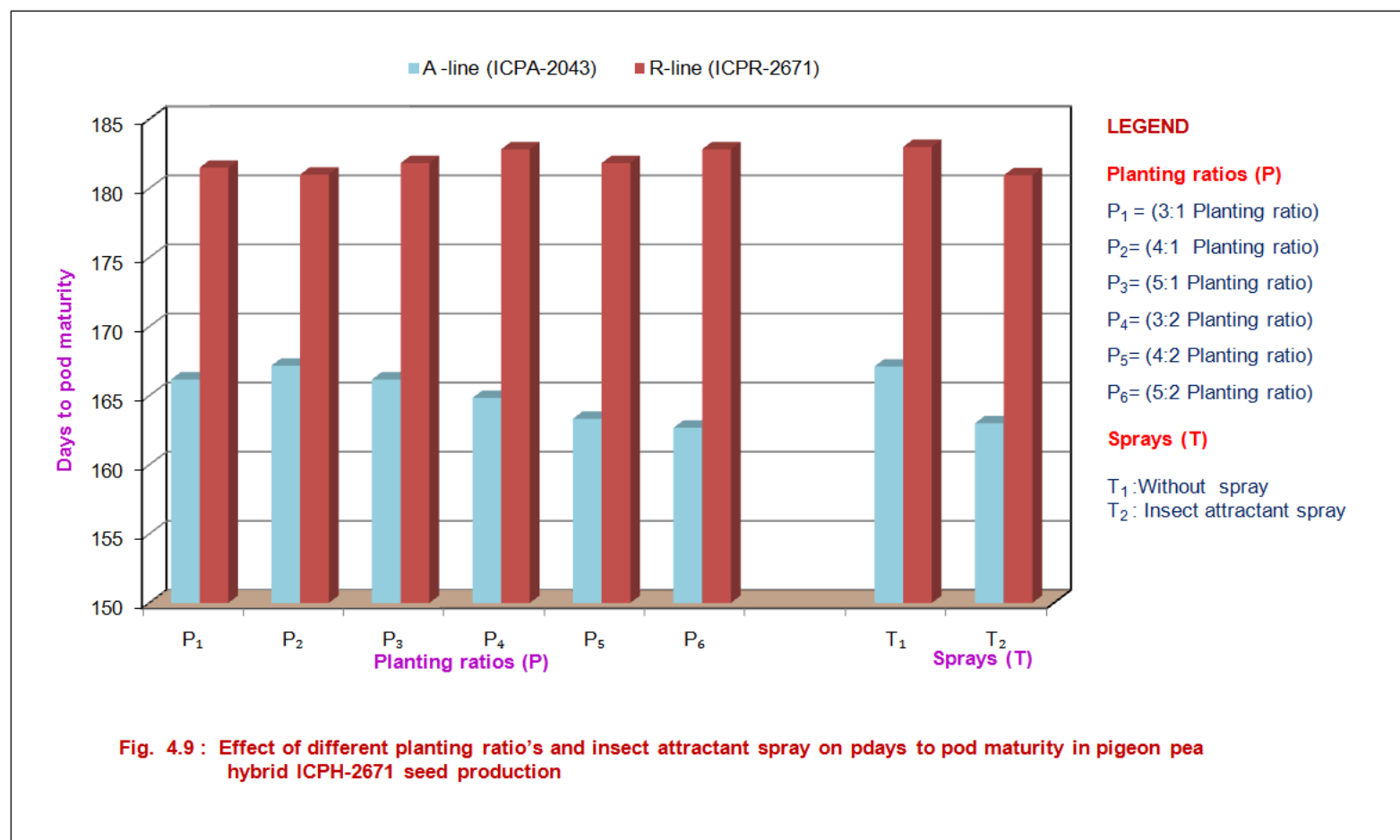


Fig. 4.9 : Effect of different planting ratio's and insect attractant spray on pdays to pod maturity in pigeon pea hybrid ICPH-2671 seed production

Table 4.18: Effect of different planting ratio's and insect attractant spray on number of pods per plant in pigeon pea hybrid ICPH-2671 seed production

Treatments	Number of pods per plant *A -line (ICPA-2043)			Number of pods per plant R-line (ICPR-2671)		
	T ₁	T ₂	Mean	T ₁	T ₂	Mean
P ₁ = (3:1 Planting ratio)	312.33	348.33	330.33	265.00	296.00	280.50
P ₂ = (4:1 Planting ratio)	310.33	350.00	330.17	264.33	297.00	280.67
P ₃ = (5:1 Planting ratio)	317.00	350.00	333.50	264.33	300.00	282.17
P ₄ = (3:2 Planting ratio)	317.67	351.00	334.33	263.00	300.00	281.50
P ₅ = (4:2 Planting ratio)	317.67	351.67	334.67	263.67	303.00	283.33
P ₆ = (5:2 Planting ratio)	318.00	354.00	336.00	262.33	304.67	283.50
Mean	315.50	350.83	333.16	263.78	300.11	281.94
For comparing means of	S.Em. ±		C.D. at 5 %	S.Em. ±		C.D. at 5 %
Insect attractant (T)	0.29		0.84	0.50		1.50
Planting ratio (P)	0.49		1.45	1.12		NS
P × T	0.70		2.05	3.20		NS

NS – Non-significant

* A-line (F₁ hybrid) = A x R line seed production

T₁ : Without spray

T₂ : Insect attractant spray

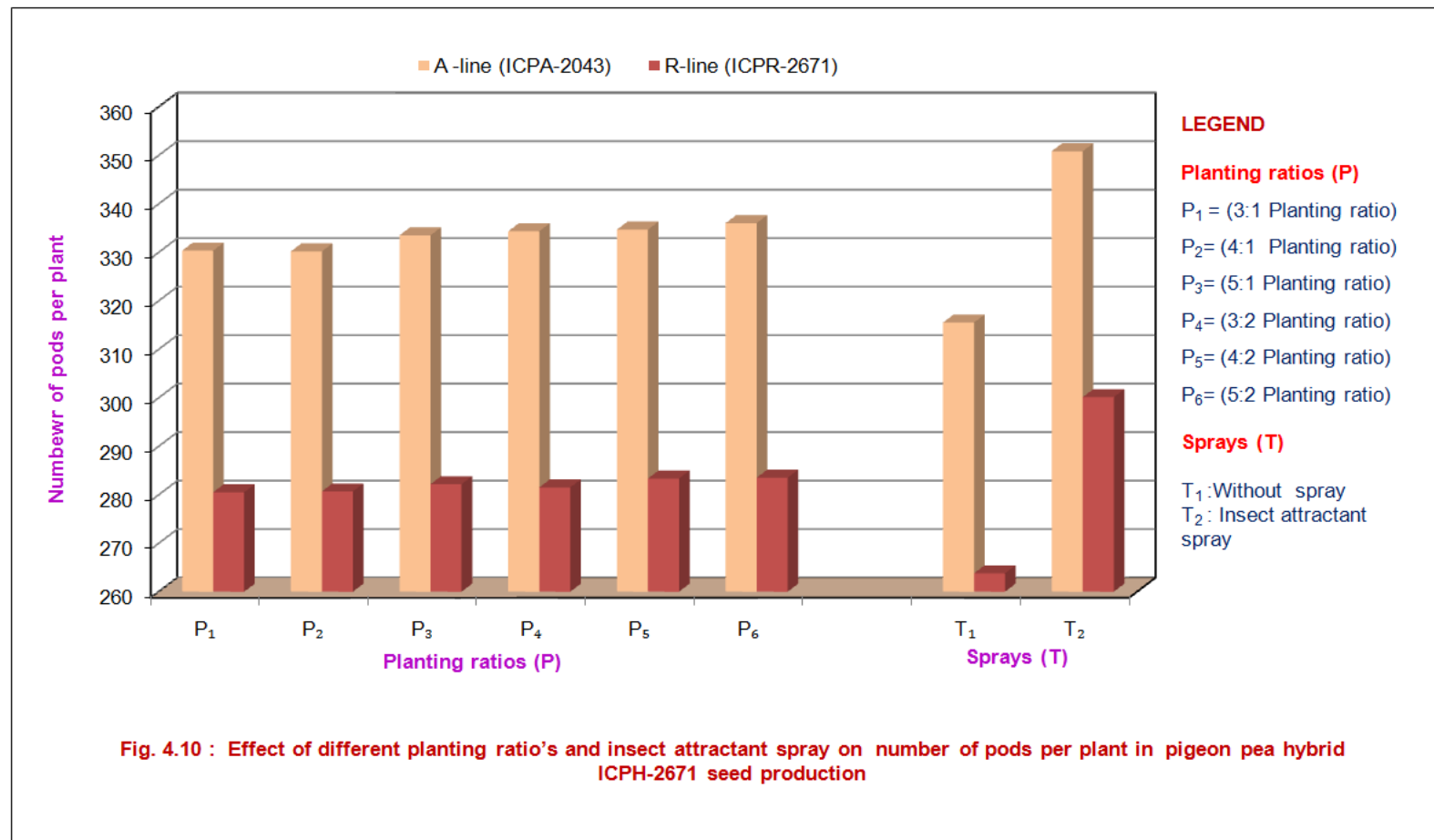


Fig. 4.10 : Effect of different planting ratio's and insect attractant spray on number of pods per plant in pigeon pea hybrid ICPH-2671 seed production

Among different planting ratio's $P_6:5:2$ and $P_5:4:2$ (female to male planting ratio) recorded the significantly highest (64.90 g & 62.76 g) pod yield per plant as compared to $P_1:3:1$ (56.38 g) and $P_2:4:1$ (57.06 g), $P_3:3:2$ (60.06 g) and $P_4:3:2$ (58.21 g).

The interaction effect due to planting ratio's (P) and insect attractant spray (T) showed significant difference with respect to pod yield (g) per plant. Among, the treatment combinations P_6T_2 : (5 :2 female to male planting ratio's with insect attractant spray) and P_5T_2 : (4:2 female to male planting ratio's with insect attractant spray) recorded significantly the higher (69.53 g) and (67.36 g) pod yield per plant as compared to all other treatment combinations in female parental line.

Male/pollen parent (ICPR -2671)

The data on pod yield per plant due to insect attractant spray exhibited significant difference and did not show any significant difference with respect to planting ratio's and their interaction effect as presented in table -4.19 and depicted in fig. 4.11. The insect attractant spray (T_2) recorded the highest pod yield per plant (44.26 g) as compared to treatment without insect attractant spray (T_1 :43.24 g) in male parental line.

4.2.7.5 Pod yield (kg) per plot

Female/seed parent (ICPA-2043 x ICPR-2671)

The results on pod yield per plant due to insect attractant spray, planting ratio's and their interaction effects showed significant difference as presented in table -4.20 and depicted in fig. 4.12.

There was significant variation in the pod yield (kg) per plot due to planting ratios, insect attractant spray and their interaction effects in female parental lines of pigeon pea hybrid ICPH-2671.

The treatment insect attractant spray (T_2) recorded significantly highest (4.97 kg) pod yield per plot as compared to T_1 (4.30 kg). Among different planting ratio's $P_6: 5:2$ and $P_3:5:1$ and $P_5: 4:2$ (female to male planting ratio) recorded the significantly highest (6.27, 5.75 & 4.89 kg) pod yield per plot as compared to $P_1:3:1$ (3.17 kg), $P_2:4:1$ (4.42 kg) and $P_4:3:2$ (3.31 kg) planting ratio's.

The interaction effect due to different planting ratio's and insect attractant spray showed significant difference due to pod yield (kg) per plot. P_6T_2 : 5:2 planting ratio's with insect attractant spray has recorded significantly highest pod yield (6.74 kg) per plot as compared to all other treatment combinations, while, it was lowest (3.01 kg) with $P_1T_1:3:1$ planting ratio's without insect attractant spray.

Male/pollen parent (ICPR -2671)

The data on pod yield (kg) per plot differed significantly due to insect attractant spray, planting ratio's and their interaction effects (table -4.20 & fig. 4.12). The insect attractant spray (T_2) recorded significantly highest (1.39 kg) pod yield per plot as compared to without insect attractant spray (T_1 :1.21 kg).

Among the different planting ratio's $P_6: 5:2$ female to male planting ratio and $P_5: 4:2$ female to male planting ratio and $P_4:4:2$ female to male planting ratio has recorded the significantly, highest (1.79, 1.78 & 1.76 kg) pod yield per plot as compared to $P_1:3:1$ (0.80 kg), $P_2:4:1$ (0.84 kg) and $P_3:3:2$ (0.85 kg) female to male planting ratio's.

Table 4.19: Effect of different planting ratio's and insect attractant spray on pod yield per plant in pigeon pea hybrid ICPH-2671 seed production

Treatments	Pod yield (g) per plant *A -line (ICPA-2043)			Pod yield (g) per plant R-line (ICPR-2671)		
	T ₁	T ₂	Mean	T ₁	T ₂	Mean
P ₁ = (3:1 Planting ratio)	54.49	58.26	56.38	43.16	44.10	43.63
P ₂ = (4:1 Planting ratio)	53.96	60.16	57.06	43.18	44.12	43.65
P ₃ = (5:1 Planting ratio)	55.96	64.16	60.06	43.20	44.15	43.68
P ₄ = (3:2 Planting ratio)	54.26	62.16	58.21	43.26	44.32	43.79
P ₅ = (4:2 Planting ratio)	58.16	67.36	62.76	43.30	44.40	43.85
P ₆ = (5:2 Planting ratio)	60.26	69.53	64.90	43.32	44.46	43.89
Mean	56.18	63.61	59.89	43.24	44.26	43.75
For comparing means of	S.Em. ±		C.D. at 5 %	S.Em. ±		C.D. at 5 %
Insect attractant (T)	0.16		0.46	0.11		0.33
Planting ratio (P)	0.27		0.80	0.20		NS
P × T	0.38		1.13	0.56		NS

NS – Non-significant

* A-line (F₁ hybrid) = A x R line seed production

T₁ : Without spray

T₂ : Insect attractant spray

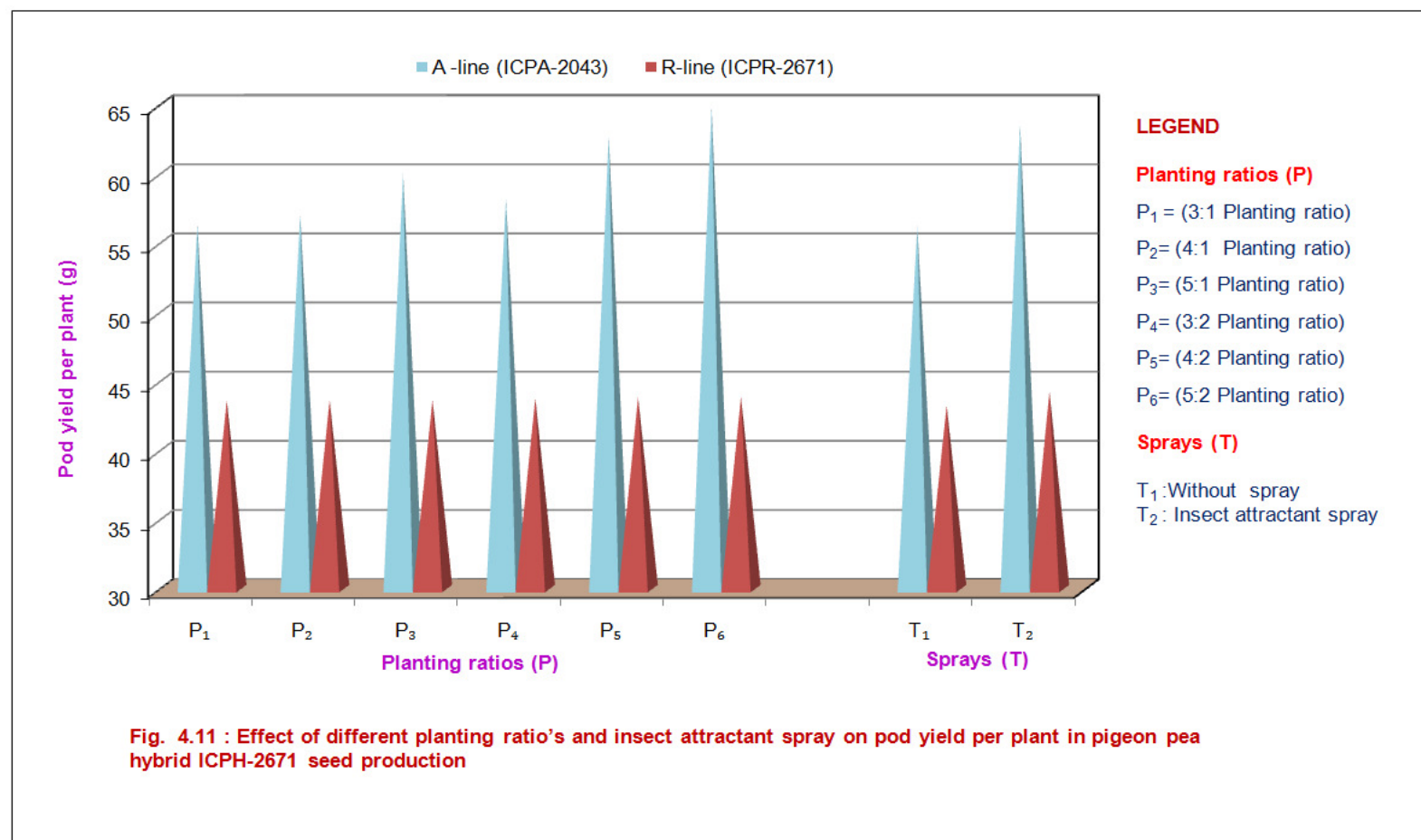


Fig. 4.11 : Effect of different planting ratio's and insect attractant spray on pod yield per plant in pigeon pea hybrid ICPH-2671 seed production

Table 4.20: Effect of different planting ratio's and insect attractant spray on pod yield per plot in pigeon pea hybrid ICPH-2671 seed production

Treatments	Pod yield (kg) per plot * A -line (ICPA-2043)			Pod yield (kg) per plot R-line (ICPR-2671)		
	T ₁	T ₂	Mean	T ₁	T ₂	Mean
P ₁ = (3:1 Planting ratio)	3.01	3.32	3.17	0.75	0.85	0.80
P ₂ = (4:1 Planting ratio)	4.12	4.71	4.42	0.78	0.89	0.84
P ₃ = (5:1 Planting ratio)	5.32	6.18	5.75	0.79	0.91	0.85
P ₄ = (3:2 Planting ratio)	3.06	3.56	3.31	1.64	1.87	1.76
P ₅ = (4:2 Planting ratio)	4.50	5.28	4.89	1.66	1.90	1.78
P ₆ = (5:2 Planting ratio)	5.80	6.74	6.27	1.66	1.92	1.79
Mean	4.30	4.97	4.63	1.21	1.39	1.30
For comparing means of	S.Em. ±		C.D. at 5 %	S.Em. ±		C.D. at 5 %
Insect attractant (T)	0.06		0.17	0.04		0.12
Planting ratio (P)	0.10		0.30	0.05		0.15
P × T	0.14		0.42	0.10		0.30

NS – Non-significant

* A-line (F₁ hybrid) = A x R line seed production

T₁ : Without spray

T₂ : Insect attractant spray

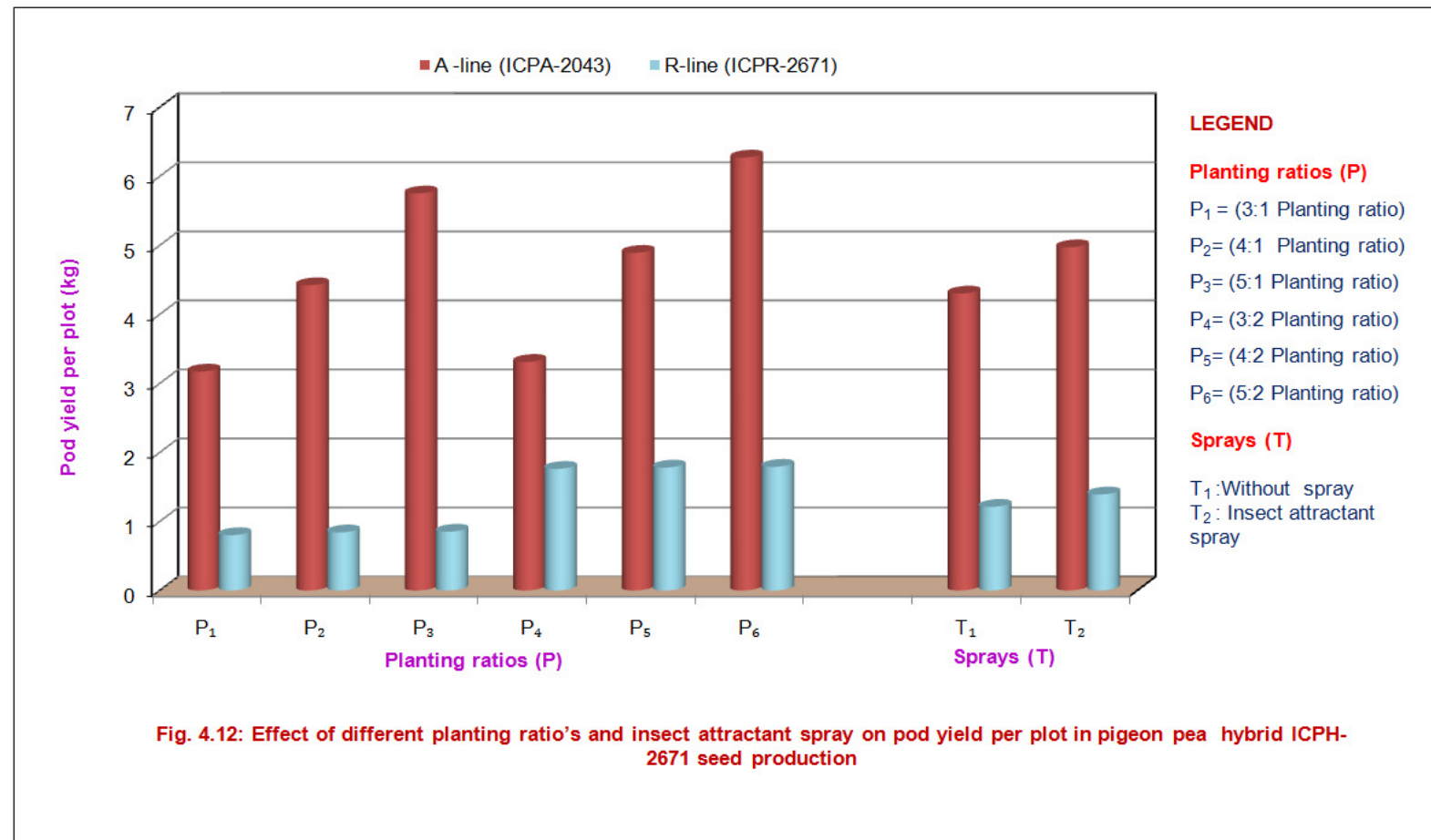


Fig. 4.12: Effect of different planting ratio's and insect attractant spray on pod yield per plot in pigeon pea hybrid ICPH-2671 seed production

The interaction effects between planting ratio's and insect attractant spray was significant. The treatment combination P_6T_2 :5:2 female to male planting ratio with insect attractant spray has recorded significantly the highest pod yield (1.92 kg) per plot as compared to all other treatment combinations, while, it was on par with P_5T_2 :4:2 female to male planting ratio with insect attractant spray (1.90 kg) and P_4T_2 :3:2 female to male planting ratio with insect attractant spray (1.87 kg).

4.2.7.6 Pod yield per ha

Female/seed parent (ICPA-2043 x ICPR-2671)

The results on pod yield per ha showed significant difference due to the effect of planting ratio's, insect attractant spray and their interaction effects in female parental line of hybrid pigeon pea ICPH-2671 (table -4.21& fig. 4.13).

The results revealed significant difference due to insect attractant spray. The treatment T_2 recorded significantly highest (2277 kg) pod yield per ha as compared to treatment without insect attractant spray (T_1 :1976 kg).

Among planting ratio's P_6 : 5:2 female to male planting ratio has recorded significantly the highest (2324 kg) pod yield per ha followed by P_5 :4:2 female lines to male planting ratio (2262 kg) and P_3 : 5:1 female to male planting ratio (2129 kg) and it was lowest (1956 kg) with P_1 :3:1 female to male planting ratio.

The interaction effects between planting ratio's and insect attractant spray was significant. The treatment combination P_6T_2 : 5:2 female to male planting ratio with insect attractant spray has recorded significantly the highest pod yield (2498 kg) per ha as compared to all other treatment combinations, while, it was lowest (1861 kg) with P_1T_1 :3:1 female to male planting ratio without insect attractant spray.

Male/pollen parent (ICPR -2671)

In male parental lines, the data on pod yield (kg) per ha significantly differed with insect attractant spray, planting ratio and their interaction effects (table -4.21 & fig.4.13). The treatment T_2 :with insect attractant spray has recorded the highest (1,696 kg/ha) pod yield per ha as compared to treatment T_1 : without insect attractant spray (T_1 : 1,481 kg/ha).

Among planting ratio's, pod yield (kg) per ha was significantly highest (1657 kg/ha) with treatment P_6 : 5:2 female to male planting ratio as compared to treatment P_1 (1,482 kg/ha), P_2 (1546 kg/ha) and P_3 (1,574 kg/ha).

Interaction effects due to the planting ratio's and insect attractant spray was found to be significant. The treatment combination P_6T_2 : 5:2 female to male planting ratio with insect attractant spray has recorded significantly the highest (1,778 kg) pod yield per ha as compared to all other treatment combinations. While, it was on par with P_5T_2 : 4:2 female to male planting ratio with insect attractant spray (1,759 kg/ha) and P_4T_2 :3:2 female to male planting ratio with insect attractant spray (1,731 kg/ha).

Table 4.21: Effect of different planting ratio's and insect attractant spray on pod yield (kg) per ha in pigeon pea hybrid ICPH-2671 seed production

Treatments	Pod yield (kg) per ha *A -line (ICPA-2043)			Pod yield (kg) per ha R-line (ICPR-2671)		
	T ₁	T ₂	Mean	T ₁	T ₂	Mean
P ₁ = (3:1 Planting ratio)	1861	2050	1956	1389	1574	1482
P ₂ = (4:1 Planting ratio)	1907	2182	2044	1444	1648	1546
P ₃ = (5:1 Planting ratio)	1969	2289	2129	1463	1685	1574
P ₄ = (3:2 Planting ratio)	1887	2200	2043	1519	1731	1625
P ₅ = (4:2 Planting ratio)	2082	2443	2262	1537	1759	1648
P ₆ = (5:2 Planting ratio)	2150	2498	2324	1537	1778	1657
Mean	1976	2277	2126	1481	1696	1589
For comparing means of	S.Em. ±		C.D. at 5 %	S.Em. ±		C.D. at 5 %
Insect attractant (T)	6.77		19.86	6.80		19.96
Planting ratio (P)	11.73		34.39	11.79		34.56
P × T	16.58		48.64	16.67		48.88

NS – Non-significant

* A-line (F₁ hybrid) = A x R line seed production

T₁ : Without spray

T₂ : Insect attractant spray

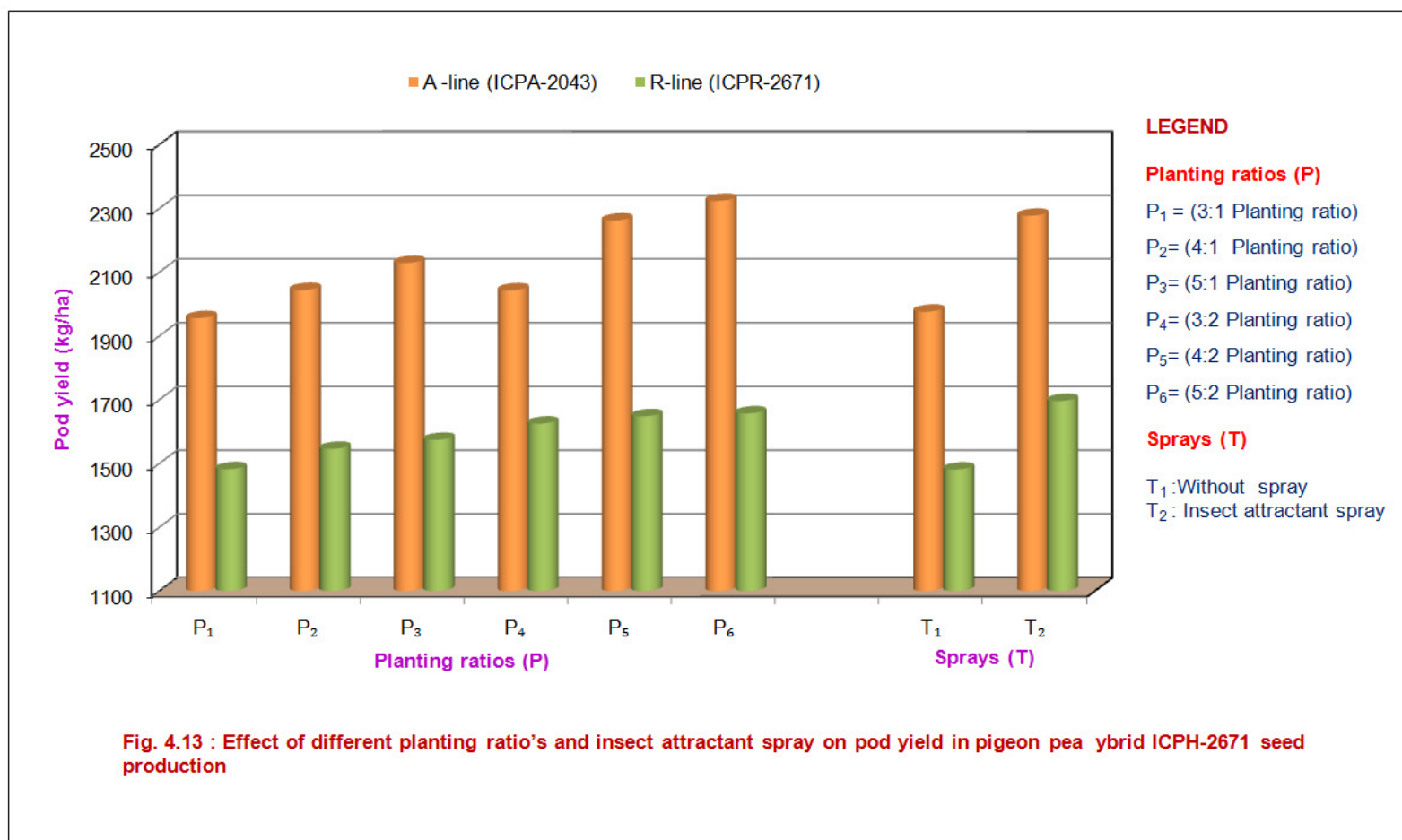


Fig. 4.13 : Effect of different planting ratio's and insect attractant spray on pod yield in pigeon pea ybrid ICPH-2671 seed production

4.2.7.7 Seed yield per plant

Female/seed parent (ICPA-2043 x ICPR-2671)

The data on seed yield (g) per plant revealed significant difference due to insect attractant spray, planting ratio's and their interaction effect in female parental lines of hybrid pigeon pea ICPH-2671 as presented in table -4.22 and depicted in fig.4.14

The statistically analyzed data revealed significant difference on seed yield (g) per plant due to insect attractant spray. The treatment insect attractant spray T_2 recorded significantly the highest (62.68 g/plant) seed yield per plant as compared to treatment without insect attractant spray (T_1 :54.81 g/plant) in female parental line of hybrid pigeon pea ICPH-2671.

There was a significant difference due to planting ratio's. Among planting ratio's P_6 :5:2 female to male planting ratio has recorded the highest (63.73 g) seed yield per plant followed by P_5 :4:2 female to male planting ratio (61.81 g/plant) and was lowest (54.57 g/plant) with P_1 : 3:1 female to male planting ratio.

Interaction effects due to the planting ratio's and insect attractant spray was found to be significant, The treatment combination P_6T_2 :5:2 female to male planting ratio + insect attractant spray has recorded significantly the highest (68.24 g/plant) seed yield per plant as compared to all other treatment combinations. While, it was on par with P_5T_2 : 4:2 female to male planting ratio + insect attractant spray (66.25 g/plant).

Male/pollen parent (ICPR -2671)

In male parental line, the data on seed yield (g) per plant showed significant difference with respect to insect attractant spray and non-significant difference was noticed with respect to planting ratio's and their interaction effect(table -4.22 & fig. 4.14). The insect attractant spray (T_2) recorded significantly highest (44.90 g/plant) seed yield per plant as compared to treatment without insect attractant spray (T_1 : 42.48 g/plant).

4.2.7.8 Seed yield (kg) per plot

Female/seed parent (ICPA-2043 x ICPR-2671)

The observations on seed yield (kg) per plot recorded significant difference due to the effect of insect attractant spray, planting ratios and their interaction effect in female parental line of hybrid pigeon pea ICPH-2671 as presented in table -4.23 and depicted in fig. 4.15.

The results on seed yield (kg) per plot in female parental line showed the significant difference due to insect attractant spray, the treatment T_2 recorded the highest (4.90 kg/plot) seed yield per plot as compared to T_1 (4.20 kg/plot).

Among planting ratio's P_6 : 5:2 female to male planting ratio's has recorded significantly highest seed yield (6.16 kg) per plot as compared to P_1 :3:1 planting ratio (3.07 kg/plot), P_2 : 4:1 planting ratio (4.34 kg/plot), P_3 :5:1 planting ratio (5.65 kg/plot) and P_4 :3:2 planting ratio (3.26 kg/plot) and P_5 :4:2 planting ratio (4.81 kg/plot).

Interaction effect due to the planting ratios (P) and insect attractant spray (T) were found to be significant, The treatment combination P_6T_2 :5:2 female to male planting ratio + insect attractant spray has recorded the significantly highest (6.62 kg/plot) seed yield per plot as compared to all other treatments. While, it was lowest with P_1T_1 :3:1 female to male planting ratio + without insect attractant spray (2.88 kg/plant).

Table 4.22: Effect of different planting ratio's and insect attractant spray on seed yield (g) per plant in pigeon pea hybrid ICPH-2671 seed production

Treatments	Seed yield (g) per plant *A -line (ICPA-2043)			Seed yield (g) per plant R-line (ICPR-2671)		
	T ₁	T ₂	Mean	T ₁	T ₂	Mean
P ₁ = (3:1Planting ratio)	52.04	57.10	54.57	38.72	44.16	41.44
P ₂ = (4:1Planting ratio)	52.96	59.20	56.08	42.32	44.32	43.32
P ₃ = (5:1Planting ratio)	54.16	63.86	59.01	42.36	44.39	43.38
P ₄ = (3:2Planting ratio)	53.11	61.45	57.28	43.26	45.21	44.24
P ₅ = (4:2Planting ratio)	57.36	66.25	61.81	44.01	45.56	44.79
P ₆ = (5:2Planting ratio)	59.21	68.24	63.73	44.21	45.76	44.99
Mean	54.81	62.68	58.74	42.48	44.90	43.69
For comparing means of	S.Em. ±		C.D. at 5 %	S.Em. ±		C.D. at 5 %
Insect attractant (T)	1.16		3.40	0.61		1.78
Planting ratio (P)	1.01		3.89	1.25		NS
P × T	1.84		3.33	1.49		NS

NS – Non-significant

* A-line (F₁ hybrid) = A x R line seed production

T₁ : Without spray

T₂ : Insect attractant spray

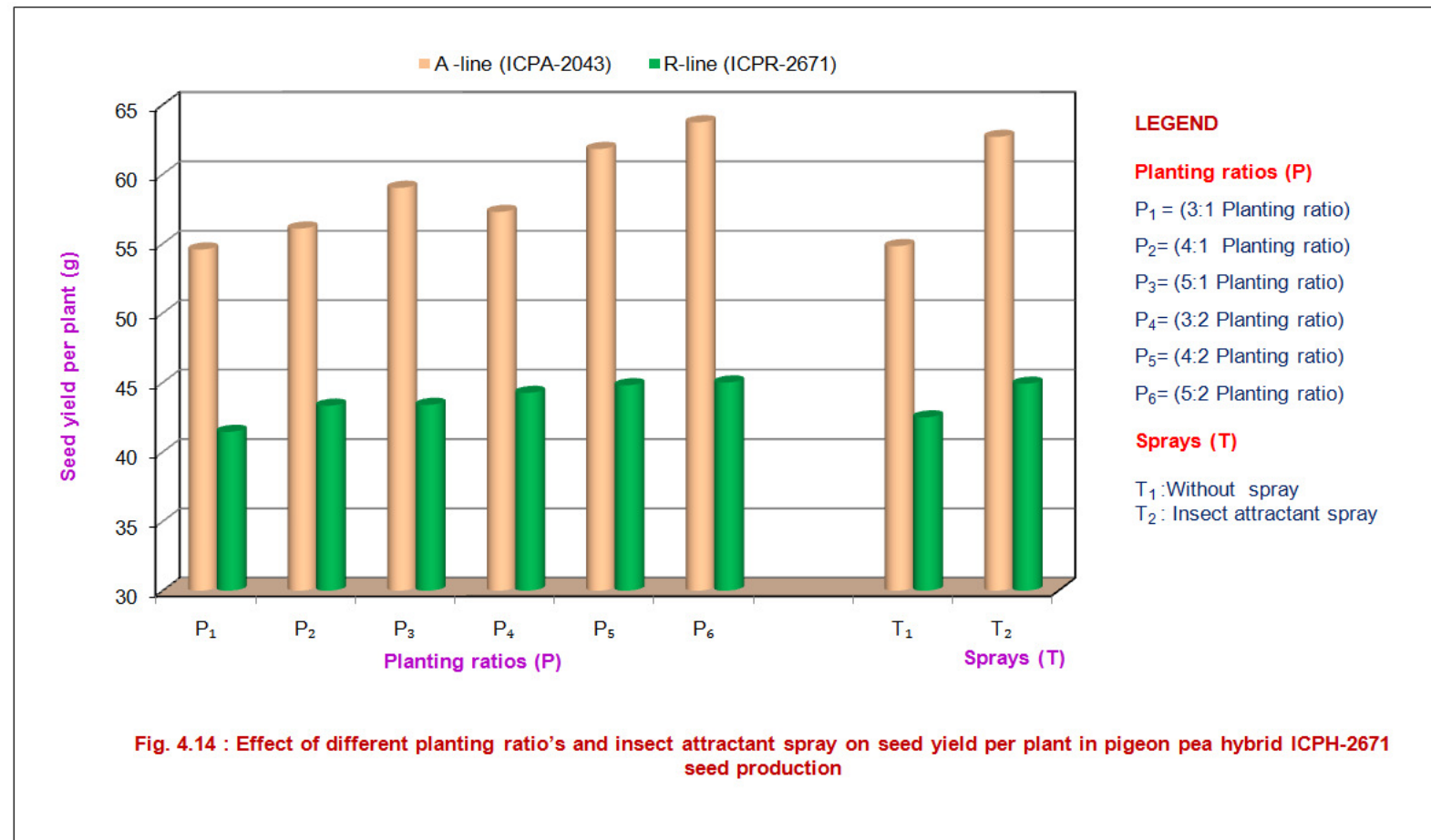


Fig. 4.14 : Effect of different planting ratio's and insect attractant spray on seed yield per plant in pigeon pea hybrid ICPH-2671 seed production

Male/pollen parent (ICPR -2671)

In male parental lines, the data on seed yield (kg/plot) per plot differed significantly due to insect attractant spray, planting ratio's and their interaction effects as presented in table -4.23 and depicted in fig.4.15. The T_2 : insect attractant spray has recorded significantly highest seed yield per plot (1.33 kg/plot) as compared to without insect attractant spray T_1 (1.18 kg/plot).

Among planting ratio's, P_6 :5:2 female to male planting ratios has recorded highest (1.74 kg/plot) seed yield per plot as compared to P_1 :3:1 (0.77 kg/plot), P_2 :4:1 (0.79 kg/plot) and P_3 :5:1 (0.83 kg/plot) planting ratio's.

The interaction effect due to planting ratio's and insect attractant spray differed significantly. The treatment combination P_6T_2 (5:2 female to male planting ratio + insect attractant spray) recorded the significantly highest (1.85 kg/plot) seed yield per plot as compared to all other treatment combinations. While, it was on par with P_5T_2 :4:2 female to male planting ratio + insect attractant spray (1.80 kg/plot) and P_4T_2 : 3:2 female to male planting ratio + insect attractant spray (1.75 kg/plot).

4.2.7.9 Seed yield per hectare

Female/seed parent (ICPA-2043 x ICPR-2671)

The results on seed yield (kg) per ha revealed significant difference due to insect attractant spray, planting ratio's and their interaction effects in female parental line of hybrid pigeon pea ICPH-2671 as presented in table -4.24 and depicted in fig.4.16

In female parental line, the treatment T_2 recorded the highest (2,243 kg/ha) seed yield per ha compared to T_1 (1,928 kg/ha). Among planting ratios P_6 : 5:2 and P_5 :4:2 female to male planting ratios has recorded significantly highest (2,282 kg/ha) and (2,228 kg/ha) seed yield per ha as compared to P_1 :3:1 planting ratio (1,892 kg/ha) P_2 : 4:1 planting ratio (2,009 kg/ha) P_3 :5:1 planting ratio (2,092 kg/ha) and P_4 :3:2 planting ratio (2,010 kg/ha).

The interaction effects due to planting ratios (P) and insect attractant spray (T) showed significant difference with respect to seed yield (kg) per ha. However, the treatment combination P_6T_2 :5:2 female to male planting ratio's with insect attractant spray has recorded significantly highest (2,452 kg/ha) seed yield per ha as compared to all other treatments and it was on par (2,402 kg/ha) with P_5T_2 :4:2 planting ratio with insect attractant spray.

Male/pollen parent (ICPR -2671)

In male parental line, the data on seed yield per ha differed significantly due to insect attractant spray, planting ratio's and their interaction effects (table -4.24 & fig. 4.16).

The treatment T_2 : insect attractant spray has recorded significantly highest (1,614 kg/ha) seed yield as compared to T_1 : without insect attractant spray (1,435 kg/ha). Among planting ratios the P_6 : 5:2 female to male planting ratio's has recorded significantly highest (1,609 kg/ha) seed yield (kg) per ha compared to P_1 : 3:1 female to male planting ratios (1,426 kg) and P_2 : 4:1 female to male planting ratios (1,454 kg) and remaining treatments were on par with each other.

The interaction effect due to planting ratio's and insect attractant spray differed significantly. The treatment combination P_6T_2 :5:2 female to male planting ratio + insect attractant spray has recorded the significantly highest (1,713 kg/ha) seed yield per ha as compared to all other treatment combinations. While, it was on par with P_5T_2 :4:2 female to male planting ratio + insect attractant spray (1,667 kg/ha).

Table 4.23: Effect of different planting ratio's and insect attractant spray on seed yield (kg) per plot in Pigeon pea hybrid ICPH-2671 seed production

Treatments	Seed yield (kg) per plot *A -line (ICPA-2043)			Seed yield (kg) per plot R-line (ICPR-2671)		
	T ₁	T ₂	Mean	T ₁	T ₂	Mean
P ₁ = (3:1 Planting ratio)	2.88	3.25	3.07	0.72	0.82	0.77
P ₂ = (4:1 Planting ratio)	4.04	4.64	4.34	0.74	0.84	0.79
P ₃ = (5:1 Planting ratio)	5.15	6.15	5.65	0.76	0.89	0.83
P ₄ = (3:2 Planting ratio)	2.99	3.52	3.26	1.61	1.75	1.68
P ₅ = (4:2 Planting ratio)	4.44	5.19	4.81	1.62	1.80	1.71
P ₆ = (5:2 Planting ratio)	5.70	6.62	6.16	1.63	1.85	1.74
Mean	4.20	4.90	4.58	1.18	1.33	1.25
For comparing means of	S.Em. ±		C.D. at 5 %	S.Em. ±		C.D. at 5 %
Insect attractant (T)	0.04		0.12	0.01		0.03
Planting ratio (P)	0.07		0.21	0.02		0.06
P × T	0.10		0.30	0.09		0.27

NS – Non-significant

* A-line (F₁ hybrid) = A x R line seed production

T₁ : Without spray

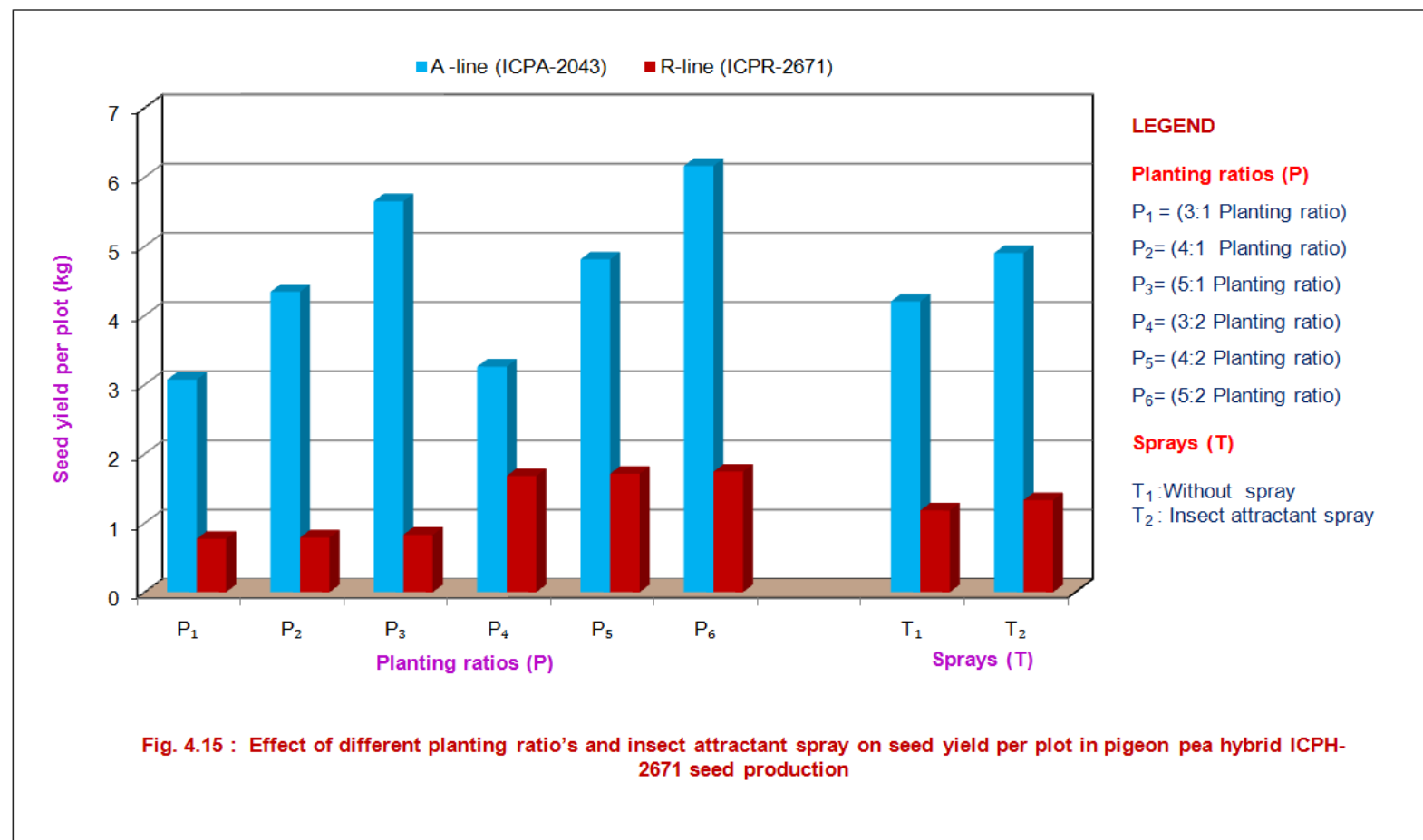


Fig. 4.15 : Effect of different planting ratio's and insect attractant spray on seed yield per plot in pigeon pea hybrid ICPH-2671 seed production

Table 4.25: Effect of different planting ratio's and insect attractant spray on number of seeds per pod in pigeon pea hybrid ICPH-2671 seed production

Treatments	Number of seeds per pod *A -line (ICPA-2043)			Number of seeds per pod R-line (ICPR-2671)		
	T ₁	T ₂	Mean	T ₁	T ₂	Mean
P ₁ = (3:1 Planting ratio)	1.67	2.67	2.17	2.33	2.67	2.50
P ₂ = (4:1 Planting ratio)	2.00	2.67	2.33	2.00	2.64	2.32
P ₃ = (5:1 Planting ratio)	2.33	3.00	2.67	2.00	3.46	2.73
P ₄ = (3:2 Planting ratio)	2.33	3.67	3.00	2.11	3.52	2.81
P ₅ = (4:2 Planting ratio)	2.67	4.33	3.50	2.67	3.77	3.22
P ₆ = (5:2 Planting ratio)	3.00	4.67	3.83	2.64	3.33	2.99
Mean	2.33	3.56	2.91	2.29	3.23	2.76
For comparing means of	S.Em. ±		C.D. at 5 %	S.Em. ±		C.D. at 5 %
Insect attractant (T)	0.17		0.49	0.23		0.67
Planting ratio (P)	0.29		0.86	0.40		NS
P × T	0.41		1.21	0.56		NS

NS – Non-significant

* A-line (F₁ hybrid) = A x R line seed production

T₁ : Without spray

T₂ : Insect attractant spray

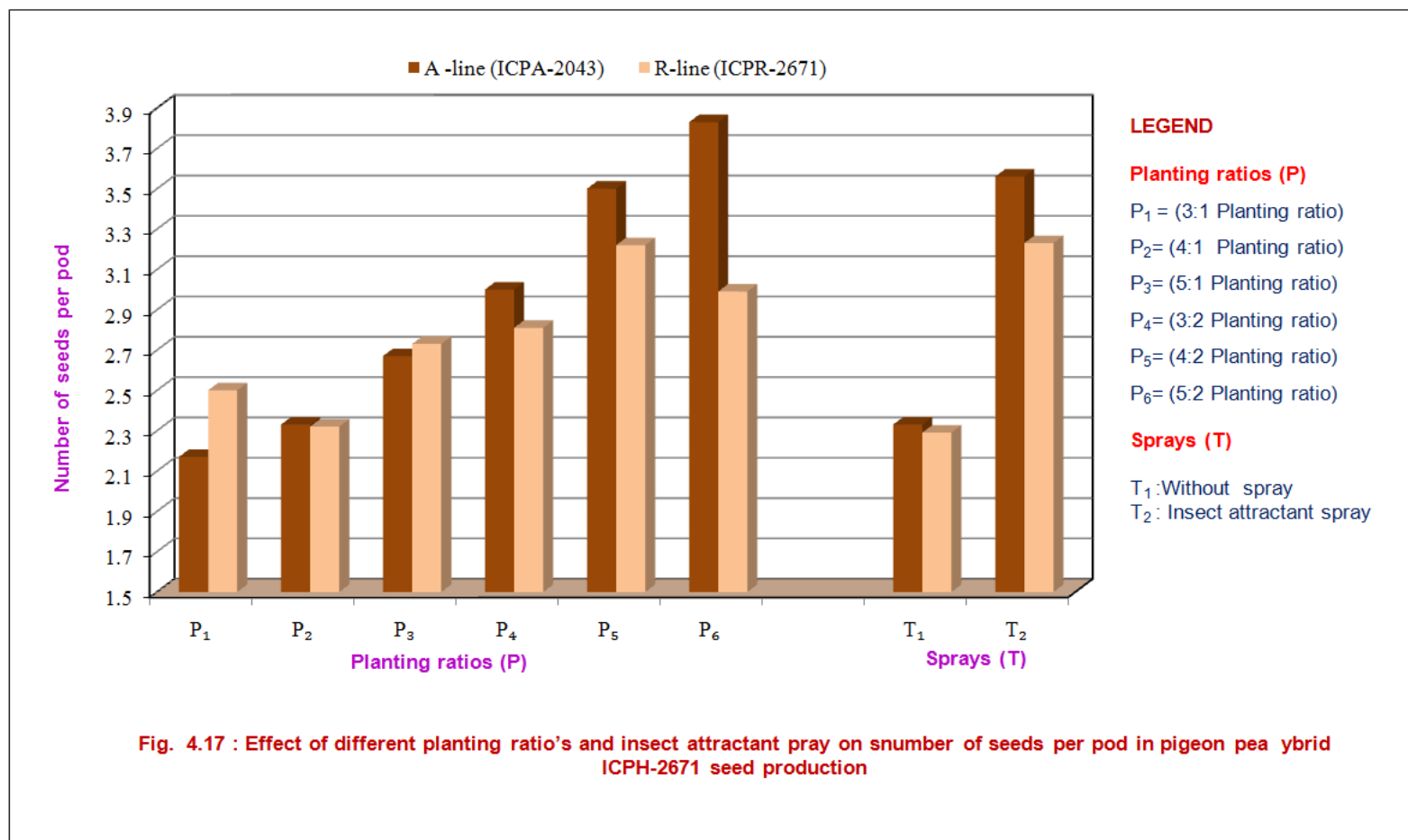


Fig. 4.17 : Effect of different planting ratio's and insect attractant pray on snumber of seeds per pod in pigeon pea ybrid ICPH-2671 seed production

4.2.7.10 Number of seeds per pod

Female/seed parent (ICPA-2043 x ICPR-2671)

The data on number of seeds per pod due to insect attractant spray, planting ratio's and their interactions effects in female parental line of hybrid pigeon pea ICPH-2671 as presented in table -4.25 and depicted in fig. 4.17

In female parental line, there was a significant difference on number of seeds per pod due to the insect attractant spray, planting ratio and their interaction effects.

The treatment T_2 : insect attractant spray has recorded significantly highest (3.56 seeds/pod) as compared to T_1 : without insect attractant spray (2.33 seeds/pod).

Among planting ratios the P_6 : 5:2 female to male planting ratio's has recorded significantly highest (3.83 seeds/pod) as compared to P_1 : 3:1 female to male planting ratios (2.17 seeds/pod) and P_2 : 4:1 female to male planting ratios (2.33 seeds/pod) and P_3 :5:1 female to male planting ratio (2.67 seeds/pod).

However, the interaction effects due to planting ratio's and insect attractant spray showed significant difference. The treatment combination P_6T_2 :5:2 female to male planting ratio + insect attractant spray has recorded significantly highest (4.67 seeds/pod) compared to rest of the treatments. While, it was on par with P_5T_2 : 4:2 female to male planting ratio + insect attractant spray (4.33 seeds/pod), and P_4T_2 : 3:2 female to male planting ratio + insect attractant spray (3.67 seeds/pod) P_3T_2 : 5:1 female to male planting ratio + insect attractant spray (3.00 seeds/pod).

Male/pollen parent (ICPR -2671)

Significant difference was noticed on number of seeds per pod due to the treatment effect of insect attractant spray and did not show any significant difference on number of seeds per pod due to planting ratio's and their interaction effect in male parental lines (table -4.25 & fig. 4.17). The treatment T_2 : with insect attractant spray has recorded the significantly highest (3.23 seeds /pod) as compared to without insect attractant spray (T_1 : 2.29 seeds/pod).

4.2.7.11 Pod length

Female/seed parent (ICPA-2043 x ICPR-2671)

The results on pod length (cm) showed significant difference due to insect attractant spray, planting ratio's and their interaction effects in female parental line of hybrid pigeon pea ICPH-2671 are presented in table -4.26.

In female parental line, there was a significant difference on pod length (cm) due to insect attractant spray, planting ratio's and their interaction effects.

The treatment T_2 with insect attractant spray has recorded significantly highest (5.33 cm) pod length as compared to without insect attractant spray (T_1 :4.89 cm). Among different planting ratio's P_6 : 5:2 female to male planting ratio has recorded significantly highest pod length (5.22 cm) as compared to P_1 :3:1 planting ratio (4.97cm) P_2 : 4:1 planting ratio (5.09 cm) P_3 :5:1 planting ratio (5.08 cm) and it was on par with P_4 :3:2 planting ratio (5.13 cm) P_5 :4:2 planting ratio (5.16 cm).

Table 4.26: Effect of different planting ratio's and insect attractant spray on pod length (cm) in pigeon pea hybrid ICPH-2671 seed production

Treatments	Pod length (cm) *A -line (ICPA-2043)			Pod length (cm) R-line (ICPR-2671)		
	T ₁	T ₂	Mean	T ₁	T ₂	Mean
P ₁ = (3:1 Planting ratio)	4.79	5.14	4.97	4.09	4.13	4.11
P ₂ = (4:1 Planting ratio)	4.87	5.31	5.09	4.09	4.17	4.13
P ₃ = (5:1 Planting ratio)	4.88	5.28	5.08	4.05	4.17	4.11
P ₄ = (3:2 Planting ratio)	4.86	5.40	5.13	4.11	4.18	4.15
P ₅ = (4:2 Planting ratio)	4.95	5.38	5.16	4.10	4.17	4.13
P ₆ = (5:2 Planting ratio)	4.96	5.49	5.22	4.07	4.19	4.13
Mean	4.89	5.33	5.11	4.09	4.17	4.12
For comparing means of	S.Em. ±		C.D. at 5 %	S.Em. ±		C.D. at 5 %
Insect attractant (T)	0.02		0.06	0.01		0.02
Planting ratio (P)	0.04		0.12	0.03		NS
P × T	0.05		0.15	0.03		NS

NS – Non-significant

* A-line (F₁ hybrid) = A x R line seed production

T₁ : Without spray

T₂ : Insect attractant spray

However, the interaction effects due to different planting ratio's and insect attractant spray showed significant difference. The treatment combination P_6T_2 :5:2 female to male planting ratio + insect attractant spray has recorded significantly highest (5.49 cm) pod length as compared to rest of the treatments. While, it was on par with P_5T_2 :4:2 female to male planting ratio + insect attractant spray (5.38 cm), and P_4T_2 :3:2 female to male planting ratio + insect attractant spray (5.40 cm).

Male/pollen parent (ICPR -2671)

In male parental line, there was a significant difference on pod length (cm) due to the effect of insect attractant spray and non-significant difference was noticed due to planting ratio's, and their interaction effects as presented in table -4.26. The treatment T_2 : insect attractant spray has recorded the significantly highest pod length (4.17 cm) as compared to without insect attractant spray T_1 (4.09 cm).

4.2.7.12 Pod breadth

Female/seed parent (ICPA-2043 x ICPR-2671)

The statistical data on pod breadth (cm) showed significant difference due to insect attractants spray, planting ratio's and their interaction effects in female parental line of hybrid pigeon pea ICPH-2671 are presented in table -4.27.

In female parental line, there was a significant difference on pod breadth (cm) due to insect attractant spray, planting ratio's, and their interaction effects. The treatment T_2 : insect attract spray has recorded significantly highest (0.95 cm) pod breadth as compared to without insect attract spray (T_1 :0.75 cm).

Among different planting ratio's P_6 :5:2 female to male planting ratio has recorded significantly highest pod breadth (0.93 cm) as compared to P_1 :3:1 planting ratio (0.79 cm), P_2 :4:1 planting ratio (0.81cm), P_3 :5:1 planting ratio (0.83 cm), P_4 :3:2 planting ratio (0.84 cm) and P_5 :4:2 planting ratio (0.90 cm).

The results of interaction effect due to different planting ratio's and insect attractant spray differed significantly on pod breadth. The treatment combination P_6T_2 : 5:2 female to male planting ratio + insect attractant spray has recorded significantly highest (1.06 cm) pod breadth as compared to all other treatments. While, it was on par with P_5T_2 :4:2 female to male planting ratio + insect attractant spray (1.04 cm).

Male/pollen parent (ICPR -2671)

In male parental lines, The significant difference was observed on pod breadth (cm) due to insect attractant spray and did not show any significant difference due to planting ratio's and their interaction effects (table -4.27). The treatment T_2 insect attractant spray has recorded significantly highest (0.55 cm) pod breadth as compared to T_1 (0.37 cm).

4.2.7.13 Pod size

Female/seed parent (ICPA-2043 x ICPR-2671)

The observations on pod size (cm) showed significant difference due to insect attractant spray, planting ratio's and their interaction effects in female parental lines of hybrid pigeon pea ICPH-2671 as presented in table -4.28.

Table 4.27: Effect of different planting ratio's and insect attractant spray on pod breadth (cm) in pigeon pea hybrid ICPH-2671 seed production

Treatments	Pod breadth (cm) *A -line (ICPA-2043)			Pod breadth (cm) R-line (ICPR-2671)		
	T ₁	T ₂	Mean	T ₁	T ₂	Mean
P ₁ = (3:1 Planting ratio)	0.73	0.86	0.79	0.31	0.55	0.34
P ₂ = (4:1 Planting ratio)	0.75	0.88	0.81	0.32	0.51	0.31
P ₃ = (5:1 Planting ratio)	0.76	0.90	0.83	0.35	0.55	0.37
P ₄ = (3:2 Planting ratio)	0.72	0.95	0.84	0.40	0.58	0.35
P ₅ = (4:2 Planting ratio)	0.76	1.04	0.90	0.42	0.56	0.35
P ₆ = (5:2 Planting ratio)	0.79	1.06	0.93	0.42	0.56	0.34
Mean	0.75	0.95	0.85	0.37	0.55	0.32
For comparing means of	S.Em. ±		C.D. at 5 %	S.Em. ±		C.D. at 5 %
Insect attractant (T)	0.03		0.06	0.01		0.03
Planting ratio (P)	0.01		0.03	0.03		NS
P × T	0.01		0.03	0.04		NS

NS – Non-significant

* A-line (F₁ hybrid) = A x R line seed production

T₁ : Without spray

T₂ : Insect attractant spray

In female parental line, there was a significant difference on pod size due to insect attractant spray, different planting ratio's, and their interaction effects. The treatment T_2 : insect attractant spray has recorded significantly highest (5.07 cm) pod size as compared to without insect attractant spray (T_1 :3.66 cm).

Among different planting ratio's P_6 :5:2 female to male planting ratio's has recorded significantly highest pod size (4.88 cm) as compared to P_1 :3:1 planting ratio (3.94 cm), P_2 : 4:1 planting ratio (4.15 cm), P_3 :5:1 planting ratio (4.23 cm), P_4 :3:2 planting ratio (4.32 cm) and P_5 :4:2 planting ratio (4.68 cm).

The data on interaction effect between different planting ratio's and insect attractant spray showed significant difference with respect to pod size. The treatment combination P_6T_2 : 5:2 female to male planting ratio + insect attractant spray has recorded significantly highest (5.83 cm) pod size as compared to all other treatment combinations.

Male/pollen parent (ICPR -2671)

In male parental line, the significant difference was observed on pod size (cm) due to insect attractant spray and non-significant difference was noticed due to different planting ratio's and their interaction effects (table -4.28). The treatment T_2 : insect attractant spray has recorded significantly highest (2.30 cm) pod size as compared to T_1 (1.51 cm).

4.2.8 Seed quality parameters

The observations on seed quality parameters viz., germination (%), root length (cm), shoot length (cm), seedling dry weight (mg), seedling vigour index and electrical conductivity (dS/m), hundred seed weight (g) and moisture content (%) as influenced by different planting ratio's , insect attractant spray and their interaction effects are presented in tables -4.29 to 4.36.

4.2.8.1 Germination percentage

Female/seed parent (ICPA-2043 x ICPR-2671)

The results on germination percentage as influenced by different planting ratios, insect attractant spray and their interaction effects showed significant difference in female parental lines of hybrid pigeon pea ICPH-2671 are presented in table -4.29 and depicted in fig.4.18.

In female parental lines, there was a significant difference on germination percentage due to the different planting ratio's, insect attractant spray and their interaction effects.

The treatment T_2 insect attractant spray has recorded significantly highest (93.39 %) germination as compared to treatment T_1 without insect attractant spray (91.06 %). Among different planting ratio's P_6 : 5:2 female to male planting ratio's has recorded significantly highest germination percentage (95.17 %) as compared to P_1 :3:1 planting ratio (89.17 %), P_2 : 4:1 planting ratio (90.83 %), P_3 :5:1 planting ratio (91.67 %), P_4 :3:2 planting ratio (93.00 %) and P_5 :4:2 planting ratio (93.50 %).

The results of interaction effect between different planting ratio's and insect attractant spray found to be significant. The treatment combination P_6T_2 :5:2 female to male planting ratio + insect attractant spray has recorded significantly highest (96.00 %) germination as compared to all other treatments. However, it was on par with P_5T_2 :5:1 female to male planting ratio + insect attractant spray (94.67 %).

Table 4.28: Effect of different planting ratio's and insect attractant spray on pod size (cm) in pigeon pea hybrid ICPH-2671 seed production

Treatments	Pod size (cm) *A -line (ICPA-2043)			Pod size (cm) R-line (ICPR-2671)		
	T ₁	T ₂	Mean	T ₁	T ₂	Mean
P ₁ = (3:1 Planting ratio)	3.48	4.41	3.94	1.27	2.27	2.27
P ₂ = (4:1 Planting ratio)	3.64	4.66	4.15	1.31	2.13	1.72
P ₃ = (5:1 Planting ratio)	3.71	4.75	4.23	1.42	2.29	1.86
P ₄ = (3:2 Planting ratio)	3.48	5.15	4.32	1.64	2.42	2.03
P ₅ = (4:2 Planting ratio)	3.76	5.60	4.68	1.72	2.34	2.03
P ₆ = (5:2 Planting ratio)	3.92	5.83	4.88	1.71	2.35	2.03
Mean	3.66	5.07	4.36	1.51	2.30	1.99
For comparing means of	S.Em. ±		C.D. at 5 %	S.Em. ±		C.D. at 5 %
Insect attractant (T)	0.03		0.09	0.06		0.20
Planting ratio (P)	0.05		0.15	0.11		NS
P × T	0.07		0.21	0.16		NS

NS – Non-significant

* A-line (F₁ hybrid) = A x R line seed production

T₁ : Without spray

T₂ : Insect attractant spray

Male/pollen parent (ICPR -2671)

The statistical data on germination percentage as influenced by insect attractant spray found to be significant. While, it was non-significant due to planting ratio's and their interaction effects in male parental lines (table -4.29 & fig.4.18). The treatment T_2 with insect attractant spray has recorded significantly highest germination (95.78 %) as compared to treatment without insect attractant spray (T_1 :94.44 %).

4.2.8.2 Shoot length

Female/seed parent (ICPA-2043 x ICPR-2671)

The statistically analyzed data on seedling shoot length (cm) revealed significant difference due to the effect of insect attractant spray, different planting ratio's and the interaction effects in female parental line of hybrid pigeon pea ICPH-2671 as presented in table -4.30.

The seedling shoot length differed significantly due to the effect of different planting ratios, insect attractant spray and their interaction effects. The treatment T_2 : insect attractant spray has recorded significantly highest (15.75 cm) seedling shoot length as compared to without insect attractant spray (T_1 :14.41 cm) in female parental lines.

Among the different planting ratios, P_6 5:2 female to male planting ratio has recorded significantly highest shoot length (17.02 cm) as compared to other different planting ratios. However, it was on par with P_5 :4:2 female to male planting ratio's (16.18 cm).

The interaction effects between planting ratio's and insect attractant spray showed significant difference on seedling shoot length. The treatment combinations P_6T_2 :5:2 female to male planting ratio + insect attractant spray has recorded the significantly highest (17.95 cm) shoot length as compared to all other treatments. However, it was on par with P_5T_2 (16.50 cm).

Male/pollen parent (ICPR -2671)

The data on shoot length found to be significant due to insect attractant spray and it did not show significant difference due to different planting ratio's and their interaction effect (table-4.30). The T_2 with insect attractant spray has recorded significantly highest (17.72 cm) seedling shoot length compared to treatment without insect attractant spray (T_1 :14.79 cm) in male parental lines.

4.2.8.3 Root length

Female/seed parent (ICPA-2043 x ICPR-2671)

The statistically analyzed data on root length (cm) revealed significant difference due to the influence of different planting ratio's, insect attractant spray and their interaction effects in female parental lines of hybrid pigeon pea ICPH-2671 as presented in table -4.31.

The data on seedling root length (cm) differed significantly due to the effect of different planting ratio's, insect attractant spray and their interaction effects. The treatment T_2 with insect attractant spray has recorded significantly highest (12.32 cm) root length as compared to treatment without insect attractant spray (T_1 :11.11 cm).

Among the planting ratio's, P_6 : 5:2 female to male planting ratio has recorded the significantly highest seedling root length (13.31 cm) as compared to other different planting ratio's. However, it was on par with P_5 :4:2 female to male planting ratio (12.62 cm).

Table 4.29: Effect of different planting ratio's and insect attractant spray on germination percentage in pigeon pea hybrid ICPH-2671 seed production

Treatments	Germination percentage * A -line (ICPA-2043)			Germination percentage R-line (ICPR-2671)		
	T ₁	T ₂	Mean	T ₁	T ₂	Mean
P ₁ = (3:1 Planting ratio)	88.33 (70.06)	90.00 (71.60)	89.17 (70.82)	93.33 (75.07)	94.67 (76.69)	94.00 (75.86)
P ₂ = (4:1 Planting ratio)	89.00 (70.67)	92.67 (74.33)	90.83 (72.41)	94.00 (75.86)	94.67 (76.69)	94.33 (76.26)
P ₃ = (5:1 Planting ratio)	90.00 (71.60)	93.33 (75.07)	91.67 (73.26)	94.33 (76.26)	95.67 (78.03)	95.00 (77.12)
P ₄ = (3:2 Planting ratio)	92.33 (73.96)	93.67 (75.47)	93.00 (74.70)	94.67 (76.69)	96.33 (79.00)	95.50 (77.79)
P ₅ = (4:2 Planting ratio)	92.33 (73.96)	94.67 (76.69)	93.50 (75.27)	95.00 (77.12)	96.67 (79.53)	95.83 (78.26)
P ₆ = (5:2 Planting ratio)	94.33 (76.26)	96.00 (78.50)	95.17 (77.34)	95.33 (77.56)	96.67 (79.53)	96.00 (78.50)
Mean	91.06 (72.64)	93.39 (75.14)	92.22 (73.89)	94.44 (76.40)	95.78 (78.19)	95.11 (77.29)
For comparing means of	S.Em. ±		C.D. at 5 %	S.Em. ±		C.D. at 5 %
Insect attractant (T)	0.18		0.53	0.31		0.91
Planting ratio (P)	0.31		0.92	0.53		NS
P × T	0.44		1.30	0.76		NS

NS – Non-significant

** Figures in the parentheses indicate arc sine transformed values.

* A-line (F₁ hybrid) = A x R line seed production

T₁ : Without spray

T₂ : Insect attractant spray

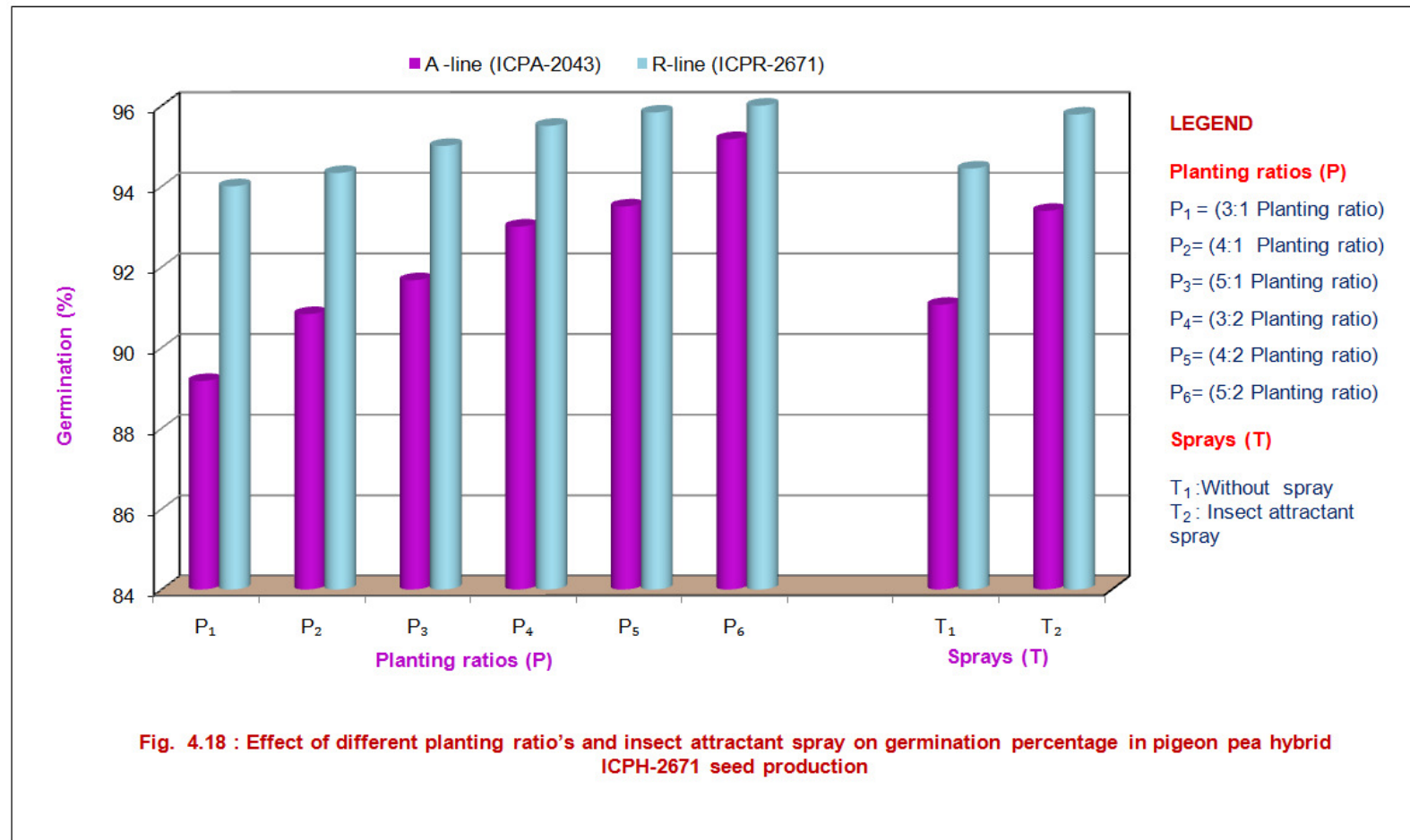


Fig. 4.18 : Effect of different planting ratio's and insect attractant spray on germination percentage in pigeon pea hybrid ICPH-2671 seed production

Table 4.30: Effect of different planting ratio's and insect attractant spray on shoot length (cm) in pigeon pea hybrid ICPH-2671 seed production

Treatments	Shoot length (cm) *A -line (ICPA-2043)			Shoot length (cm) R-line (ICPR-2671)		
	T ₁	T ₂	Mean	T ₁	T ₂	Mean
P ₁ = (3:1 Planting ratio)	12.17	13.46	12.81	14.25	16.94	15.59
P ₂ = (4:1 Planting ratio)	13.42	14.67	14.04	14.32	17.24	15.78
P ₃ = (5:1 Planting ratio)	14.41	15.50	14.96	14.24	17.59	15.91
P ₄ = (3:2 Planting ratio)	14.53	16.42	15.48	15.23	17.95	16.59
P ₅ = (4:2 Planting ratio)	15.87	16.50	16.18	15.34	18.27	16.80
P ₆ = (5:2 Planting ratio)	16.08	17.95	17.02	15.38	18.31	16.84
Mean	14.41	15.75	15.08	14.79	17.72	16.25
For comparing means of	S.Em. ±		C.D. at 5 %	S.Em. ±		C.D. at 5 %
Insect attractant (T)	0.22		0.64	0.18		0.52
Planting ratio (P)	0.38		1.10	0.40		NS
P × T	0.53		1.56	0.43		NS

NS – Non-significant

* A-line (F₁ hybrid) = A x R line seed production

T₁ : Without spray

T₂ : Insect attractant spray

Table 4.31: Effect of different planting ratio's and insect attractant spray on root length in pigeon pea hybrid ICPH-2671 seed production

Treatments	Root length (cm) *A -line (ICPA-2043)			Root length (cm) R-line (ICPR-2671)		
	T ₁	T ₂	Mean	T ₁	T ₂	Mean
P ₁ = (3:1 Planting ratio)	10.34	10.73	10.54	10.09	12.00	11.05
P ₂ = (4:1 Planting ratio)	10.39	10.83	10.61	10.26	12.33	11.30
P ₃ = (5:1 Planting ratio)	10.63	11.26	10.95	10.12	12.33	11.23
P ₄ = (3:2 Planting ratio)	11.47	13.13	12.30	10.76	12.33	11.55
P ₅ = (4:2 Planting ratio)	11.57	13.67	12.62	10.79	12.67	11.73
P ₆ = (5:2 Planting ratio)	12.28	14.33	13.31	11.30	12.67	11.99
Mean	11.11	12.32	11.72	10.55	12.38	11.47
For comparing means of	S.Em. ±		C.D. at 5 %	S.Em. ±		C.D. at 5 %
Insect attractant (T)	0.07		0.22	0.11		0.33
Planting ratio (P)	0.13		0.37	0.40		NS
P × T	0.18		0.53	0.58		NS

NS – Non-significant

* A-line (F₁ hybrid) = A x R line seed production

T₁ : Without spray

T₂ : Insect attractant spray

The interaction effects between planting ratio's and insect attractant spray found to be differed significantly. The treatment combination P_6T_2 : 5:2 female to male planting ratio + insect attractant spray has recorded significantly highest (14.33 cm) seedling root length as compared to all other treatments.

Male/pollen parent (ICPR -2671)

The results on seedling root length (cm) showed significant difference due to insect attractant spray and non-different difference was noticed due to planting ratio's and interaction effects (table - 4.31). The treatment T_2 with insect attractant spray has recorded significantly highest (12.38 cm) root length as compared to treatment without insect attractant spray (T_1 :10.55 cm).

4.2.8.4 Seedling vigour index

Female/seed parent (ICPA-2043 x ICPR-2671)

The results on seedling vigour index as influenced by insect attractant spray, different planting ratio's and their interaction effect showed significant difference in female parental lines of hybrid pigeon pea ICPH-2671 (table -4.32 & fig. 4.19).

In female parental lines, the treatment T_2 with insect attractant spray has recorded significantly highest (2627) seedling vigour index as compared to treatment T_1 without insect attractant spray (2328).

Among different planting ratio's P_6 :5:2 female to male planting ratio has recorded highest (2887) seedling vigour index followed by P_5 :5:2 female to male planting ratio (2695). While, it was lowest (2083) with P_1 : 3:1 female to male planting ratio.

The interaction between planting ratio's and insect attractant spray differed significantly on seedling vigour index. Among different treatments P_6T_2 :5:2 female to male planting ratio + insect attractant spray has recorded highest (3099) seedling vigour index. However, it was lowest (1988) and (2119) with P_1T_1 : 3:1 female to male planting ratios without insect attractant spray and P_2T_1 :4:1 female to male planting ratio's without insect attractant spray respectively.

Male/pollen parent (ICPR -2671)

The results on seedling vigour index revealed that significant difference due to insect attractant spray. Among different planting ratio's and their interaction effects non-significant difference was observed on seedling vigour index (table -4.32 & fig.4.19).

The treatment T_2 insect attractant spray has recorded significantly highest (2874) seedling vigour index as compared to T_1 without insect attractant spray (2394).

4.2.8.5 Seedling dry weight (g/10 seedlings)

Female/seed parent (ICPA-2043 x ICPR-2671)

The data on seedling dry weight (g) as influenced by different planting ratio's, insect attractant and their interaction effects showed significant difference in female parental lines of hybrid pigeon pea ICPH-2671 (table -4.33).

The insect attractant spray (T_2) recorded significantly the highest (0.91 g) seedling dry weight as compared to without insect attractant spray (T_1 :0.80 g).

Table 4.32: Effect of different planting ratio's and insect attractant spray on seedling vigour index in Pigeon pea hybrid ICPH-2671 seed production

Treatments	Seedling vigour index *A -line (ICPA-2043)			Seedling vigour index R-line (ICPR-2671)		
	T ₁	T ₂	Mean	T ₁	T ₂	Mean
P ₁ = (3:1 Planting ratio)	1988	2177	2083	2272	2740	2506
P ₂ = (4:1 Planting ratio)	2119	2363	2241	2311	2799	2555
P ₃ = (5:1 Planting ratio)	2254	2498	2376	2298	2862	2580
P ₄ = (3:2 Planting ratio)	2401	2768	2584	2460	2917	2689
P ₅ = (4:2 Planting ratio)	2534	2856	2695	2482	2991	2737
P ₆ = (5:2 Planting ratio)	2675	3099	2887	2543	2995	2769
Mean	2328	2627	2478	2394	2884	2639
For comparing means of	S.Em. ±		C.D. at 5 %	S.Em. ±		C.D. at 5 %
Insect attractant (T)	21.64		63.46	63.00		184.76
Planting ratio (P)	37.48		109.91	109.11		NS
P × T	53.00		155.44	154.31		NS

NS – Non-significant

* A-line (F₁ hybrid) = A x R line seed production

T₁ : Without spray

T₂ : Insect attractant spray

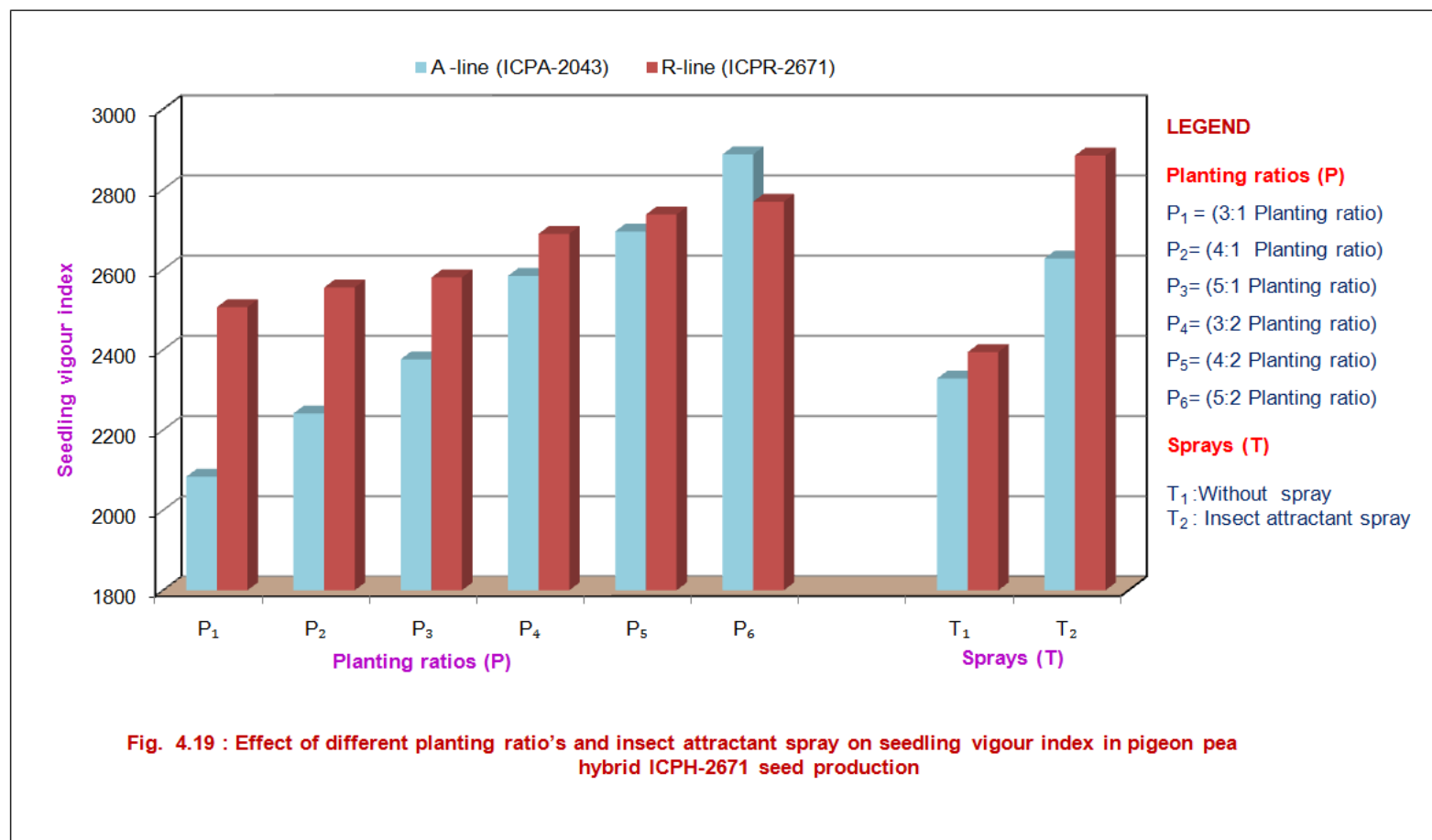


Fig. 4.19 : Effect of different planting ratio's and insect attractant spray on seedling vigour index in pigeon pea hybrid ICPH-2671 seed production

Among different planting ratios $P_6:5:2$ female to male planting ratio has recorded the highest (0.93 g) seedling dry weight, followed by $P_5: 5:2$ female to male planting ratio (0.91 g). While, it was lowest (0.80 g) with $P_1:3:1$ female to male planting ratio.

Among different treatment combinations $P_6T_2 :5:2$ female to male planting ratio + insect attractant spray has recorded the highest (0.97 g) seedling dry weight. While, it was lowest (0.73 g) and (0.73 g) with $P_1T_1:3:1$ female to male planting ratios without insect attractant spray and $P_2T_1 :4:1$ female to male planting ratio's without insect attractant spray respectively.

Male/pollen parent (ICPR -2671)

The results on seedling dry weight showed significant difference due to insect attractant spray and there was non- significant difference due to planting ratio's and their interaction effects in male parental line (table -4.33).

The T_2 insect attractant spray has recorded significantly the highest (0.97 g) seedling dry weight as compared to treatment T_1 : without insect attractant spray (0.89 g).

4.2.8.6 Electrical conductivity

Female/seed parent (ICPA-2043 x ICPR-2671)

The data on electrical conductivity revealed significant difference due to different planting ratio, insect attractant spray and their interaction effects in female parental lines of hybrid pigeon pea ICPH-2671 (table -4.34).

The treatment T_2 insect attractant spray has recorded lowest (0.39 dS/m) electrical conductivity as compared to without insect attractant spray (T_1 : 0.57 dS/m). Among different planting ratio's $P_6:5:2$ female to male planting ratio has recorded significantly lowest (0.40 dS/m) electrical conductivity over $P_1: 3:1$ female to male planting ratio (0.57 dS/m).

Interaction effects due to the different planting ratio and insect attractant spray were found to be significant, $P_6T_2 :5:2$ female to male planting ratio + insect attractant spray has recorded significantly lowest (0.31 dS/m) electrical conductivity as compared to all other treatment combinations. While, it was highest (0.68 dS/m) with $P_1T_1 :3:1$ female to male planting ratio + without insect attractant spray.

Male/pollen parent (ICPR -2671)

In male parental lines, the data on electrical conductivity differed significantly due to insect attractant spray and it was did not showed significantly difference with respect to different planting ratio's and their interaction effects.(table -4.34)

The treatment T_2 insect attractant spray has recorded the lowest (0.33 dS/m) electrical conductivity as compared to without insect attractant spray (T_1 :0.41 dS/m).

4.2.8.7 Hundred seed weight

Female/seed parent (ICPA-2043 x ICPR-2671)

The data recorded on hundred seed weight showed significant difference due to different planting ratio's, insect attractant spray and their interaction effects in female parental lines of hybrid pigeon pea ICPH-2671 (table -4.35 & fig. 4.20).

Table 4.33: Effect of different planting ratio's and insect attractant spray on seedling dry weight (in pigeon pea hybrid ICPH-2671 seed production

Treatments	Seedling dry weight (g) *A -line (ICPA-2043)			Seedling dry weight (g) R-line (ICPR-2671)		
	T ₁	T ₂	Mean	T ₁	T ₂	Mean
P ₁ = (3:1 Planting ratio)	0.73	0.86	0.80	0.86	0.92	0.89
P ₂ = (4:1 Planting ratio)	0.73	0.88	0.81	0.86	0.97	0.92
P ₃ = (5:1 Planting ratio)	0.75	0.89	0.82	0.90	0.97	0.94
P ₄ = (3:2 Planting ratio)	0.85	0.92	0.88	0.90	0.98	0.94
P ₅ = (4:2 Planting ratio)	0.87	0.95	0.91	0.91	0.98	0.95
P ₆ = (5:2 Planting ratio)	0.88	0.97	0.93	0.92	0.99	0.96
Mean	0.80	0.91	0.85	0.89	0.97	0.93
For comparing means of	S.Em. ±		C.D. at 5 %	S.Em. ±		C.D. at 5 %
Insect attractant (T)	0.03		0.09	0.01		0.03
Planting ratio (P)	0.02		0.06	0.04		NS
P × T	0.04		0.12	0.03		NS

NS – Non-significant

* A-line (F₁ hybrid) = A x R line seed production

T₁ : Without spray

T₂ : Insect attractant spray

Table 4.34: Effect of different planting ratio's and insect attractant spray on electrical conductivity (dS/m) in pigeon pea hybrid ICPH-2671 seed production

Treatments	Electrical conductivity (dS/m) *A -line (ICPA-2043)			Electrical conductivity (dS/m) R-line (ICPR-2671)		
	T ₁	T ₂	Mean	T ₁	T ₂	Mean
P ₁ = (3:1 Planting ratio)	0.68	0.45	0.57	0.47	0.35	0.41
P ₂ = (4:1 Planting ratio)	0.64	0.44	0.54	0.44	0.34	0.39
P ₃ = (5:1 Planting ratio)	0.62	0.42	0.52	0.40	0.33	0.37
P ₄ = (3:2 Planting ratio)	0.52	0.40	0.46	0.40	0.33	0.37
P ₅ = (4:2 Planting ratio)	0.50	0.35	0.43	0.39	0.32	0.36
P ₆ = (5:2 Planting ratio)	0.48	0.31	0.40	0.38	0.30	0.34
Mean	0.57	0.39	0.48	0.41	0.33	0.37
For comparing means of	S.Em. ±		C.D. at 5 %	S.Em. ±		C.D. at 5 %
Insect attractant (T)	0.01		0.03	0.01		0.03
Planting ratio (P)	0.02		0.06	0.04		NS
P × T	0.02		0.06	0.05		NS

NS – Non-significant

* A-line (F₁ hybrid) = A x R line seed production

T₁ : Without spray

T₂ : Insect attractant spray

Table 4.35: Effect of different planting ratio's and insect attractant spray on hundred seed weight in pigeon pea hybrid ICPH-2671 seed production

Treatments	Hundred Seed weight (g) *A -line (ICPA-2043)			Hundred Seed weight (g) R-line (ICPR-2671)		
	T ₁	T ₂	Mean	T ₁	T ₂	Mean
P ₁ = (3:1 Planting ratio)	10.67	11.78	11.23	10.20	11.24	10.72
P ₂ = (4:1 Planting ratio)	10.79	11.85	11.32	10.24	11.25	10.75
P ₃ = (5:1 Planting ratio)	10.91	11.96	11.44	10.26	11.27	10.77
P ₄ = (3:2 Planting ratio)	11.12	12.43	11.82	10.26	11.28	10.77
P ₅ = (4:2 Planting ratio)	11.26	12.53	11.85	10.27	11.31	10.79
P ₆ = (5:2 Planting ratio)	11.37	12.58	11.97	10.29	11.38	10.84
Mean	11.02	12.19	11.60	10.25	11.29	10.77
For comparing means of	S.Em. ±		C.D. at 5 %	S.Em. ±		C.D. at 5 %
Insect attractant (T)	0.02		0.07	0.01		0.04
Planting ratio (P)	0.04		0.12	0.02		NS
P × T	0.06		0.17	0.03		0.09

NS – Non-significant

* A-line (F₁ hybrid) = A x R line seed production

T₁ : Without spray

T₂ : Insect attractant spray

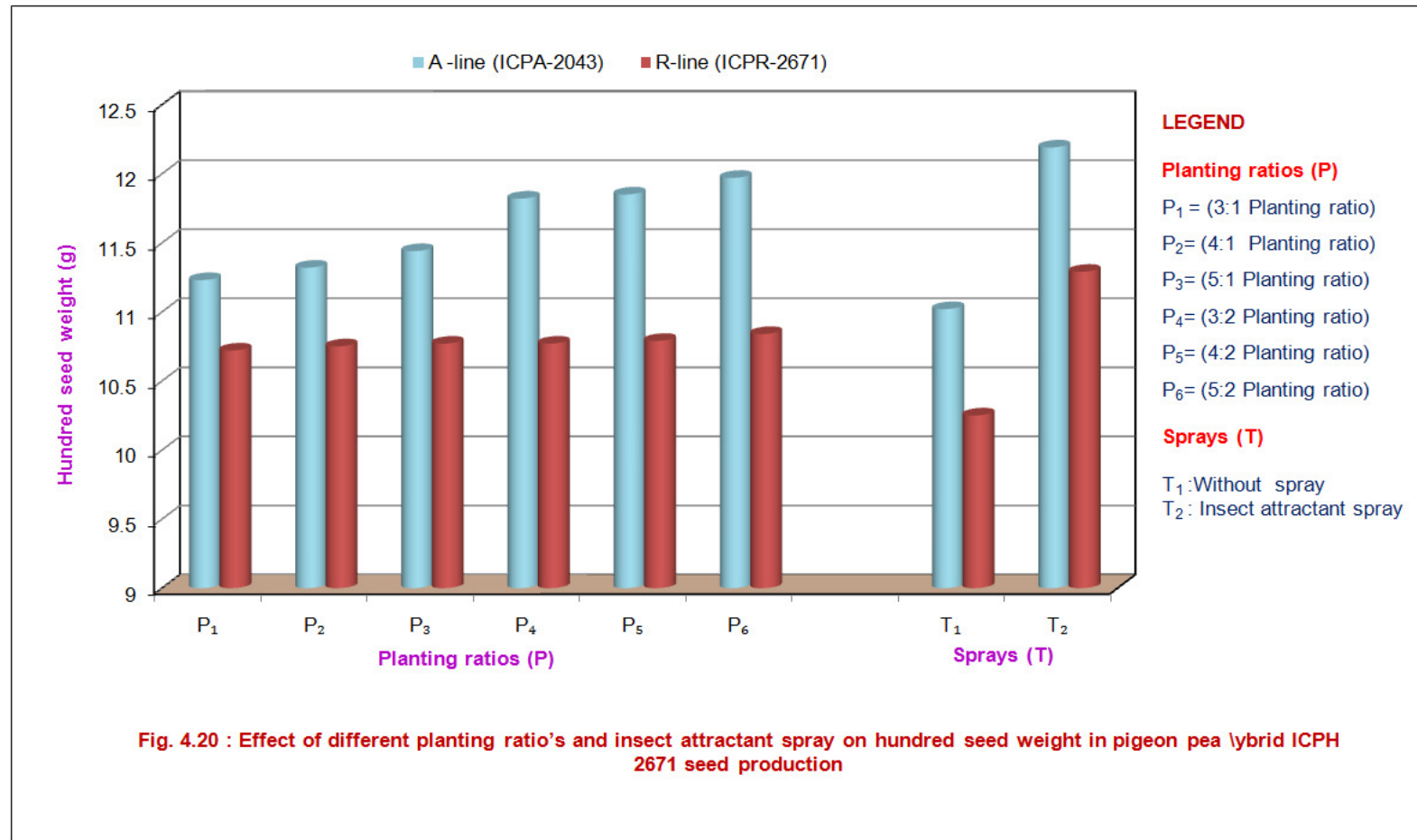


Fig. 4.20 : Effect of different planting ratio's and insect attractant spray on hundred seed weight in pigeon pea \ybrid ICPH 2671 seed production

Table 4.36: Effect of different planting ratio's and insect attractant spray on moisture content (%) in pigeon pea hybrid ICPH-2671 seed production

Treatments	Moisture content (%) *A -line (ICPA-2043)			Moisture content (%) R-line (ICPR-2671)		
	T ₁	T ₂	Mean	T ₁	T ₂	Mean
P ₁ = (3:1 Planting ratio)	7.60	7.63	7.61	7.65	7.95	7.80
P ₂ = (4:1 Planting ratio)	7.62	7.67	7.65	7.56	7.87	7.72
P ₃ = (5:1 Planting ratio)	7.63	7.64	7.63	7.95	7.62	7.79
P ₄ = (3:2 Planting ratio)	7.60	8.12	7.86	7.97	8.01	7.74
P ₅ = (4:2 Planting ratio)	7.65	8.65	8.15	7.91	8.15	7.83
P ₆ = (5:2 Planting ratio)	7.65	8.92	8.28	7.70	8.67	7.69
Mean	7.62	8.10	7.86	7.79	8.04	7.76
For comparing means of	S.Em. ±		C.D. at 5 %	S.Em. ±		C.D. at 5 %
Insect attractant (T)	0.24		NS	0.12		NS
Planting ratio (P)	0.75		NS	0.26		NS
P × T	0.86		NS	0.52		NS

NS – Non-significant

* A-line (F₁ hybrid) = A x R line seed production

T₁ : Without spray

T₂ : Insect attractant spray

The treatment T_2 : insect attractant spray recorded significantly highest hundred seed weight (12.19 g) as compared to without insect attractant spray (T_1 :11.02 g). Among different planting ratio's P_6 :5:2 female to male planting ratio has recorded significantly highest (11.97 g) hundred seed weight over P_1 :3:1 (11.23 g), P_2 :4:1 (11.32 g) and P_3 :5:1 (11.44 g) and P_4 : 3:2 (11.82 g) different planting ratio's. While, it was on par with P_5 :4:2 (11.85 g).

Interaction effects due to the different planting ratio's and insect attractant spray was found to be significant, P_6T_2 :5:2 female to male planting ratio + insect attractant spray has recorded significantly highest (12.58 g) hundred seed weight as compared to all other treatment combinations. While, it was on par with P_5T_2 : 4:2 female to male planting ratio + with insect attractant spray (12.53 g) and P_4T_2 :3:2 female to male planting ratio + with insect attractant spray (12.43 g).

Male/pollen parent (ICPR-2671)

The results on hundred seed weight showed significant difference due to insect attractant spray and their interaction effects and it was not showed any significant difference due to different planting ratio's in male parental line (table -4.35 & fig. 4.20).

The treatment T_2 insect attractant spray recorded the significantly highest hundred seed weight (11.29 g) as compared to without insect attractant spray (T_1 :10.25 g).

Interaction effects due to the different planting ratio's and insect attractant were found to be significant, P_6T_2 :5:2 female to male planting ratio + insect attractant spray has recorded significantly highest (11.38 g) hundred seed weight as compared to all other treatment combinations. While, it was on par with P_5T_2 :4:2 female to male planting ratio + with insect attractant spray (11.31 g) and P_4T_2 :3:1 female to male planting ratio + with insect attractant spray (11.28 g).

Moisture content (Female and Male)

The results on moisture content did not differ significantly due to different planting ratio's, insect attractant spray and their interaction effects in both female and male parental lines of hybrid pigeon pea ICPH-2671 as presented in table -4.36.

4.3 Experiment-III: Morphological characterization of parental lines of hybrid Pigeon pea ICPH-2671

4.3.1 Seedling morphological characters

Based on the seedling morphological characters viz., anthocyanin pigmentation on hypocotyl present or absent on A, B & R lines of hybrid Pigeon pea ICPH-2671 are grouped into different categories as presented in table -4.37.

4.3.1.1 Anthocyanin pigmentation on hypocotyls

The observations on Anthocyanin pigmentation on hypocotyl of parental lines of hybrid pigeon pea ICPH-2671 present or absent was studied by visual assessment by observation of individual plants or parts of plants at seedling stage.

The parental lines of hybrid pigeon pea ICPH-2671 had good variation for seedling hypocotyl colour. The female parental line ICPA-2043 and maintainer line ICPB-2043 having green colour hypocotyl while male parental line ICPR-2671 had purple colour pigmentation on hypocotyl of the seedling.

4.3.2 Plant morphological characters

The different plant morphological characters *viz.*, plant height (cm) at harvest, plant branching pattern, time of flowering, growth habit, stem colour, leaf shape, pubescence on lower surface of the leaf, colour of base of petal, pattern on streaks on petal, flower colour, pollen grain colour were considered to differentiate the chosen parental lines of hybrid pigeon pea ICPH-2671 and results of these characters are presented in table-4.38 and depicted in plates 4.3, 4. 4, 4.5 and 4.6

4.3.2.1 Plant height at harvest

Based on plant height parental lines of hybrid pigeon pea ICPH- 2671 are grouped into three categories *viz.*, Short (<100 cm), Medium (100-150 cm), Tall (>150cm). All parental lines of pigeon pea hybrid ICPH -2671 were categorized into tall plant height and hence no grouping was made for this character.

4.3.2.2 Plant Branching pattern

All parental lines of pigeon pea hybrid ICPH-2671 were grouped into three categories as erect (<30 °C), semi-spreading (30 °C- 60 °C), spreading (>60 °C) group. The genotype ICPA-2043 semi spreading type, ICPB -2043 and ICPR-2671 were considered as erect type of plant branching pattern.

4.3.2.3 Time of flowering (Number of days)

The data on time of flowering in terms of days to 50 per cent flowering in parental lines of pigeon pea hybrid ICPH 2671 were grouped into five groups for time of flowering as very early (<60 days), early (61-90 days), medium (91-130 days), late (131-160 days) and very late (>160 days).

All the parental lines of pigeon pea hybrid ICPH 2671 *viz.*, ICPA-2043, ICPB-2043 and ICPR-2671 were grouped under medium category for time of flowering.

4.3.2.4 Growth habit

The observations on plant growth habit of pigeon pea was categorized into Indeterminate and determinate. All the parental lines of pigeon pea hybrid ICPH 2671 *viz.*, ICPA-2043, ICPB-2043 and ICPR-2671 were grouped under indeterminate type of growth habit.

4.3.2.5 Stem colour

The observations on stem colour of pigeon pea was categorized into green and purple. All the parental lines of pigeon pea hybrid ICPH-2671 *viz.*, ICPA-2043, ICPB-2043 and ICPR-2671 were grouped under green stem colour. Hence, no group was made for this character.

4.3.2.6 Leaf shape

The parental lines of hybrid pigeon ICPH-2671 were grouped into two categorized as narrow oblong and lanceolate type of leaf shape.

The genotypes, ICPA-2043 and ICPB-2043 were categorized as narrow oblong leaf shape and ICPR-2671 was categorized under the lanceolate leaf shape.

4.3.2.7 Pubescence on lower surface of the leaf

The pigeon pea genotypes were grouped into groups for pubescence on lower surface of the leaf as present and absent. ICPA-2043, ICPB-2043 and ICPR-2671 were grouped under absent of pubescence on lower surface of the leaf. Hence, no group was made for this character.

Table 4.37: Morphological characterization of parental lines of hybrid pigeon pea-ICPH-2671

Characterization	ICPA -2043	ICPB-2043	ICPR-2671	Stage of observation	Type of assessment
Anthocyanin colouration of Hypocotyl	Green	Green	Purple	Seedling stage	VS
Plant height (cm)					
Range	180-190	185-195	195-215	At harvest	MS
Average	185	190	205		
Group	Tall	Tall	Tall		
Plant Branching pattern (°C)				First flowering	VS
Range	25-35 °C	23-30 °C	20-30 °C		
Average	30.15	25.25	23.25		
Group	Semi-spreading	Semi-spreading	Spreading		
Time of flowering (Number of days)				First flowering	VG
Range	95-110 days	110-115	110-125		
Average	100	105	115		
Group	Medium	Medium	Medium		
Growth habit	Indeterminate	Indeterminate	Indeterminate	50 % flowering	VG
Stem colour	Green	Green	Green	50 % flowering	VG
Leaf shape	Narrow oblong	Narrow oblong	Lanceolate	50 % flowering	VG
Pubescence on lower surface of the leaf	Absent	Absent	Absent	50 % flowering	VG
Colour of base of petal	Yellow	Yellow	Orange yellow	50 % flowering	VG
Pattern of streaks on petal	Absent	Absent	Sparse	50 % flowering	VG
Flower colour	Light yellow	Light yellow	Yellow with light streaks	50 % flowering	VG
Pollen grain colour	Light yellow	Light yellow	Dark yellow	50 % flowering	VG

MS : Measurement of a number of individual plants or parts of plants

VG : Visual assessment by a single observation of a group of plants or parts of plants

VS: Visual assessment by observation of individual plants or parts of plants

Plant height : Short (<100 cm), Medium (100-150 cm), Tall (>150 cm)

Branching pattern : Erect (<30 °C), Semi-spreading (30 °C-60 °C), Spreading(>60 °C)

Time of flowering: Very early (<60 days), Early (61-90 days), Medium (91-130 days), Late (131-160 days), Very late(>160 days)



Plate 4.3: View of maintainer line of pigeon pea hybrid ICPH-2671

Plate 4.3: View of maintainer line of pigeon pea hybrid ICPH-2671



Plate 4.4: View of male parental line of pigeon pea hybrid ICPH-2671

Plate 4.4: View of male parental line of pigeon pea hybrid ICPH-2671

4.3.2.8 Colour of base of petal

The colour of base of petal of ICPA-2043 and ICPB-2043 were yellow, where as genotype ICPR-2671 was orange yellow base of petal colour.

4.3.2.9 Pattern of streaks on petal

The pigeon pea genotypes were grouped into two groups for pattern of streaks on petal as absent and sparse. The genotype ICPA-2043 and ICPB-2043 were grouped under absent where as ICPR-2671 was categorized under sparse pattern of streaks on petal.

4.3.2.10 Flower colour

The genotype ICPA-2043 and ICPB-2043 were grouped under light yellow colour flower where as ICPR-2671 was categorized under yellow with light streaks on flower and depicted in plate - 3.

4.3.2.11 Pollen grain colour

The genotype ICPA-2043 and ICPB-2043 were grouped under light yellow colour pollen grains where as ICPR-2671 was categorized under dark yellow with light colour pollen grains.

4.3.2.12 Based on pod characteristics

The different pod characters viz., pod colour, pod pubescence, pod waxiness, pod surface stickiness, pod constriction, pod form, pod size (cm) and number of seeds per pod were considered to differentiate the chosen parental lines of hybrid pigeon pea ICPH-2671 and results of these characters are presented in table -38 and depicted in plates-4, 5 and 6.

4.3.2.13 Pod colour

The data on pod colour at premature stage of pigeon pea genotypes were classified as green and green with brown streaks. The genotype ICPA-2043 and ICPB-2043 were having green colour where as ICPR-2671 having green with brown streaks.

4.3.2.14 Pod pubescence

The data on pod pubescence of pigeon pea genotypes were classified as present and absent. The genotype ICPA-2043 and ICPB-2043 pod pubescence was present where as ICPR-2671 pod pubescence was absent.

4.3.2.15 Pod waxiness

All parental lines of hybrid pigeon pea ICPH-2671 has the waxiness on their pods hence no grouping was made for this character.

4.3.2.16 Pod surface stickiness

The genotypes ICPA-2043, ICPB-2043 and ICPR-2671 showed waxiness on their pods hence no grouping was made for this character.

4.3.2.17 Pod constriction

The genotypes were grouped into two groups for pod constriction as slight and prominent. The genotype ICPA-2043 and ICPB-2043 were prominent where as ICPR-2671 was slight pod constriction.

4.3.2.18 Pod form

All genotypes viz., ICPA-2043, ICPB-2043 and ICPR-2671 were cylindrical form at mature pod stage.



Plate 4.5: Flower colour of the female and male parental lines of hybrid pigeon pea (ICPH-2671)

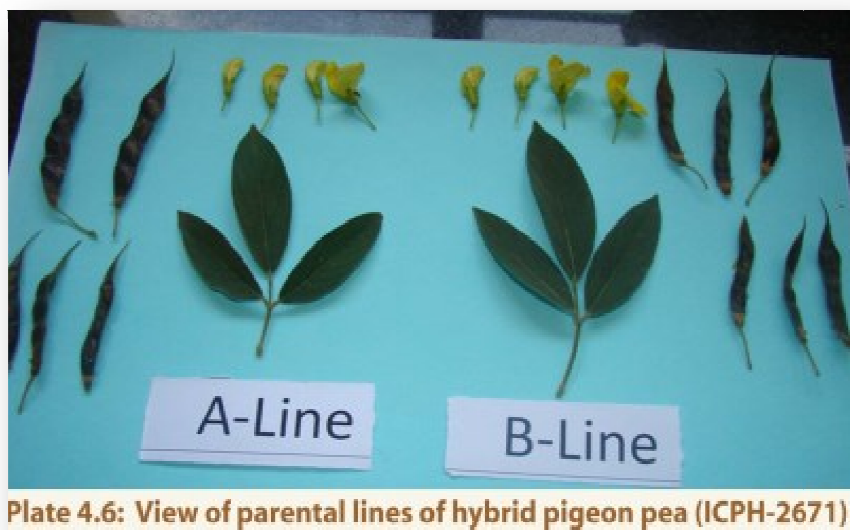


Plate 4.6: View of parental lines of hybrid pigeon pea (ICPH-2671)

Table 4.38: Morphological characterization of parental lines of hybrid pigeon pea ICPH-2671

Characterization	ICPA -2043	ICPB-2043	ICPR-2671	Stage of observation	Type of assessment
Pod colour	Green	Green	Green with brown streaks	Premature or pod dough stage	VG
Pod pubescence	Present	Present	Absent	Fully developed green pods	VG
Pod waxiness	Present	Present	Present	Premature or pod dough stage	VG
Pod surface stickiness	Present	Present	Present	Premature pod	VG
Pod constriction	Prominent	Prominent	Slight	Mature pods	VG
Pod form	Cylindrical	Cylindrical	Cylindrical	Mature pods	VG
Pod size (cm)				Mature pods	VG
Range	5.5-6.0	5.0-5.5	4.5-5.0		
Average	5.5	5.0	4.5		
Group	Large	Medium	Medium		
Number of seeds per pod				Mature pods	MS
Range	3.5-4.0	3.4-4.0	3.2-3.5		
Average	3.5	3.4	3.3		
Group	Medium	Medium	Medium		
Seed size (100 seed weight (g))				Dry seeds	MG
Range	11.2-11.8	10.5-11.5	10.7-12.3		
Average	11.5	11.05	10.08		
Group	Large	Large	Large		
Seed coat colour	Brown colour	Brown colour	Brown colour	Dry seeds	VG
Seed colour pattern	Uniform	Uniform	Uniform	Dry seeds	VG
Seed shape	Oval	Oval	Oval	Dry seeds	VG

MG : Measurement by a single observation of a group of plants or parts of plants

MS : Measurement of a number of individual plants or parts of plants

VG : Visual assessment by a single observation of a group of plants or parts of plants

Pod size) : < 4 cm (Small), 4-5 cm (Medium), > 5 (Large)

Number of seeds per pod: 2(Less), 3-4 (Medium), >4 (High)

Hundred Seed weight (g): Small : <7 , Medium : 7-9, Large: >9-11, Very large : >11

4.3.2.19 Pod size

The pigeon pea genotypes were categorized into three types of pod size viz., small (< 4 cm), medium (4-5 cm) and large (> 5 cm). The genotype ICPA-2043 was comes under large and ICPB-2043 and ICPR-2671 were comes under medium pod size.

4.3.2.20 Number of seeds per pod

The pigeon pea genotypes were classified as three categories less (2 seeds/pod), medium (3-4 seeds/pod) and high (> 4 seeds/pod). The pigeon pea genotypes ICPA-2043, ICPB-2043 and ICPR-2671 were grouped under medium number of seeds per pod.

4.3.3 Seed morphological characters

Based on seed morphological characters viz., seed coat colour, seed colour pattern, seed shape, and 100 seed weight (g) the parental lines of hybrid pigeon pea ICPH-2671 were grouped into different groups as below. The details of seed morphological characters of each genotype are presented in table -4.38.

4.3.3.1 Seed coat colour

The genotypes viz., ICPA-2043, ICPB-2043 and ICPR-2671 grouped under brown seed coat colour.

4.3.3.2 Seed colour pattern

The genotypes viz., ICPA-2043, ICPB-2043 and ICPR-2671 grouped under uniform seed colour pattern. The stage of observation was dry seeds.

4.3.2.3 Seed shape

The genotypes viz., ICPA-2043, ICPB-2043 and ICPR-2671 grouped under oval seed shape. The stage of observation was dry seeds.

4.3.2.4 Seed size

Hundred seed weight (g) of pigeon pea genotypes were grouped as small (>7 g), medium (7-9 g), large (>9-11 g) and very large (>11 g). The genotypes viz., ICPA-2043, ICPB-2043 and ICPR-2671 were grouped under very large seed weight (g). The stage of observation was dry seeds.

4.4 Experiment-IV: Molecular characterization of pigeon pea hybrid ICPH-2671 and its parental lines by using SSR markers

The results of the study pertaining to molecular characterization of hybrid pigeon pea ICPH-2671 and their parental lines are presented in this chapter and depicted in plate-4.7.

The present study utilized the hybrid pigeon pea ICPH-2671, female parent ICPA-2043, maintainer line ICPB-2043 and restorer male parent line ICPR-2671. For varietal characterization of these genotypes the SSR primers were used.

In the present study pigeon pea hybrid ICPH-2671 and its parental lines were characterized on the basis of SSR profile. Based on the DNA amplification banding pattern between the hybrid and their parents (plate- 4.7) The five SSR primers viz., CcM 0021, CcM 0724, CcM 1707, CcM 2626 and CcM 2300 were utilized for identification of pigeon pea hybrid (F_1) and its parental lines. Out of the

five SSR primers assessed only two primers i.e CcM 0021, CcM 1707 showed DNA amplification pattern between F₁ hybrid and its parental lines. The SSR primer CcM 0021 showed the DNA amplification of about 298 base pairs specific to ICPA-2043 and ICPB-2043 and 301 base pairs to specific to male parental line ICPR-2671 and 298 base pair and 301 base pairs are specific to the hybrid pigeon pea ICPH -2671.

The SSR primer CcM 1707 showed the DNA amplification of about 210 base pairs specific to ICPA-2043 and ICPB-2043 and 213 base pairs to specific to male parental line ICPR-2671 and 210 base pair and 213 base pairs are specific to the hybrid pigeon pea ICPH 2671. Therefore, the presence of DNA banding amplification banding pattern at 298 base pair and 301 base pair were noticed in both F₁ hybrid and its parental lines, thus confirm the hybridity of pigeon pea ICPH-2671.



Plate 4.7: SSR Marker CcM0021 and CcM1707 profile confirming hybridity of pigeon pea ICPH-2671 obtained on agarose gel

5. DISCUSSION

Among the pulse crops of the tropics and sub-tropics red gram or pigeon pea [*Cajanus cajan* (L.) Mill sp.] is recognized as an important crop for subsistence agriculture due to its drought tolerance, ability to recover from the losses caused by various stresses, high-protein (20–22 %) grains, quality fodder, and fuel wood. Its ability to enrich soils further adds to the value of the crop, and therefore, pigeon pea finds a valuable place among small holding farmers in a number of developing Asian and African countries. It is one of the major staple foods providing quality proteins to complement carbohydrate rich vegetarian diet in India.

The productivity of pigeon pea has remained low and stagnant over the last few decades thus this prompted scientists to search for novel ways of crop improvement. To tackle this challenge, ICRISAT and IIPR are working on number of innovative ideas like, development of CGMS hybrids with 30 to 40 % yield advantage over traditional varieties.

The level of realized heterosis for seed yield in pigeon pea is comparable to other crops where commercial hybrids have already made a mark in global agriculture. The hybrid breeding program at ICRISAT and ICAR in recent past focused towards developing a more efficient cytoplasmic-genetic male-sterility (CGMS) system.

The low productivity of pigeon pea may be related to the several constraints like absence of suitable hybrid seed production technology, improved agronomic practices, incidence of insect, pest, diseases and climatic conditions that prevail during crop season. One of the important aspect of low productivity is lack of quality seeds at economically feasible cost.

Since pigeon pea exhibits natural out-crossing, a safe isolation distance is always essential to produce quality seed of parental lines and hybrids, rouging and strict crop monitoring is a critical aspects of hybrid seed production and rouging should be done at seedling, flowering and before harvesting stages.

The research investigation is required to conduct on maintenance of parental lines to improve seed quality. The different pollination methods viz., selfing, isolated random mating (mass selection) and plant to progeny row selection methods were practiced for maintenance of parental lines of hybrid pigeon pea ICPH-2671 for assessment of seed quality parameters. Further, the morphological characterization of parental lines of hybrid pigeon pea ICPH-2671 is pivotal and essential as it is necessary to identify and distinguish the A, B & R lines of hybrid pigeon pea ICPH-2671 based on seed, seedling and plant morphological characters for carrying out the effective rouging operations during seed production and maintenance of parental lines to get quality seed with high productivity. In addition to these genetic parameters studies were carried out for parental lines of hybrid pigeon pea.

However, there is a need to understand the hybrid seed production constraints in a particular area. One of the major constraints for poor harvests could be low population of pollinating insects. This needs monitoring before investing large sums in seed production in a particular area. The commercial exploitation of hybrids is known to be directly linked to the ease with which their hybrid seeds could be produced and delivered economically to the farmers.

Pollination is an important and essential stage in the sexual reproduction of flowering plants. It involves the transfer of pollen from anther to the receptive stigma of the flower. As such pollination is an essential prerequisite to seed and fruit set.

The efficiency of mass pollen transfer from male to female parent through air or insects to affect cross-fertilization plays an important role in commercializing the hybrids in pigeon pea crop. In most of the pulses, the absence of natural cross-fertilization is the major bottleneck in exploiting hybrid vigour at commercial scale because their flower structure forces high level of self pollination (Saxena *et al.*, 1992).

One of the important consideration in pigeon pea hybrid seed production is to maximize the yield with high quality seeds and it is influenced by several factors such as different planting ratio's of female to male parental lines and application of insect attractants to the crop at flowering period to enhance the cross pollination through honey bees. The insect attractant spraying to the female and male parental lines at flowering stage assumes a greater significance as the seed set on female parents depend on the pollen supplied from the male parent during flowering period. Hence, insect attractant spray Bee-Q (12.5 g/l) enhances honeybee visitation rates on pigeon pea flowers thus improves pollination performance as compared to that in control plots. Enhanced bee visit in this crop is translated into marginal increase in yield parameters e.g. number of seeds/pod and hundred seed weight.

In pigeon pea, Grow Out test takes more time due to the long duration nature of the crop. Therefore a simple, rapid, and cost effective hybrid seed quality testing approach in pigeon pea based on molecular markers assay is needed. SSR base purity assessment kits are developed which can be used for assessing the purity of the hybrids. Hence, the molecular characterization of parental lines and hybrid through SSR markers are required for hybrid purity assessment.

By keeping these aspects, field and laboratory investigations were conducted at university of Agricultural Sciences, Dharwad during *kharif*- 2011 and results of these experiments are discussed as below.

5.1 Experiment I: Maintenance of parental lines (A, B & R line) of hybrid Pigeon Pea ICPH-2671 on seed quality

The parental lines A-line (Female parent ICPA-2043), B-line (Maintainer line-ICPB-2043), and R- line (Restorer/male parent ICPR-2671) were grown in an isolated block separately. The different pollination methods *viz.*, selfing, random mating and plant to progeny row selection were followed to maintain the parental lines for the assessment of seed quality parameters.

The maintenance of varieties in an organized and systemic way is a big task, as it has to retain the relationship of small amount of nucleus seed with that originally selected by plant breeders and the certified seed marketed to the farmers. Growing environment also has major effect on behaviour of pollination of varieties and well important consequences of varietal maintenance.

5.1.1 Maintenance of parental lines by different pollination methods and effect of selfing, random mating and environment on genotype

Selfing increases the homozygosity with corresponding decrease in heterozygosity. Inbreeding also increases homozygosity and reduces the heterozygosity. The effect of selfing on homozygosity and heterozygosity may be illustrated by an individual heterozygous for a single gene (Aa) and the successive generations derived from it are self-pollination. Every generation of self pollination will reduce the frequency of heterozygote Aa to 50 per cent of that in the previous generation. There is corresponding increase in the frequency of the two homozygotes AA and aa. As a result, after 10 generations of selfing, virtually all the plants in the population would be homozygous i.e. AA and aa. On the other hand, the frequency of heterozygote Aa would be only 0.097 per cent. It is assumed here that the three genotypes AA, Aa and aa have equal survival. When a number of genes are segregating together, each gene would become homozygous at the same rate as Aa. Thus the number of genes segregating does not affect the percentage of the homozygosity. The effect of selfing is so strong that even if 100 genes are segregating, more than 95 per cent of the population would be completely homozygous after only 12 generations. Linkage would increase the proportion of homozygous plants since, it would reduce the number of genes segregating independently. Unequal survival of different genotypes would have the same effect as in the case of homozygosity. Thus continued selfing has two main effects on the population: first, all the plants in the population become completely homozygous and second, the population of a self-pollinated species represents a mixture of several homozygous genotypes.

In maintenance breeding the following factors are very important and the process of maintenance varies with these factors i) genetic makeup of the genotype ii) mode of reproduction and mating system.

The breeder has two basic tools to change the genetic composition of a variety 1) selection and 2) mating system. There are five basic mating systems: random mating, genetic assortative mating, genetic disassortative mating, phenotypic assortative mating, phenotypic disassortative mating.

In random mating, each female gamete is equally likely to unite with any male gamete and the rate of reproduction of each genotype is equal, i.e. there is no selection. In such a situation gene frequencies remain constant, variances for the character remain constant and the correlation between relatives or prepotency does not change. However, in plant breeding some form of selection is practiced such a mating system is random mating with selection. Further, the mating is usually not completely random. Differences in flowering time, position of plants in the field, self-incompatibility and prevalent wind direction limit the randomness of mating.

Random mating with selection changes the mean of the character and increases the frequency of alleles for which selection is practiced. It would tend to increase the variance when selection is in favour of rare alleles, but would tend to decrease it when a predominant allele is selected for. These changes are more pronounced when the character is moderately heritable. Random mating is useful in plant breeding in several ways e.g. Progeny testing, production and maintenance of synthetic and composites.

The phenotype is the sum effect of genotype, environment and their interactions $P = G + E + GE$. The basic tool of plant breeders, the selection and rejections are dependent on phenotype. Whereas, the phenotype is the most unstable entity since, it is dependent on environment and its interactions. Hence, any decisions on selection and rejection, the two facets of the same coin will be considerably affected. In maintenance breeding too the rejections that are done mainly depend on phenotypes. There is nothing or no process like selection in maintenance. Hence, any procedure adopted should consider this point also. The gene expressions are affected due to environmental conditions such as air and soil temperature, humidity, rainfall, soil type fertility status etc. Therefore, any practical procedure of maintenance should be able to check these processes and retain the gene and genotypic frequencies at the original level (Patil, 2002). The research on this issue is very meager and there is urgent need for it. By keeping these views thus, the present investigation aimed to study the effect of pollination methods on seed yield component and quality parameters in parental lines of hybrid Pigeon pea ICPC-2671.

The results on pod length, pod breadth, pod size, germination (%), seedling dry weight (mg), electrical conductivity (dS/m) and field emergence (%) as influenced by pollination methods effects are did not show significant difference. Further, the seeds used were fresh, consequently germination was on par with each other pollination methods. These results are in conformity with the findings of (Gurumurthy, 2005) in maize open pollinated varieties.

Among pollination methods P_3 : plant to progeny row selection recorded the highest number of seeds per pod (4.23 seeds) and hundred seed weight (12.01g) as compared to compared to P_1 : selfing method (2.38 seeds, 10.94 g) and P_2 : random mating method (3.56 seeds, 11.85 g) respectively. The genotype G_1 : ICPC-2043 recorded the highest hundred seed weight (12.12 g) and number of seeds per pod (3.83) as compared to G_2 : ICPC-2043 (11.72 g and 3.41) and G_3 : ICPC-2671 (11.86 g and 2.93) respectively.

Among pollination methods showed significant difference was observed with respect to seedling root length, shoot length and seedling vigour index. The P_3 plant to progeny selection recorded the highest root length (14.59 cm), shoot length (16.89 cm) and vigour index (2963) as compared to P_1 selfing method (12.53 cm, 13.54 cm and 2428) and P_2 random mating method (13.46 cm, 16.62 cm and 2832) respectively.

Between genotypes, significant difference was observed with respect seed quality parameters viz., ICPC-2671 recorded significantly the highest (14.66 cm) root length and (16.57 cm) shoot length and seedling vigour index (2941). The genotypes ICPC-2043 recorded the lowest root length (12.52 cm), shoot length (15.23 cm) and seedling vigour index (2581). This may be attributed to genotypic difference or genetic constitution of a genotype. These results are in conformity with the findings of Gurumurthy (2005) in maize open pollinated varieties.

However, among pollination methods P_3 (plant to progeny row selection) recorded significantly the highest (4.23) number of seeds per pod, hundred seed weight (12.01g) and seedling vigour index (2937.83) as compared to P_1 and P_2 . This may be progenitor bulk of the seeds. These results are close to the findings of Gupta *et al.*, 1981b. He concluded that a comparison of the number of pod set per plant on the selfed plant with nonselfed plant (open pollinated) led to the conclusion

that selfing done by a large muslin bag gave 225 pods per plant against, 35 pods per plant with a small muslin bag, and 9 pods per plant with a glassine bag. The non selfed plants produced 240 pods per plant (Gupta *et al.*, 1981b). Therefore, depending on seed requirements, a large- or small-muslin bag is useful for selfing an individual plant. On an average the pod set inside the selfing bags is not very encouraging and it is determined by the extent of foliage growth and availability of sunlight inside the bags.

In plant to progeny row selection and random mating methods, each plant receives a blend of pollen from a large number of individuals each having different genotypes. Such populations are characterized by a high degree of heterozygosity with tremendous free and potential genetic variation, which is maintained in a steady state by free gene flow among individuals within the populations. In the development of hybrid varieties, the aim is to identify the most productive heterozygote from the population, which then is produced with the exclusion of other members of the population.

It is the simplest, easiest and oldest method of selection where individual plants are selected based on their phenotypic performance and bulk seed selection proved to be quite effective in improvement at the initial stages but its efficacy especially for improvement of yield, soon came under severe criticism that culminated in the refinement of the method of mass selection. The selection after pollination does not provide any control over the pollen parent as result of which effective selection is limited only to female parents. The heritability estimates are reduced by half, since only parents are used to harvest seed whereas, the pollen source is not known after the cross pollination has taken place. Therefore, the seeds obtained from plant to progeny row methods and random mating techniques have good seed quality parameters as compared to selfed seed.

5.1.2 Estimation of genetic parameters of parental lines of hybrid pigeon pea ICPH-2671

Pigeon pea though predominantly a self pollinated crop has cross pollination ranging from 5 % to 70 % (Saxena *et al.*, 1990). The choice of an appropriate selection/breeding method and its success for improvement of quantitative traits largely depends on the extent of genetic variability present in segregating material and gene action. Knowledge on genetic architecture of yield and related traits plays an important role in deciding breeding strategies and methodologies for crop improvement. In comparison to other economically important crops, relatively less effort has been made to understand the genetics of important quantitative traits in pigeon pea. Both additive and dominant/ non-additive effects have been reported to be important in determining yield, plant height, and protein content (Saxena and Sharma, 1990). Pleiotropic effects of gene, physiological changes, and highly sensitive nature of pigeon pea towards the environmental changes make it difficult to interpret the inheritance of yield and associated traits (Byth *et al.*, 1981). Information about nature and magnitude of gene action can be useful for breeding program (Satishkumar *et al.*, 2006). Yield and its component characters that are quantitative in nature exhibit all the three types of gene action (Saxena, 2008). Knowledge of gene action, interaction effect and heritability involved in several quantitatively inherited traits helps in deciding appropriate breeding program. Thus, the present investigation aimed to study genetic variability generated from the parental lines of hybrid pigeon pea ICPH-2671 to make effective selections for improving productivity.

The data on genetic variability and heritability parameters with respect to Plant height (cm), number of primary branches, number of secondary branches, days to 50 % flowering, days to flower initiation, days to pod initiation, days to pod maturity, number of pods per plant, Pod yield per plant and number of seeds per pod showed significant difference. The Phenotypic co-efficient of variation (PCV) was higher than genotypic co-efficient of variation (GCV) for all the characters mentioned above. Number of seeds per pod exhibited high PCV (12.04) and GCV (6.75). Whereas, days to pod maturity also exhibited high heritability (0.98) with genetic advance (14.94).

The results on genetic variability and heritability parameters pertaining to seed quality parameters viz., germination, shoot length, root length, seedling dry weight, seedling vigour index, and electrical conductivity showed significant difference. The Phenotypic co-efficient of variation (PCV) was higher than genotypic co-efficient of variation (GCV) for all the characters mentioned above. Number of seeds per pod exhibited high PCV (12.04) and GCV (6.75). Whereas, germination percentage also exhibited high heritability (0.90) with genetic advance (4.49). These results were also reported by Ali *et al.*, 2008; Adewale *et al.*, 2010; Rahim *et al.*, 2010; Riaz-Ud-Din *et al.*, 2010) which support the present findings. Heritability and genetic advance are important selection parameters. The estimate of genetic advance is more useful as a selection tool when considered jointly with heritability estimates (Johnson *et al.*, 1955). The estimates of genetic advance help in understanding the type of gene action involved in the expression of various polygenic characters. High values of genetic advance are indicative of additive gene action whereas low values are indicative of non-additive gene action (Singh and Narayanan, 1993). Thus, the heritability estimates will be reliable if accompanied by high genetic advance.

5.2 Experiment II: Effect of planting ratio's and insect attractant on seed yield and quality

5.2.1 Effect of insect attractant spray on seed yield and quality of hybrid pigeon pea ICPH-2671

The experimental material consisted of two parental lines that include female parent (ICPA-2043) and male parent (ICPR-2671) with insect attractant spray (T_2) and without insect attractant spray (T_1) sown in an spacing of 3 x 1 feet distance. The results of the study are discussed as below.

Pollination is an important and essential stage in the sexual reproduction of flowering plants. It involves the transfer of pollen from anther to the receptive stigma of the flower. As such pollination is an essential prerequisite to seed and fruit set.

The role of insects as pollinators was first reported by Kolreuter in 1763. It was estimated that in nature 5 % of flower self-pollinated and 95 % are cross-pollinated, out of which 10 % depends upon wind and 85 % upon the insects.

Cross-pollination needs a biological vector for the transfer of pollen from anthers of one flower to the stigma of another flower, to accomplish the process of pollination. Only the potential biological vector can fulfill this job (Free, 1993). Bees are considered to be the most important pollinators because they are the only insects whose immature stages are reared exclusively on pollen and nectar (Crane, 1990).

Honey bees are important for effecting qualitative and quantitative improvement in crop yields (Deodikar and Suryanarayana, 1977; Suryanarayana *et al.*, 1987; Rao and Suryanarayana, 1988 and Panda *et al.*, 1988).

Flower being a source of nectar and pollen attract the bee pollinators. Bees effectively and advertently or inadvertently carry the pollen from one flower to the other flower helping in an accomplishment of successful pollination. This transfer of pollen is found to be useful for genetic variability in plants. It also helps to increase viability and certain new characters appear in offspring. Bees are most plentiful pollinating insects available in agricultural areas. In case of highly self-sterile crops, presence of bees during their flowering periods can influence their yield significantly (Deodikar and Suryanarayana, 1977).

The timing of sexual reproduction is a critically important determinant of plant reproductive success. Flowering at the optimum time ensures fecundity and good development of seeds and fruits (Mazer, 1987). Flowering phenology is mediated by the interaction of internal factors (Murfet, 1977 and Putterill *et al.*, 2004) with external environmental signals such as temperature (Hollister *et al.*, 2005), day length (Imaizumi and Kay, 2006) or drought (Fox, 1990). In general, plant species in their native ranges have coupled the sensitive flowering period to the optimal climatic conditions through natural selection, thus maximizing their reproductive success.

In most pulses, the genetic contamination of varieties through natural out-crossing is not an issue as their flowers are cleistogamous with high levels of self-pollination, and the genetic drifts due to natural out-crossing are minimum. In contrast, pigeon pea is grossly different in its pollination behaviour and a considerable level (25–30 %) of natural out-crossing is observed. Since pigeon pea is a perennial plant, its flowering continues until an optimum pod load on an individual plant under specific environmental conditions is achieved. This characteristic behaviour of pigeon pea exposes its flowers for a longer period to insect visits. The large yellow flowers of pigeon pea attract various insect species particularly bees. The frequent visits of pollen-carrying insects across various genotypes lead to natural crosspollination, resulting in crop pollination is the most important economic outcome of honeybee activities. Planned honeybee pollination is practiced widely to ensure maximized high quality crop yield. Pollination is closely associated with fruit set, yield and seed quality. Yields of agricultural crops can be significantly increased through good management practices and by using effective pollinators (Mecnichenko 1977). By keeping these view the investigation on Effect of insect attractants on seed yield and quality of hybrid pigeon pea ICPH-2671 was conducted at Seed unit, University of Agricultural Sciences, Dharwad.

The results on growth parameters *viz.*, plant stand, plant height (cm), number of primary and secondary branches per plant at harvest, days to flower initiation, duration of flowering did not differed significantly in both female and male parental lines of hybrid pigeon pea ICPH-2671 with insect attractant spray.

The results of the present investigation revealed that, the treatment (T₂) Bee-Q sprayed to the female and male parental lines of hybrid pigeonpea ICPH-2671 significantly superior in seed yield parameters *viz.*, days to 50 percent flowering (109.89 days), days to pod initiation (129.11 days), days to pod maturity (163 days), number of pods per plant (350.83), pod yield per plant (63.61 g), pod yield

per plot (4.97 kg), pod yield (2277.00 kg/ha), seed yield per plant (62.68 g/plant), seed yield per plot (4.90 kg) and seed yield (2243.00 kg/ha), number of seeds per pod (3.56), pod length (5.33 cm), pod breadth (0.95 cm), pod size (5.07 cm) in female parental line and days to 50 percent flowering (122.61 days), days to pod initiation (134.22 days), days to pod maturity (180.94 days), number of pods per plant (300.11), pod yield per plant (44.26 g), pod yield per plot (1.39 kg/plot), pod yield per ha (1696.00 kg/ha), seed yield per plant (44.90 g/plant), seed yield per plot (1.33 kg/plot) and seed yield (1614.00 kg /ha), number of seeds per pod (3.23), pod length (4.17 cm), pod breadth (0.55cm), pod size (2.30 cm) in male parental line as compared to treatment T₁ control without insect attractant spray. This may be due to the effect of insect attractant spray to the crop. Saxena *et al.* (2006) indicated that the successful hybrid seed production in pigeon pea depends on the efficacy of mass pollen transfer from restorer line (R-line) to male-sterile line (A-line) by pollinators mainly bees. Increase of yield parameters was due to spraying of Bee-Q with its Pheromonal effect attracted more pollinators, i.e., honey bees transferring pollen grains from one flower to another and providing the most favourable conditions for better selectivity and higher stigmatic receptivity. Bee-Q is rich in carbohydrate content and has a phagostimulatory competing with pigeon pea for honey visits which attracted more pollinators to the flowers. Bee-Q increased bee-visits on both staminate and pistillate flowers, which resulted in adequate pollination and seed set. Research on pollination ecology of seed production suggests the possibility of increasing seed yield with quality improvement by using insect attractant for honeybee pollination. As a result of bee pollination, somatic, reproductive, adaptive behaviour or hybrid effects occur in the progeny either in single or in different combinations, which brings quantitative as well as qualitative changes in seed quality. Such changes include improved fertilization, increased viability of seed, formation of more nutritious seeds, early growth of embryo, increased seed set, increased seed yield and decreased flower drop and enhanced resistance to disease/pest or adverse environmental conditions. These results are in agreement with the findings of Jayaramappa and Bhargava (2015) in water melon; Anitha *et al.* (2012) in Guava and Sivaram and Jayaramappa (2013) in pigeon pea.

The seed quality parameters viz. germination (93.39 %), shoot length (15.75 cm), root length (12.32cm), seedling vigour index (2627), seedling dry weight (0.91 g), electrical conductivity (0.39 ds/m) and hundred seed weight (12.19 g) in hybrid seeds ICPH-2671 obtained from female parental line ICPA-2043 and germination (95.78 %), shoot length (17.72 cm), root length (12.38 cm), seedling vigour Index (2884), electrical conductivity (0.33 ds/m), seedling dry weight (0.97 g) and hundred seed weight (11.29) in male parental line recorded the significantly superior seed quality with respect to Bee-Q spraying to the crop as compared to without insect attractant spray. The seeds obtained from the plants sprayed with Bee-Q at 12.5 g/l recorded the highest germination percentage root length, shoot length, vigour index as compared to the without insect attractant spray. The increase in germination percentage was obviously attributed to the increased bee visitation on crop treated with insect attractant at 5 to 50 per cent flowering.

The average weight of 100 seeds was significantly greater from effective pollination. Higher seed quality may be because of bee pollination, flowers are pollinated in the phase of fully functional generative organs producing better quality seeds. Hence, the honey bee pollination not only increased

the yield but also improved the quality of the seed. These findings are in line with the results of Kalmath (2002) who observed highest germination per cent in onion seeds obtained from plot sprayed with cacambe (10 %), jaggery solution (10 %) and Guruprasad (2001) in nizer and Chandrashekar and sattagi (2005) in radish

5.2.2 Effect of different female to male planting ratio's on seed yield and quality of hybrid pigeon pea ICPH-2671

The experimental material consisted of two parental lines that include female parent (ICPA-2043) and male-fertile restorer line (ICPR-2671) with different female to male planting ratios viz., $P_1:3:1$, $P_2:4:1$, $P_3:5:1$ and $P_4:3:2$, $P_5:4:2$, $P_6:5:2$ sown in an spacing of 1 x 3 feet distance at seed unit, UAS, Dharwad.

The results on growth parameters viz., plant height (cm), number of primary and secondary branches per plant at harvest, days to flower initiation, days to 50 per cent flowering duration of flowering and days to pod initiation did not differed significantly in both female and male parental lines of hybrid pigeon pea with different female and male planting ratio's. The major effect of female to male planting ratio's (5:2 and 4:2) was found to be significant on seed yield and quality parameters in pigeon pea hybrid seed production of ICPH-2671.

In hybrid seed production of (A x R) line, the female to male planting ratio's of $P_6:5:2$, $P_5:4:2$ and $P_4:3:2$ recorded the significantly less number of days (162.67, 163.33 & 164.83) to pod maturity and highest number of pods per plant (336.00, 334.67 & 334.33 pods) respectively as compared to $P_1:3:1$ (166.17) $P_2:4:1$ (167.17) and $P_3:5:1$ (166.17) planting ratio's.

The planting ratio's $P_6:5:2$ and $P_5:4:2$ & $P_3:5:1$ (female to male planting ratio), the female parent ICPA-2043 recorded the significantly highest (64.90, and 62.76 and 60.06 g) pod yield per plant (6.27, 4.89 and 5.75 kg) pod yield per plot (2324, 2262 and 2129 kg/ha) pod yield per ha, (63.73, 61.81 and 59.01) seed yield per plant (6.16, 4.81 and 5.65 kg) seed yield per plot and (2,282, 2,228 and 2,092 kg/ha) seed yield per ha respectively as compared to $P_1:3:1$, $P_2:4:1$ and $P_4:3:2$ planting ratio's. In hybrid production of A x R line seed production, The female parent ICPA-2043, the planting ratio's of $P_6:5:2$ and $P_5:4:2$, $P_4:3:2$ recorded significantly highest number of seeds per pod (3.83, 3.50 & 3.00 seeds), pod length (5.22, 5.16 and 5.13 cm), pod breadth (0.93, 0.90 and 0.84 cm), pod size (4.88, 4.68 and 4.32 cm) respectively as compared to $P_1:3:1$, $P_2:4:1$ and $P_3:5:1$ planting ratio's. This may be due to the population density is more in case of 5:2, 4:2 and 5:1 (i.e. more number of rows of male sterile line) planting ratio's and availability of potential and fertile pollen grains were more in two rows of pollen parent resulting in effective pollination and fertilization leads to more number of pods and seed setting. Since, the hybrid seed set on the male-sterile plants is chiefly determined by the availability of bee population in the vicinity, pod set is generally poor in the locations with less number of pollinators. Therefore, to harvest good hybrid yields, it is imperative to select suitable seed production sites with good insect pollinator activity. These results are supported by the findings of Saxena (2006) and Mula *et al.*, (2010a) where 4:1 was acknowledged as the best row ratio of female to male parent lines for producing optimum seed yield of pigeon pea.

Mula *et al.*, (2011) reported that growth and yield of female parent ICPA-2043 were significantly affected by row ratio, plant spacing and soil moisture availability. Female to male row ratio of 4:1 produced the highest seed yield (1306.29 kg/ha) due to more number of rows of female lines than in 3:1. Moreover, spacing of 75 cm x 30 cm accorded the highest seed yield (3254.9 kg/ha) as compared to the other treatments. Because of wider spacing, individual plant attributes showed significant advantage on the growth and yield traits over closer spacing, although, this advantage has not influenced the increase in total seed yield of ICPA-2043 due to lesser plant population. Furthermore, the application of irrigation whether at 21 days interval (2 times) or at 14 days interval (3 times) during flower initiation till pod development is crucial for seed growth. In Alfisols, the spacing of 75 cm x 30 cm with 3 : 1 row ratio and irrigation at every 14 days produced A-line seed yield of 1872 kg/ha. In vertisols, the seed yield of 2357 kg/ha was recorded at Patancheru at the spacing of 75 cm x 30 cm with 3:1 row ratio and irrigation at every 21 days. To harvest good hybrid seed yield, it is imperative to select suitable seed production sites with good insect pollinator activity. To achieve this select the sites with abundance of bees were selected for hybrid seed production.

Large scale hybrid seed production in central India was successful with hybrid yields ranging between 1333 and 3040 kg/ha. Similarly, a few high-yielding seed production sites were also identified in the Indian states of Andhra Pradesh, Maharashtra and Gujarat (Saxena, 2007).

For effective hybrid seed production, it is important that flowering of the male and female parents synchronizes well to affect cross-pollination. The other issues related to productivity of seed parents have to be looked into by considering the interaction of genotype with biophysical factors such as spacing and irrigation. For hybrid seed production of A x R-lines are planted using a female: male row ratio of 4:1. In the production areas where greater bee activity is observed, a higher row ratio can be used for getting high yields.

In seed quality parameters, F₁ hybrid ICPH-2671 recorded the significantly highest germination (95.17, 93.50 and 93.00 %), seedling shoot length (17.02, 16.08 & 15.48 cm), seedling root length (13.31, 12.62 and 12.30 cm), seedling vigour index (2887, 2695 and 2584), seedling dry weight (0.93, 0.91 and 0.88 g) and electrical conductivity (0.40, 0.43 and 0.46 dS/m) in case of P₆: 5:2 and P₅: 4:2, P₄: 3:2 as compared to P₁: 3:1, P₂:4:1 and P₃: 5:1 planting ratio's. This may be due to the effect of pollination percentage is more because the availability of pollen grains and pollen potentiality is more in case of two rows of male parent as compared to one row of male parent. The effective pollination leads to a efficient seed set and increase the seed quality parameters.

In male parental line ICPR-2671, among different planting ratio's P₆: 5:2 and P₅: 4:2, P₄: 3:2 recorded the significantly highest (1.79, 1.78 and 1.76 kg) pod yield per plot, (1657, 1648 and 1625 kg) pod yield per ha, (1.74, 1.71 and 1.68 kg) seed yield per plot and (1609, 1586 and 1556 kg) seed yield per ha respectively as compared to P₁: 3:1, P₂:4:1 and P₃: 5:1 planting ratio's. This may be due the population density is more in case of 5:2, 4:2 and 3:2 row ratio compared to 5:1, 4:1 and 3:1 female to male planting ratio. Two rows of male parental lines are optimum for getting sufficient pollen grains to increase the pollination and seed set percentage as compared to one row of pollen parent. The location of the seed production area also influences the seed production. This is because of Bee-Q increased bee-visits on both staminate and pistillate flowers, which resulted in adequate pollination

and productivity. The bee visit on this plant translated into marginal increase of yield parameters viz. increase in seed weight, pod breadth, pod length and pod size and seed quality. These findings are in conformity with Bee-Q (based on carbohydrate rich) have the most promising effect (Currie *et al.* 1992, Naumann *et al.* 1994).

The seed yield components viz., number of seeds per pod, pod length, Pod breadth, pod size and seed quality parameters did not differed significantly among the different planting ratio's in male parental line ICPR-2671. However, the seed quality parameters viz., germination percentage and seedling vigour index was more in male parental line (ICPR-2671) as compared to female parent ICPA-2043. This may be due to the genetic factor of the male parent.

5.2.3 Interaction effect of insect attractant spray and different female to male planting ratio

The results on growth parameters viz., field emergence (%), plant height (cm) and number of primary and secondary branches per plant, days to flower initiation, days to 50 per cent flowering, duration of flowering and days to pod initiation did not differed significantly in both female and male parental lines of hybrid pigeon pea by interaction effects between planting ratio's and insect attractant spray to the crop.

Interaction effects between planting ratio's and insect attractant spray was found to be significant, P_6T_2 : 5:2 female to male planting ratio + insect attractant spray has recorded the significantly highest seed yield parameters viz., days to pod maturity (160.33), number of pods per plant (354), pod yield per plant (69.53 g), pod yield per plot (6.74 kg), pod yield (2,498.00 kg/ha), seed yield per plant (68.24 g), seed yield per plot (6.62 kg), seed yield (2,452.00 kg), number of seeds per pod (4.67 seeds), pod length (5.49 cm), pod breadth (1.06 cm) and pod size (5.83 cm) as compared to other treatment combinations in female parental line ICPA-2043. This is because of availability of the pollen grains and pollen potentiality was more in two rows of pollen parent and pollination percentage is more in case of insect attractant spray this increases the seed set. Many crops require an insect pollinator to help insure pollination. Having enough pollinators during flowering stage is essential to produce hybrid seeds. Proper pollination increases pod size at yield, hastens maturity. The transfer of compatible pollen results in fertilization and the formation of seeds. The seeds stimulate growth of the surrounding pod walls inside the locules. Flowers with higher levels of fertilization produce more seeds and thus bigger pods. Improperly pollinated seeds will often be smaller and misshapen because the ovarian tissue is not stimulated to grow around the areas without seeds.

These results supported the findings of Saxena (2006), Mula *et al.* (2010a) in planting ratio's and Sivaram and Jayaramappa (2013) in pigeon pea due to insect attractant.

Seed quality parameters viz., differed significantly with respect to treatment combinations of P_6T_2 (5:2 female to male planting ratio + insect attractant spray) recorded the significantly highest germination (96.00 %), shoot length (17.95 cm), root length (14.33 cm), seedling vigour index (3099), seedling dry weight (0.97 g), electrical conductivity (0.31 ds/m) and hundred seed weight (12.58 g) in ICPA-2043 female parental line.

In male parental line ICPR-2671, the treatment combination $P_6T_2:5:2$, $P_5T_2:4:2$ and $P_4T_2:3:2$ female to male planting ratio's with insect attractant spray has recorded the significantly highest seed yield parameters viz., pod yield per plot (1.92, 1.90 and 1.87 kg), pod yield per ha (1778, 1759 and 1731 kg/ha) seed yield per plot (1.85, 1.80 and 1.75 kg), seed yield per ha (1713, 1667 and 1620 kg/ha) as compared to other treatment combinations. This might be due to the plant stand/ population density is more in two rows of male parental line as compared to one row of male parental line. The seed quality parameters did not differ significantly due to the interaction effect of planting ratio and insect attractant spray in male parental line.

This might be due to the interaction effect between insect attractant spray and female to male planting rows. This investigation indicated that the full pod set is obtained if two pollinator rows are planted after every five rows of male sterile lines. The ratio of pollinator row and male sterile row may have to be changed. The recommended 6:1 female to male ratio is not optimal because there are insufficient pollinating insects vectors or plant growth is variable. For instance at ICRISAT six rows of female and one row of male at S.K. Nagar five female: one male rows at Ludhiana four female and one male row ratio found to be optimum (Srivastava and Jayaramappa, 1993). By this investigation at Dharwad condition 5:2 female to male rows along with insect attractant spray found to be optimum to get good seed yield and quality.

Bee-Q spray at the time of 5 to 50 % flowering stage increased bee-visits on both staminate and pistillate flowers which resulted in adequate pollination and productivity. The bee visit on this plant translated into marginal increase of yield parameters viz., increase in seed weight, pod breadth, pod length and pod size and seed quality. These findings are in conformity with Bee-Q (based on carbohydrate rich) have the most promising effect (Currie *et al.*, 1992a and Naumann *et al.*, 1994). Impact of bee attractants in enhancing pollination and yield has been reported in cucumber (Viraktamath and Anagoudar, 2002 and Pateel and Sattigi 2007); onion (Kalamath and Sattigi 2002) and radish (Chandrashekar and Sattigi 2009). Influence of Bee-attractants on yield parameters of Ridge Gourd (*Luffa acutangula* L.) (*Cucurbitaceae*) (Jayaramappa *et al.*, 2011), Jayaramappa and Bhargava (2015) in water melon; Anitha *et al.* (2012) in Guava and Sivaram and Jayaramappa (2013) in pigeon pea.

5.3 Experiment III: Studies on morphological characterization of parental lines of hybrid pigeon pea ICPH -2671

In recent years, several high yielding pigeon pea hybrids have been released by pigeon pea breeders and as such genetic identification has become more essential for maintenance of genotype, seed certification and seed quality. The present study, the A, B, & R line morphological characterization and identification of parental lines will help for rouging operation during hybrid seed production. The parental lines viz., ICPA-2043, ICPB-2043 and ICPR-2671 were characterized based on seed, seedling and plant morphological factors as well as molecular techniques by using SSR primers. The results of this study are discussed as detailed below.

5.3.1 Seedling morphological characters

The parental lines of hybrid pigeon pea ICPH-2671 were good variation for seedling hypocotyls colour. The female parental line ICPA-2043 and maintainer line ICPB-2043 having green colour hypocotyls while male parental line ICPR-2671 was purple colour pigmentation on hypocotyl of the seedling. The similar varietal characterization based on seedling morphology were made by Arun kumar *et al.*, (2004) in pearl millet; Karivartharaju (2005) in cotton and Chandrashekar (2005) in French bean.

5.3.2 Plant morphological characters

Based on plant height parental lines of hybrid pigeon pea ICPH- 2671 were grouped into three categories *viz.*, Short (<100 cm), Medium (100-150 cm), Tall (>150cm). All parental lines of pigeon pea hybrid ICPH-2671 were categorized into tall plant height and hence no grouping was made for this character. All parental lines of pigeon pea hybrid –ICPH 2671 were grouped into three categories as erect (<30 °C), Semi-spreading (30 °C – 60 °C), Spreading (>60 °C) group. The genotype ICPA-2043 semi spreading type, ICPB -2043 and ICPR-2671 were considered as erect type of plant branching pattern. These results are conformity with the findings of Sreelakshmi *et al.* (2010) and Naima *et al.* (2014).

The data on time of flowering in terms of days to 50 per cent flowering in parental lines of pigeon pea hybrid ICPH 2671 were grouped into five groups for time of flowering as very early (<60 days), early (61-90 days), medium (91-130 days), late (131-160 days) and very late (>160 days). All the parental lines of pigeon pea hybrid ICPH 2671 *viz.*, ICPA-2043, ICPB-2043 and ICPR-2671 were grouped under medium category for time of flowering.

The observations on plant growth habit of pigeon pea was categorized into indeterminate and determinate. All the parental lines of pigeon pea hybrid ICPH 2671 *viz.*, ICPA-2043, ICPB-2043 and ICPR-2671 were grouped under indeterminate type of growth habit. The observations on stem colour of pigeon pea was categorized into green and purple. All the parental lines of pigeon pea hybrid ICPH 2671 *viz.*, ICPA-2043, ICPB-2043 and ICPR-2671 were grouped under green stem colour. Hence, no group were made for this character.

The genotypes *viz.*, ICPA-2043 and ICPB-2043 were categorized as narrow oblong leaf shape and ICPR-2671 was categorized under the lanceolate leaf shape. The pigeon pea genotypes were grouped into groups for pubescence on lower surface of the leaf as present and absent. ICPA-2043, ICPB-2043 and ICPR-2671 were grouped under absent of pubescence on lower surface of the leaf. Hence, no group were made for this character.

The colour of base of petal of ICPA-2043 and ICPB-2043 were yellow where as genotype ICPR-2671 was orange yellow base of petal colour. The pigeon pea genotypes were grouped into two groups for pattern of streaks on petal as absent and sparse. The genotype ICPA-2043 and ICPB-2043 were grouped under absent where as ICPR-2671 was categorized under sparse pattern of streaks on petal. The genotype ICPA-2043 and ICPB-2043 were grouped under light yellow colour flower where as ICPR-2671 was categorized under yellow with light streaks on flower.

The genotype ICPA-2043 and ICPB-2043 were grouped under light yellow colour pollen grains where as ICPR-2671 was categorized under dark yellow colour pollen grains. The data on pod colour at premature stage of pigeon pea genotypes were classified as green and green with brown streaks. The genotype ICPA-2043 and ICPB-2043 were having green colour where as ICPR-2671 having green with brown streaks. The data on pod pubescence of pigeon pea genotypes were classified as present and absent. The genotype ICPA-2043 and ICPB-2043 pod pubescence was present where as ICPR-2671 pod pubescence was absent.

All parental lines of hybrid pigeon pea hybrid ICPH-2671 waxiness on their pods was present hence no grouping was made for this character. The genotypes ICPA-2043, ICPB-2043 and ICPR-2671 showed waxiness on their pods hence no grouping was made for this character.

The genotypes were grouped into two groups for pod constriction as slight and prominent. The genotype ICPA-2043 and ICPB-2043 were prominent where as ICPR-2671 was slight pod constriction.

All genotypes viz., ICPA-2043, ICPB-2043 and ICPR-2671 were cylindrical form at mature pod stage. The pigeon pea genotypes were categorized into three types of pod size viz., small (< 4 cm), medium (4-5 cm) and large (> 5 cm). The genotype ICPA-2043 was comes under large, ICPB-2043 and ICPR-2671 were comes under medium pod size.

The pigeon pea genotypes were classified as three categories less (2 seeds/pod), medium (3-4 seeds/pod) and high (> 4 seeds/pod). The pigeon pea genotypes ICPA-2043, ICPB-2043 and ICPR-2671 were grouped under medium number of seeds per pod. These results are in conformity with the findings of Khare *et al.* (2002) and Jain and Khare (2002a) in green gram and Ravi Kumar *et al* (1999) in soyabean.

5.3.3 Seed morphological characters

Based on seed morphological characters viz., seed coat colour, seed colour pattern, seed shape and 100 seed weight (g) the parental lines of hybrid pigeon pea ICPH-2671 were grouped into different groups as below. The genotypes viz., ICPA-2043, ICPB-2043 and ICPR-2671 were grouped under brown seed coat colour.

The genotypes viz., ICPA-2043, ICPB-2043 and ICPR-2671 were grouped under uniform seed colour pattern. The stage of observation was dry seeds. The genotypes viz., ICPA-2043, ICPB-2043 and ICPR-2671 were grouped under oval seed shape. These findings are in conformity with the findings of Arun kumar *et al.*, (2004) in pearl millet, Karivartharaju (2005) in cotton and Chandrashekar (2005) in French bean.

Hundred seed weight (g) of pigeon pea genotypes were grouped as small (>7 g), medium (7-9 g), large (>9-11 g) and very large (>11 g). The genotypes viz., ICPA-2043, ICPB-2043 and ICPR-2671 were grouped under very large seed weight (g). The stage of observation was dry seeds. These results were conformity with the findings of Arun kumar *et al.*(2004) in pearl millet, Karivartharaju (2005) in cotton and Chandrashekar (2005) in French bean.

5.4 Experiment-IV: Molecular characterization of pigeon pea hybrid ICPH-2671 and its parental lines by using SSR markers

For sustaining the productivity of hybrids, it is important to produce and supply genetically pure hybrid seed to farmers. In general, the purity of hybrid seed is assessed through standard 'grow-out test' using simply inherited morphological traits (Saxena 2006). In pigeon pea, such tests take more time due to the long duration of the crop and feasibility to raise a single crop in a year, particularly in medium and long duration of pigeon pea hybrids. The traditional grow out test (GOT) is done to determine the seed genetic purity test based on morphological markers are time consuming and are environmental dependence. To overcome this disadvantage, the biochemical markers are being used in many crops. However, repeatability and accuracy of these results on biochemical markers are subject to question. This made a way for the use of DNA molecular markers particularly the co-dominant markers. Therefore, a simple, rapid and cost-effective hybrid seed quality testing approach in pigeon pea based on molecular markers assay is very much needed. Saxena *et al.* (2010) and Bohra *et al.* (2011) identified a set of simple sequence repeat (SSR) markers for testing the hybridity of pigeon pea hybrid ICPH-2671.

However, the SSR markers require identification of heterozygosity of the pigeon pea hybrid and distinguishing the hybrid in question from its parental lines and other hybrids. Therefore, the present study was conducted to identify a specific SSR marker to discriminate the pigeon pea hybrid from its parental lines.

In this study, 5 primer pairs of SSR's primer associated with hybrid and parental lines were assessed on 4.0 percent agarose. Although the PCR products of the DNA samples on the agarose did appear DNA amplification but they failed to show any polymorphism among the hybrid and their parental lines. Probably the differences in the base pair sizes are too small to resolve and identify on the agarose. The primers which were not able to resolve and show polymorphism in agarose gel were found slight differentiation between DNA banding pattern among hybrid and parental lines of pigeon pea in agarose gel of six per cent.

Among the pigeon pea hybrid and parental line studied, hybrid ICPH-2671 could be distinguishable from their parental lines using a specific SSR marker. Based on the complementary banding patterns between the hybrid and their parents, the SSR marker CcM 1707 and CcM 0021 was identified as the two specific markers distinguish F₁ hybrid ICPH-2671 from their parental lines. These results are in conformity with the findings of Bohra *et al.*, (2011) hybrid purity assessment of hybrid ICPH-2671 with the CcM-0021 marker. The parental lines ICPA-2043 (A-line) and ICPR-2671 (R-line) show 298- and 301-bp alleles respectively on screening with a diagnostic simple sequence repeat (SSR) marker (CcM 0021), while ICPH-2671 seed showing the presence of both alleles (298 and 301 bp) represents true hybrid. As expected degree of marker polymorphism was lower in intra-specific populations than in inter-specific mapping population (ICP 28 × ICPW 94). The frequency of marker polymorphism increased with SSR locus longer than 200 bp. PIC values for SSR markers were also analyzed in relation to repeat length and unit type. This can be attributed to use of only one

wild species genotype in this studies. Occurrence of a very low level of DNA polymorphism among pigeon pea cultivars is not unexpected as several studies have documented such results (Saxena *et al.* 2010; Sivaramakrishnan *et al.* 1997 and Yang *et al.*, 2006). This assay can now be used by both the public and private seed companies for reliable detection of seed purity within the commercial seed lots of hybrids ICPH 2671 to ensure the supply of high-quality seeds. For assessing the genetic purity of a large number of farmers samples.

Practical utility of the findings

1. Among different pollination methods selfing, random mating and plant to progeny row selection can be conveniently adopted for maintenance of parental lines of hybrid pigeon pea ICPH-2671.
2. The insect attractant spray Bee-Q (12.5g/l) can be used in hybrid pigeon pea seed production to get higher seed yield and quality.
3. Among different planting ratio's 5:2 female to male planting ratio was found superior under Dharwad condition in order to get better seed yield and quality of the hybrid pigeon pea ICPH-2671.
4. The study of morphological characterization of parental lines of the hybrid pigeon pea ICPH-2671 can be utilized for identification of off types in hybrid pigeon pea seed production seed plot to carry out the rouging operation to get genetically pure F1 hybrid seed as per the requirements of seed norm.
5. The molecular characterization of hybrid and its parental lines by using SSR markers can be utilized for identification of pigeon pea hybrid and its parental lines and assessment of hybrid purity.
6. The SSR molecular markers CcM 0021 and CcM 1707 showed the DNA amplification banding pattern between hybrid and its parental lines of pigeon pea.

Future line of work

1. The progenitor obtained through different pollination methods *viz.*, selfing, random mating and plant progeny row selection can be further utilized for conduct of grow out test (GOT) to know the genetic purity of the parental lines of hybrid pigeon pea ICPH-2671.
2. The study may be conducted over generations to assess the truly superior method of pollination for the maintenance of parental lines.
3. The insect attractants other than Bee-Q *viz.*, fruit boost, cinnamon leaf extract, tuberose floral scented water, bee scent can also be utilized for enhancing the bee visits to increase the seed yield and quality of hybrid pigeon pea.
4. The planting ratio's 5:2 :female to male was found superior in order to get better seed yield and quality of the hybrid pigeon pea ICPH-2671 yet to be confirmed by other locations of the Karnataka and there is a scope for increasing the planting ratio's depending upon the location of the hybrid seed production.
5. Optimization of female to male planting ratio to increase the seed yield and quality of hybrid pigeon pea ICPH-2671.

6. SUMMARY AND CONCLUSION

The investigations were carried out with the objective to study the effect of pollination methods on the maintenance of parental lines, influence of planting ratio's, insect attractant on growth, seed yield and seed quality of pigeon pea hybrid ICPH-2671 and morphological characterization of parental lines of hybrid pigeon pea ICPH-2671 was carried out at Seed unit farm, University of Agricultural Sciences, Dharwad. Further, laboratory studies on seed quality parameters in pigeon pea hybrid ICPH-2671 and its parental lines were conducted at department of Seed Science and Technology, College of Agriculture, Dharwad. Molecular characterization of A, B, R & F₁ of Pigeon pea hybrid ICPH-2671 by using SSR markers was carried out at the laboratory of Bio-technology, UAS, Dharwad. The results obtained from these studies are summarized in this chapter under following different headings.

Experiment I: Maintenance of parental lines (A, B & R line) of hybrid pigeon pea ICPH-2671 on seed quality

The experiment was carried out with the parental lines of hybrid pigeon pea ICPH-2671 viz., G₁ : A- line (female parent ICPA-2043), G₂ : B-line (Maintainer line- ICPB-2043) and G₃ :R-line (Restorer/male parent ICPR-2671) and three pollination methods viz., P₁: Selfing, P₂: Isolated Random mating and P₃: Plant to progeny row selection for maintenance of parental lines on seed quality.

Among the pollination methods the data on seed yield and quality parameters viz., pod length (cm), pod breadth (cm), pod size (cm), germination (%), field emergence (%), seedling dry weight (g) and electrical conductivity (d/sm) did not differ significantly.

Among pollination methods P₃: plant to progeny row selection recorded significantly the highest number of seeds per pod (4.23), hundred seed weight (12.01 g) and seedling vigour index (2963) as compared to P₁: selfing and P₂: random mating pollination methods.

The results on germination percentage revealed non-significant difference due to pollination methods, genotypes and their interactions. However, significant difference was showed with respect to root length and shoot length.

Among genotypes G₁: ICPA-2043 recorded significantly the highest (3.83) number of seeds per pod, hundred seed weight (11.81g). Whereas G₃: ICPR-2671 recorded significantly the highest seedling vigour index (2941) as compared to G₁: ICPA-2043 and G₂:ICPB-2043.

The interaction effect due to pollination methods and genotypes found significant for shoot length. However, the treatment combination G₃P₃: ICPA-2671 in plant to progeny selection recorded significantly the highest (17.56 cm) shoot length and it was lowest (12.73cm) with G₁ P₁: ICPA-2043 in selfing method.

The interaction effects due to pollination methods and genotypes found significant for root length. However, the treatment combination G₃P₃ recorded significantly the highest (16.27 cm) root length and it was lowest (11.73cm) with G₁ P₁.

The data on genetic variability parameters of parental lines of ICPH-2671 pigeon pea hybrid with respect to plant height (cm), number of primary branches, number of secondary branches, days to 50 % flowering, days to flower initiation, days to pod initiation, days to pod maturity, number of pods

per plant, pod yield per plant and number of seeds per pod showed significant difference. The phenotypic co-efficient of variation (PCV) was higher than genotypic co-efficient of variation (GCV) for all the characters mentioned above. Number of seeds per pod exhibited high PCV (12.04) and GCV (6.75). Whereas, days to pod maturity also exhibited high heritability (0.98) with genetic advance (14.94).

Experiment-II: Effect of different planting ratio's and insect attractant on growth, Seed yield and quality of hybrid pigeon pea ICPH-2671

Female/seed parent (F₁ hybrid = ICPA-2043 x ICPR-2671)

The data on growth parameters viz., plant stand, plant height (cm) and number of primary and secondary branches per plant at harvest, days to flower initiation and duration of flowering as influenced by different planting ratio's and insect attractant spray and their interaction effect did not show any significant difference in female parental lines.

The treatment T₂: insect attractant spray Bee-Q at the rate of 12.5 g/liter of water recorded the significantly less number of days to 50 percent flowering (109.89 days), pod initiation (129.11 days), days to pod maturity (163.00 days) and significantly the highest number of pods per plant (350.83 pods), pod yield per plant (63.61g), pod yield per plot (4.97 kg), pod yield per ha (2277.00 kg), pod length (5.33 cm), pod breadth (0.95 cm), pod size (5.07cm), number of seeds per pod (3.56 seeds /pod), seed yield per plant (62.68 g/plant), seed yield per plot (4.90 kg/plot) and seed yield per ha (2243.00 kg/ha) as compared to T₁: control (Without insect attractant spray). Irrespective of planting ratio, without insect attractant spray (T₁) took significantly more number of days to 50 percent flowering (116.89 days), days for pod initiation (133.50 days) days to pod maturity (167.11 days) and significantly less number of pods per plant (315.50 pods), pod yield per plant (56.18 g/plant), pod yield per plot (4.30 kg), Pod yield per ha (1976.00 kg), pod length (4.89 cm), pod breadth (0.75 cm), pod size (3.66 cm), number of seeds per pod (2.33 seeds/pod), seed yield per plant (54.81 g/plant), seed yield per plot (4.20 kg/plot) and seed yield per ha (1,928.00 kg) respectively in female parental line.

The treatment T₂ : Bee-Q insect attractant spray has recorded the significantly highest germination (93.39 %), shoot length (15.75 cm), root length (12.32cm), seedling vigour Index (2627), seedling dry weight (0.91 g), hundred seed weight (12.19 g) and low electrical conductivity (0.39 dS/m) while T₁: control (without insect attractant spray) recorded less values for germination (91.06 %), shoot length (14.41 cm), root length (11.11 cm), seedling vigour Index (2328), seedling dry weight (0.80 g), high electrical conductivity (0.57 dS/m) and low hundred seed weight (11.02 g) in female parental line.

Among the different planting ratios the P₆: 5:2 female to male planting ratio's recorded the significantly less number of days for pod maturity (162.67) and significantly highest pod yield per plant (64.90 g), pod yield per plot (6.27 kg), pod yield per ha (2324 kg), pod length (5.22 cm), pod breadth (0.93 cm), pod size (4.88 cm), number of seeds per pod (3.83 seeds), seed yield per plant (63.73 g), seed yield per plot (6.16 kg), seed yield per ha (2282 kg/ha) as compared to P₁:3:1, P₂:4:1, P₃:5:1 and P₄:3:2 planting ratio's.

P₆: 5:2 female to male planting ratio's has recorded significantly highest seed quality parameters viz., germination (95.17 %), shoot length (17.02 cm), root length (13.31 cm), seedling vigour index (2887), seedling dry weight (0.93 g) and lowest electrical conductivity (0.40 dS/m), higher hundred seed weight (11.97 g) as compared to P₁, P₂, P₃ and P₄ in ICPA-2043 female parental lines.

The Interaction effect between different planting ratio's and insect attractant were found to be significant. The treatment combination P₆T₂ :5:2 female to male planting ratio + insect attractant spray has recorded significantly highest seed yield parameters viz., days to pod maturity (160.33 days), pod yield per plant (69.53 g), pod yield per plot (6.74 kg), pod yield per ha (2,498 kg), seed yield per plant (68.24 kg), seed yield per plot (6.62 kg/plot) and seed yield per ha (2452 kg/ha) in female parental line ICPA-2043.

6.2.2 Male parent/pollen parent (ICPR -2671)

The treatment T₂: insect attractant spray Bee-Q @ of 12.5 g/liter of water has recorded the significantly less number days for 50 percent flowering (122.61 days), days to pod initiation (134.22 days), days to pod maturity (180.94 days) and significantly higher number of pods per plant (300.11), pod yield per plant (44.26 g), pod yield per plot (1.39 kg/plot), pod yield (1696 kg/ha), pod length (4.17 cm), pod breadth (0.55 cm), pod size (2.30 cm), number of seeds per pod (3.23), seed yield per plant (44.90 g/plant), seed yield per plot (1.33 kg/plot) and seed yield (1614 kg /ha) in male parental line as compared to T₁: control (without insect attractant spray).

The data on seed quality parameters viz., germination (95.78 %), shoot length (17.72 cm), root length (12.38 cm), seedling vigour index (2884), electrical conductivity of seed leachate (0.33 ds/m), seedling dry weight (0.97 g), hundred seed weight (11.29) in male parental line recorded the significantly superior values with treatment T₂:Bee-Q spray as compared to T₁: without insect attractant spray.

In male parental line ICPR-2671, among planting ratio's P₆:5:2, P₅:4:2 & P₄:3:2 were recorded significantly the highest seed yield parameters as compared to P₁:3:1, P₂:4:1 & P₃:5:1 planting ratio's. Seed quality parameters did not show any significant difference among different planting ratio's and their interaction effects.

Experiment III: Studies on morphological characterization of parental lines of hybrid pigeon pea ICPH -2671

The parental lines of hybrid pigeon pea ICPH-2671 viz., ICPA-2043, ICPB-2043 and ICPR-2671 were characterized based on seed, seedling and plant morphological characters.

- ❖ The parental lines of hybrid pigeon pea ICPH-2671 has good variation for seedling hypocotyl colour. The female parental line ICPA-2043 and maintainer line ICPB-2043 having green colour hypocotyl. While, male parental line ICPR-2671 has purple colour pigmentation on hypocotyl of the seedling.
- ❖ Both the parental lines of pigeon pea hybrid ICPH 2671 recorded more than 150 cm plant height hence, they are categorized as tall plant height.

- ❖ The genotype ICPA-2043 is semi spreading type, while, ICPB -2043 and ICPR-2671 are considered as erect type of plant branching pattern.
- ❖ Both the parental lines of pigeon pea hybrid ICPH-2671 took (91-130 days) for flower initiation hence, ICPA-2043, ICPB-2043 and ICPR-2671 are grouped under medium category for time of flowering.
- ❖ Both the parental lines of pigeon pea hybrid ICPH 2671 viz., ICPA-2043, ICPB-2043 and ICPR-2671 are grouped under indeterminate type of growth habit and green stem colour.
- ❖ The parental lines ICPA-2043 and ICPB-2043 are categorized as narrow oblong leaf shape and ICPR-2671 is categorized under the lanceolate leaf shape. ICPA-2043, ICPB-2043 and ICPR-2671 are grouped as absent pertaining to pubescence on lower surface of the leaf.
- ❖ The colour at the base of the petal of ICPA-2043 and ICPB-2043 are yellow where as ICPR-2671 has orange yellow at the base of the petal.
- ❖ The genotypes ICPA-2043 and ICPB-2043 were grouped under pattern of streaks on petal absent where as ICPR-2671 sparse pattern of streaks on petal was present.
- ❖ The parental lines ICPA-2043 and ICPB-2043 are grouped under light yellow colour flower where as ICPR-2671 is categorized under yellow with light streaks on flower.
- ❖ The parental lines ICPA-2043 and ICPB-2043 are grouped under light yellow colour pollen grains where as ICPR-2671 is categorized under dark yellow colour pollen grains.
- ❖ The pod colour at premature stage in ICPA-2043 and ICPB-2043 are having green colour where as ICPR-2671 is having green with brown streaks.
- ❖ In parental lines ICPA-2043 and ICPB-2043 pod pubescence is present where as in ICPR-2671 pod pubescence was absent.
- ❖ The waxiness on the pod was present in ICPA-2043, ICPB-2043 and ICPR-2671.
- ❖ The parental lines ICPA-2043 and ICPB-2043 have prominent pod constriction where as ICPR-2671 has slight pod constriction.
- ❖ The parental lines viz., ICPA-2043, ICPB-2043 and ICPR-2671 have cylindrical form pod at mature pod stage.
- ❖ The parental lines ICPA-2043, ICPB-2043 and ICPR-2671 are grouped under (3-4 seeds/pod) medium number of seeds per pod.
- ❖ The parental lines viz., ICPA-2043, ICPB-2043 and ICPR-2671 are grouped under oval seed shape.
- ❖ The parental lines viz., ICPA-2043, ICPB-2043 and ICPR-2671 are grouped under large seed weight (11-12 g).

Experiment-IV: Molecular characterization of pigeon pea hybrid ICPH-2671 and its parental lines by using SSR markers

The results on the varietal characterization of parental lines and its hybrid pigeon pea viz., female parent ICPA-2043, maintainer line ICPB-2043 and restorer male parent line ICPR-2671 and F₁ hybrid ICPH-2671 by using SSR primers summarized. The two SSR primers CcM-0021, CcM-1707 showed DNA amplification pattern between F₁ hybrid and parental lines of hybrid pigeon pea ICPH-2671.

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Appendix I : Preparation of stock solutions

1	Extraction buffer	10 mM Tris HCl,pH8.0 50 mM EDTA,Ph8.0 500 mM NaCl
2	Iso –propanal Ammonium acetate mixture	Three volumes of 10 M Iso-propanal and 1 Volume of 10M Ammonium acetate
3	T ₁₀ E ₁	Tris 10 m containing 1 mM EDTA
4	Chloroform: Isoamyl alcohol(24:1)	Chloroform 240 ml + Isoamyl alcohol 10 ml (24:1) and store in dark at room temperature. Make up and dispense the solution in a fumed cupboard.
5	Ethanol (70%)	Absolute alcohol (70 ml) Distill water (30 ml)
6	NaCl 5M	Dissolve 292.2 g NaCl in 750 ml water. Make up to 1 litre with water, filter and autoclave.
7	Phenol /chloroform	Mix equal volumes of buffered phenol and chloroform : Isoamyl alcohol (24:1) and store at 4°C.
8	Tris HCl (1M,pH8.0)	Dissolve 121.1 g Tris in 800 ml water. Adjust pH 8.0 with concentrated HCl make up the volume to 1 litre and autoclave.
9	Ethidium bromide (10 mg/ml)	Dissolve 100 mg ethidium bromide in 10ml of distilled water in tube cover with aluminum foil and store at 4°C.

Preparations of Reagents and buffer :

A) 1 M Tris (Molecular wt. : 121.14 g)

121.14 g → 1000 ml → 1 M

12.14 g → 100 ml → 1 M

Dissolve 12.14 g of Tris in distilled water and make up the volume to 100 ml.

B) 0.5 M EDTA (Molecular wt. : 292.25 g)

292.25 g → 1000 ml → 1 M

146.125 g → 1000 ml → 0.5 M

14.613 g → 100 ml → 0.5 M

C) 5 M NaCl (Molecular wt. : 121.14 g)

58.44 g → 1000 ml → 1 M

292.20 g → 1000 ml → 5 M

29.2 g → 100 ml → .5 M

Dissolve 29.2 g of NaCl in distilled water and make up the volume to 100 ml

i) **Extraction buffer (100 ml)** : The extraction buffer was prepared by mixing 15 ml of CTAB (20%), 10 ml Tris (pH 8.0, 1M), 4 ml EDTA(0.5 M), 46.6 ml NaCl (3M), 24.4 ml of distilled water and 100 µl 2- mercaptoethanol.

ii) **TE buffer (0.01 M, pH8.0, EDTA 0.001 M)**: It was prepared by mixing 1 ml of Tris (0.1 M pH 8.0) and 0.2 ml of EDTA (0.5 M pH 8.0) and the volume was made to 100 ml.

iii) **TBE buffer (10 X) (500 ml)**: 60.5 g Tris base, 25.6 g Boric acid and 1.85 g EDTA was dissolved in 300 ml of sterile distilled water and the volume was made up to 500 ml.

SEED TECHNOLOGICAL RESEARCH ON HYBRID PIGEON PEA (ICPH-2671)

CHANDRAVATHI B

2016

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ABSTRACT

The field and laboratory experiments were conducted to study the effect of pollination methods, planting ratio's, insect attractants and morphological and molecular characterization of parental lines of hybrid pigeon pea ICPH-2671 at Seed Science and Technology, Department, College of Agriculture, University of Agricultural Sciences, Dharwad during *kharif*-2011 and 2015.

Among pollination methods, P₃: plant to progeny row selection recorded significantly the highest number of seeds per pod (4.23), hundred seed weight (12.01 g) and seedling vigour index (2963) as compared to P₁: selfing and P₂: random mating pollination methods.

The treatment T₂: insect attractant spray Bee-Q at the rate of 12.5 g/liter of water recorded significantly the highest number of seeds per pod (3.56 seeds /pod), seed yield per plant (62.68 g/plant), seed yield per plot (4.90 kg/plot) and seed yield per ha (2243.00 kg/ha), germination (93.39 %), shoot length (15.75 cm), root length (12.32 cm), seedling vigour Index (2627), seedling dry weight (0.91 g), hundred seed weight (12.19 g) and low electrical conductivity (0.39 dS/m) as compared to T₁: control (Without insect attractant spray) in F₁ hybrid ICPH-2671 seed production.

Among the different planting ratio's the P₆: 5:2 and P₅: 4:2 female to male planting ratio's recorded significantly higher seed yield and quality as compared to P₁: 3:1, P₂: 4:1, P₃: 5:1 and P₄: 3:2 planting ratio's in F₁ hybrid 'ICPH-2671' seed production.

The study of morphological characterization of parental lines of the hybrid pigeon pea 'ICPH-2671' is useful for identification of off types in hybrid pigeon pea seed production mainly to carry out the rouging operation. In molecular markers characterization study the SSR primers CcM-0021 and CcM-1707 showed DNA amplification pattern between F₁ hybrid and its parental lines of hybrid pigeon pea 'ICPH-2671' these primers can be employed for detection of genetic purity.