

# Development and Commercialization of CMS Pigeonpea Hybrids

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## ABSTRACT

The role of heterosis in enhancing productivity in food crops is well known. Legume breeders have not been able, however, to take advantage of this genetic phenomenon for a long time, due to biological restrictions, such as the requirement of high seeding rate and the inability to produce large quantities of  $F_1$  hybrid seed. Recently, in pigeonpea (*Cajanus cajan* (L.) Millsp.), a breakthrough has been realized with the development and marketing of the world's first legume hybrid, ICPH 2671. The key for this achievement was breeding and using a stable cytoplasmic nuclear male sterility (CMS) system obtained from the cross between *C. cajanifolius*, a wild relative of pigeonpea, and the cultivated type. The inherent partial natural out-crossing of pigeonpea was knitted with this CMS system to facilitate economically-viable large-scale hybrid seed production.

These developments provided opportunities to overcome the historic stagnant low yield ( $0.6\text{--}0.8\text{ t ha}^{-1}$ ) through heterosis breeding. Among hundreds of hybrid combinations tested, a cross between ICPA 2043 and ICPL 87119 (=ICPR 2671), designated as ICPH 2671, was the most promising, with >40% yield superiority (reaching yields above  $3\text{ t ha}^{-1}$ ) over the prevalent cultivar 'Maruti', in multi-location, multi-year, on-station trials, as well as on-farm evaluations.

The outstanding performance of ICPH 2671 led to its release in 2010 as the first medium duration commercial pigeonpea hybrid in India. Subsequently, two additional pigeonpea hybrids, ICPH 3762 and ICPH 2740 were also released

for commercial cultivation in India in 2014 and 2015, respectively. According to recent estimates, in 2015 the CMS-based pigeonpea hybrids were grown over 150,000 hectares in central and southern India. In this review, we summarize the research efforts that led to the milestone of developing the first commercial hybrid in food legumes.

**KEYWORDS:** *Cajanus cajan*; cytoplasmic nuclear male sterility; heterosis; host plant resistance; hybrid seed production; pulses; legumes; yield.

## OUTLINE

### ABBREVIATIONS

- I. INTRODUCTION
- II. REPRODUCTIVE CYCLE AND MORPHOLOGY OF PIGEONPEA
  - A. Induction of Flowering
  - B. Maturity Range
  - C. Flower Structure
  - D. Flowering Pattern
  - E. Pollination and Fertilization
  - F. Natural Cross-pollination
    1. Cross-pollinating Agents
    2. Extent of Out-crossing
- III. CROP PRODUCTION
  - A. General Agronomy
  - B. Major Production Constraints
    1. Diseases
    2. Insect Pests
    3. Waterlogging
- IV. EXTENT AND NATURE OF HETEROSIS IN PIGEONPEA
- V. GENETIC MALE STERILITY-BASED HYBRID TECHNOLOGY
  - A. Genetic Male Sterility Systems
  - B. Heterosis in GMS-based Hybrids
  - C. Release of the First GMS-based Pigeonpea Hybrid
  - D. Hybrid Seed Production Technology
  - E. Assessment of GMS-based Hybrid Technology
- VI. TEMPERATURE-SENSITIVE MALE STERILITY
- VII. CYTOPLASMIC NUCLEAR MALE STERILITY-BASED HYBRID TECHNOLOGY
  - A. Early Efforts to Produce CMS System
  - B. Breakthrough in Breeding Stable CMS Systems
  - C. Diversification of Cytoplasm
    1. A<sub>1</sub> CMS System from *Cajanus sericeus* (Benth. ex Bak.) van der Maesen
    2. A<sub>2</sub> CMS System from *Cajanus scarabaeoides* (L.) Thou
    3. A<sub>3</sub> CMS System from *Cajanus volubilis* (Blanco) Blanco

4. A<sub>4</sub> CMS System from *Cajanus cajanifolius* (Haines) Maesen
  5. A<sub>5</sub> CMS System from *Cajanus cajan* (L.) Millsp
  6. A<sub>6</sub> CMS System from *Cajanus lineatus* (W & A) van der Maesen
  7. A<sub>7</sub> CMS from *Cajanus platycarpus* (Benth.) van der Maesen
  8. A<sub>8</sub> CMS System from *Cajanus reticulatus* (Aiton) F. Muell
  9. A<sub>9</sub> CMS System from *Cajanus cajan* (L.) Millsp
- D. Effect of Pigeonpea Cytoplasm on Yield
- E. Fertility Restoration of A<sub>4</sub> CMS System
- VIII. BREEDING NEW HYBRID PARENTS
- A. Fixing Priorities
  - B. Selection of Hybrid Parents from Germplasm and Breeding Populations
  - C. Isolation of Fertility Restoring Inbred Lines from Heterotic Hybrids
  - D. Breeding Dwarf Parental Lines
  - E. Breeding Determinate/Non-determinate Parental Lines
  - F. Disease-resistant Parental Lines
  - G. Use of a Naked-eye Polymorphic Marker in Hybrid Breeding
  - H. Formation of Heterotic Groups
  - I. Inbreeding Depression
- IX. APPLICATION OF GENOMICS IN BREEDING HYBRIDS
- A. Understanding the Molecular Genetics Basis of the A<sub>4</sub> CMS System
  - B. Tagging Fertility-restoring Genes
  - C. Assessment of Genetic Purity
  - D. Potential Role in Breeding Two-line Hybrids
- X. COMMERCIALIZATION OF HYBRID PIGEONPEA TECHNOLOGY
- A. Standard Heterosis
    1. Early Maturing Hybrids
    2. Medium- and Late-maturing Hybrids
  - B. Release of the World's First Commercial Legume Hybrid
  - C. Hybrid Seed Production Technology
  - D. Economics of Hybrid Seed Production
- XI. OUTLOOK
- ACKNOWLEDGEMENTS
- LITERATURE CITED

## ABBREVIATIONS

ATP	adenosine triphosphate
BC	backcross
CMS/CGMS	cytoplasmic nuclear/genetic male sterility
DES	Directorate of Economics and Statistics
DV	daily value

FAO	Food and Agriculture Organization on the United Nations
GMS	genetic male sterility
ICAR	Indian Council of Agricultural Research
ICRISAT	International Crops Research Institute for the Semi-Arid Tropics
Mb	megabase pair
mRNA	messenger ribonucleic acid
ORF	open reading frame
Person. commun.	personal communication
QTL	quantitative trait loci
rpm	revolutions per minute
SSR	simple sequence repeat
TGMS	temperature-sensitive male sterility system
USDA	United States Department of Agriculture

## I. INTRODUCTION

Most farmers in the developing world earn their livelihoods from small land holdings using subsistence level agricultural systems. In those areas, malnutrition is spreading fast, mainly due to a steady decline in the availability of protein-rich foods (NIN, 2010). Several factors, including expanding family size, limited growth in the production of protein-rich pulses, and escalating prices, are contributing to this problem (Shalendra *et al.*, 2013).

In this context, pigeonpea (*Cajanus cajan* (L.) Millsp.) is a nutritious legume rich in carbohydrates (62.8 g per 100 g of raw mature grain, 21% DV), fibre (15 g per 100 g, 60% DV), protein (22 g per 100 g, 43% DV), containing the important amino acids methionine, lysine and tryptophan), vitamins (Thiamine 43% of DV, Folate 114% of DV), minerals (manganese 90% of DV, magnesium 46% of DV and phosphorus 37% of DV) and low fat (1.5 g per 100, 2% of DV) (USDA-ARS National Nutrient Database). It is highly appreciated in the semi-arid tropics, due to its resilience and role in subsistence agricultural systems. It grows well under diverse environments, cropping systems and it has capacity to tolerate various biotic and abiotic stresses.

In rural settings, pigeonpea is considered a multi-purpose crop; it is used as food (fresh as vegetable and dry as processed split peas), fodder, feed, fuel wood, and even construction material (Saxena, 2008; Saxena *et al.*, 2010b). Besides these benefits, the cultivation of this environmentally friendly crop also helps in improving general soil health (composition and structure) by providing around 40 kg ha<sup>-1</sup> of residual

**Table 3.1.** Area, production, and grain yield of the main pigeonpea growing countries.

Country	Area (000 ha)	Production (000t)	Yield (kg ha <sup>-1</sup> )
<b>Asia</b>			
India	5,602	3,290	587
Myanmar	611	575	940
Nepal	17	16	965
<b>Africa</b>			
Kenya	276	274	994
Malawi	81	34	420
Tanzania	250	248	990
Uganda	33	13	406
Congo	11	7	636
<b>Caribbean</b>			
Dominican Republic	23	24	1,066
Haiti	110	90	814
<b>Global</b>	<b>7,033</b>	<b>4,890</b>	<b>695</b>

Source: <http://www.faostat3.fao.org>. Reproduced with permission of Food and Agriculture Organization of the United Nations (FAOSTAT), 2017.

nitrogen, releasing soil-bound phosphorus, adding organic matter, and facilitating water infiltration. Considering these advantages, pigeonpea has become an important crop of the tropics and sub-tropics of Asia, Africa, and South America. According to FAOSTAT (2017), the estimated globally-sown pigeonpea area is around 7.03 million ha with a total production of 4.89 million t, and average yield of 0.695 t ha<sup>-1</sup>. India has the largest (75%) share of the global pigeonpea production area (Table 3.1). According to DES (2015), the national production of 3.29 million t is insufficient to meet the domestic requirements and about 500,000 t of pigeonpea are imported annually from Myanmar and Africa.

Pigeonpea has been under cultivation for more than 3,500 years, but some botanists believe it is far from true domestication, because it still carries certain survival and evolutionary traits of its wild ancestors, such as perennial growth habit, poor harvest index, deep root system, natural out-crossing, ability to recover from various stresses, and shattering of mature pods. Genetic improvement of this crop began in India in 1931 at the Imperial Agricultural Research Institute, Pusa (Bihar), with pure line selection within phenotypically promising landraces for simply inherited traits, with enhanced focus on disease resistance and plant type.

Subsequently, the Indian Council of Agricultural Research (ICAR) launched a long-term 'All India Coordinated Pigeonpea Improvement Programme' to develop high-yielding cultivars and their production

technology for different regions. This endeavour led to the release of over 100 cultivars (Singh *et al.*, 2005) through pedigree selection from among and within landraces and breeding populations. A perusal of these cultivars revealed that, although significant advances were made with respect to earliness, plant type, seed type, and resistance to diseases, their on-farm yield remained stagnant (0.6–0.8 t ha<sup>-1</sup>), which has been a matter of concern for decades. Partial out-crossing of pigeonpea could somewhat explain the lack of success in increasing grain yield, despite releasing new promising cultivars over time. Certain important traits (such as host plant resistance to pathogens) in commercial cultivars can be easily lost when farmers save seed (a mix of selfing and out-crossing) for the next season. Efforts to preserve cultivar identity, recently popularized, include the implementation of the concept of *One Village One Variety*, which guarantees physical separation between cultivars.

Green *et al.* (1981) reviewed global pigeonpea improvement programs, and concluded that '*Almost all the traditional breeding methods of self-pollinated crops were tried by pigeonpea breeders, but without significant gains in its productivity.*' Moreover, Khan (1973) proposed the use of partial natural out-crossing in breeding high yielding pigeonpea populations. Onim (1981) used this approach in Kenya, and obtained encouraging results with 2% yield gain in each cycle of selection. However, despite the encouraging results, this breeding approach failed to take off. Hence, pedigree breeding has remained the preferred pigeonpea breeding method in India and elsewhere.

The discovery of male sterility systems, and the existence of partial natural out-crossing in pigeonpea, encouraged breeders both at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT, Patancheru, Telangana, India) and ICAR to explore the possibility of developing hybrids in this food legume. Overall, these developments generated a lot of optimism among breeders towards breaking the decades-old productivity barrier in pigeonpea. In this review article, we summarize the breeding and seed production research efforts that led to the milestone of developing the first commercial hybrid in food legumes, and we also discuss the potential role of pigeonpea hybrids in achieving food and nutritional security in the semi-arid tropics.

## II. REPRODUCTIVE CYCLE AND MORPHOLOGY OF PIGEONPEA

### A. Induction of Flowering

By nature, most pigeonpea landraces are of long duration, highly photoperiod sensitive, and short-lived (4–5 years) perennials. In such

landraces, the induction of flowering takes place at the onset of short days (around ten hours of light). This photoperiod requirement restricts their adaptation between 15–35° latitudes. Breeders successfully bred short-duration cultivars that exhibited relatively less sensitivity to day length. Wallis *et al.* (1981) demonstrated that pigeonpea earliness was closely related to photoperiod insensitivity. The less sensitive genotypes produce less biomass, show wide adaptation (up to 45° latitude), and provide flexible sowing date options (Saxena, 2008). Saxena *et al.* (1981) found three dominant genes that controlled the photoperiod reaction and were expressed in hierarchical order ( $Ps_3 > Ps_2 > Ps_1$ ), with  $ps_1 ps_1$  being the earliest to flower and the least photoperiod sensitive. At ICRISAT photoperiod sensitive genotypes flowered under a ten-hour photoperiod and continued flowering even under relatively longer (14 hours) days.

### B. Maturity Range

Pigeonpea shows continuous variation for maturity, from < 90 to >250 days. This range has allowed farmers to choose the cultivars best suited to their local agro-ecological conditions and production systems. For practical purposes, four broad maturity groups have been recognized in India. These are: extra-short/early (91–120 days), short/early (121–150 days), medium (161–200 days), and long/late (>250 days).

Recently, Vales *et al.* (2012) bred pigeonpea genotypes that matured in < 90 days at ICRISAT. These super-early types are useful in diversifying pigeonpea cultivation in the areas characterized by a short growing season or low temperature, such as high latitudes and altitudes. Furthermore, to assist breeders in planning and selection, they established 11 maturity groups in pigeonpea (Table 3.2). From the adaptation and commercial points of view, medium duration pigeonpea occupies the larger area (65%) followed by the long duration group (30%). These two groups are invariably cultivated as intercrops with short-aged cereals or pulses. In contrast, the early types are typically cultivated under high density and pure stands in cropping systems that alternate cereals and legumes, and occupy < 5% of the total cropped area.

Recently, the super-early pigeonpea group has been attracting increased attention, due to the adoption of multiple cropping systems and enhanced mechanized agriculture. Traditionally, all the pigeonpea cultivars are planted as annual crops, but some genotypes with long pods and large seeds are also grown as perennials, mostly in backyards for vegetable purposes, where plants easily survive for 3–5 years.

**Table 3.2.** Pigeonpea maturity groups based on days to flowering and maturity of genotypes planted by mid-June at ICRISAT in Patancheru, India (17°N 30', 78°16'E, 545 m.a.s.l.) Table based on Vales *et al.* (Vales *et al.*, 2012) and Green *et al.* (Green *et al.*, 1979). Expanded to show divisions based on days to 75% maturity.

ICRISAT maturity group	Popular maturity group	Days to 50% flowering <sup>z</sup>	Days to 75% maturity <sup>z</sup>	Reference cultivars
00	Super-early	<50	<90	MN5
0	Extra-short	51–60	91–100	ICPL 88039
I	Extra-short	61–70	101–120	Prabhat
II	Short	71–80	121–130	UPAS 120, ICPL 87
III	Short	81–90	130–140	Pusa Ageti, T 21
IV	Short	91–100	141–150	ICP 6
V	Short-medium	101–110	151–160	BDN 1, Maruti
VI	Medium	111–130	161–180	Asha
VII	Medium	131–140	181–200	ICP 7035
VIII	Medium-long	141–160	201–220	ICP 7065, Bahar
IX	Long	>160	>250	NP (WR) 15, MAL 13

<sup>z</sup> days after planting

### C. Flower Structure

Pigeonpea belongs to the *Fabaceae* family. It has typical papilionaceous flowers, borne in bunches on short or long racemes. The peduncles are 1–8 cm long, while the pedicel length varies between 7 and 15 mm. The calyx is campanulate (bell-shaped), with glandular hairs and bulbous bases. The corolla is zygomorphic and petals (mainly yellow, but could also be red, purple, with streaks, or a combination of colours, depending on the genotype) are imbricate in buds. The standard petal is erect, 14–22 mm long and 14–20 mm wide, with clawed base. The wing petals are asymmetrically biauriculate, 15–20 mm long and 6–7 mm wide, obovate with a straight upper margin and clawed base. Keel petals are boat-shaped, 14–17 mm long, 5–7 mm wide, c-clawed and dorsally split, and cover androecium and gynoecium (Reddy, 1990). There are ten stamens, oriented in a diadelphous (9 + 1) format.

Bahadur *et al.* (1981) reported that pigeonpea has two types of stamens: the long stamens are antisepalous and the short ones are antipetalous. The odd stamen has a groove that provides passage for nectar that is secreted at the base of filaments. The anthers are ellipsoid, about 1–2 mm long, dorsified, and yellow, tapering towards the top and flattening towards the base. In general, the pollen grains produced by short stamens are larger than those produced by long stamens. The style is long, filiform, glabrous, and attached to a thick, incurved and capitate



(swollen) stigma. The ovary is superior and sessile, with short stalk and marginal placenta. The ovule number varies from 2 to 9, and the pods with 8–9 seeds often exhibit some degree of ovule abortion.

#### **D. Flowering Pattern**

Based on flowering pattern, pigeonpea genotypes are broadly divided into determinate and non-determinate groups. For induction of flowering in the determinate type, the terminal vegetative buds are transformed into reproductive buds and flowers are borne at the top of the canopy. The determinate plants do not grow much in size after flowering. In contrast, in the non-determinate type, the growing buds remain vegetative and flowers are borne in the axils. This allows plants to continue their vegetative growth during their reproductive phase. Pigeonpea plants produce numerous flowers for extended periods in more than one flush, but only 10–20% of them convert into pods.

According to Shelldrake (1979), the factors that lead to floral abscission in pigeonpea are primarily physiological in nature. He hypothesized that the supply of assimilates and nutrients within the plants is adjusted in such a way that fewer pods are set than the plants are capable of filling. Likewise, there seems to be a threshold level of nutrient supply, below which pod set does not take place. Due to the perennial nature of the plants, soon after floral abscission, new flowers emerge and set some pods. This process continues until the capacity pod load on a plant is achieved. Consequently, at a given time, one can observe different sizes of floral buds, flowers and pods on the same inflorescence per plant.

#### **E. Pollination and Fertilization**

Pigeonpea flowers are not truly cleistogamous. It has been observed that the floral buds remain cleistopetalous for the first 2–3 days only and, during this period, 70–80% of the buds are self-pollinated. Subsequently, the buds open and remain so for the next 24–48 hours, with the stigma still remaining receptive. Such open flowers attract insects to effect out-crossing. Lord (1981) defined such floral morphology as ‘pre-anthesis cleistogamy’, and this situation leads to both self- as well as cross-pollination on the same plant.

Stigma receptivity in pigeonpea is another key factor responsible for out-crossing. To validate this, Dalvi and Saxena (2009) conducted a detailed study in an insect-proof net house, using a male sterile line as pollen recipient. They demonstrated that the stigma becomes receptive

one day prior to the flower opening. The receptivity peaks for the next three days, including the day of flower opening. Soon the petals start unfolding, but the stigma still remains receptive (with 35% pod set) and, during this period, a number of nectar-hunting insects visit the flowers and effect cross-pollination.

According to Bahadur *et al.* (1981), the ten pigeonpea stamens are organized in two whorls. Four of these have short filaments, and their maturity coincides with peak stigma receptivity, so the majority of the flowers get self-pollinated. The remaining six stamens, including the odd posterior one, have relatively longer filaments, and their maturity is delayed by a couple of days. This coincides with unfolding of petals and insect visitation, and they play a significant role in pollen collection and cross-pollination. Besides extended stigma receptivity, the advantage of foreign pollen over self-pollen with respect to their germination (Onim *et al.*, 1979), pollen tube growth (Dutta and Deb, 1970), and fertilization (Reddy and Mishra, 1981) also encourages out-crossing in pigeonpea.

## F. Natural Cross-pollination

Kumar and Saxena (2001) conducted an experiment to understand the role of wind in cross-pollination in pigeonpea. They grew male fertile plants as the pollen source (contaminator) and male sterile plants as the pollen recipient in an insect-proof glasshouse. At full flowering, an attempt was made to blow pollen grains from the fertile plants towards the male sterile plants using an electric wind blower, operating between 920 and 1,425 rpm, which was installed on the opposite side to allow wind to pass through the fertile plants towards the rows of male sterile plants. In this exercise, no pod set was observed, even on the plants placed nearest (100 cm) to the pollen source. This experiment concluded that wind had no role in cross-pollination in pigeonpea, and the entire process of natural cross-pollination observed under field conditions is mediated by some other external factors.

**1. Cross-pollinating Agents.** It has been observed that pigeonpea flowers attract a variety of flying insects. During the process of foraging and nectar collection, a load of pollen gets stuck to the bodies of the insects and, when they visit other flowers and repeat tripping, this results in cross-pollination. Pathak (1970) was the first to identify *Apis mellifera*

and *A. dorsata* as the primary cross-pollinating agents in pigeonpea. Williams (1977) reported that, although a variety of insects visited pigeonpea flowers, only *A. mellifera* and *Megachile lanata* participated in pollen transfer at Patancheru (India). She further reported that each insect carried an estimated 5,500–107,333 pollen grains, and > 90% of these belonged to pigeonpea.

In Kenya, Onim (1981) reported that also *Xylocopa* spp. (carpenter bee) and *Bombus* spp. (bumble bee) affected cross-pollination in pigeonpea. Brar *et al.* (1992) and Verma and Sandhu (1995) reported that *M. lanata*, *A. dorsata*, and *Xylocopa* spp. were responsible for cross-pollinating pigeonpea in Ludhiana (India). Similarly, Zeng-Hong *et al.* (2011) also observed that in Yuanmou (China) *Megachile* spp., *Xylocopa* spp., and *Apineae* spp. actively participated in the collection and transfer of pollen grains to effect cross-pollination.

Onim (1979, 1981) reported very high levels (>70%) of natural outcrossing in Kenya. They also observed that each insect visit to pigeonpea flowers lasted from 15–55 seconds. Zheng-Hong *et al.* (2011) reported that the pollinating insects were more frequent on male fertile plants, with a mean of 4.8 visits 10 minutes<sup>-1</sup>, compared with male sterile counterparts recording 2.8 visits 10 minutes<sup>-1</sup>. They attributed that this behaviour of the pollinators was due to differences in the production of:

- (i) chemicals such as flavone and flavonol;
- (ii) nectar;
- (iii) some specific scent emitted by pollen grains.

They further reported that even with 50% fewer insect visitations, the male sterile plants produced cross-pollinated yield (384 g plant<sup>-1</sup>), similar to that of more frequently visited fertile plants (357 g plant<sup>-1</sup>). They also concluded that, for good pod seed set on the male sterile plants, a very high level of insect activity was not essential to produce reasonably good quantities of hybrid seed in the production plots.

An experiment conducted by ICRISAT in Patancheru during the 2010 and 2011 seasons showed that seed production of a male sterile line (A × B) outdoors using net houses containing *Apis mellifera* bee hives was unacceptably low (<250 kg ha<sup>-1</sup>), compared with open field conditions with natural insect pollinators (>800 kg ha<sup>-1</sup> and reaching ≈ 1,200 kg ha<sup>-1</sup> when sequential planting (three weekly plantings) was used). The controlled system (net houses containing beehives) had the potential to

produce pure seed of fertile plants ( $>1,000 \text{ kg ha}^{-1}$  of B lines), but not the seed on the male sterile plants. Thus, it could be recommended for producing seed of fertile parental lines and varieties, but not for maintenance of the A parent ( $A \times B$ ) or hybrid seed ( $A \times R$ ) (Vales, unpubl.).

**2. Extent of Out-crossing.** Natural out-crossing in pigeonpea was first recorded by Howard *et al.* (1919). A review on this subject by Saxena *et al.* (1990) revealed a large variation (0–70%) in the extent of natural out-crossing in all the pigeonpea growing sites. The primary factor responsible for the variation in out-crossing is the population of insect pollinators in a particular field during the flowering period. Besides this, there are some biological and physical factors that also influence cross-pollination in pigeonpea.

The genotypic variability in floral morphology, such as the presence of wrapped flowers (Byth *et al.*, 1982), cleistogamy (Saxena *et al.*, 1993) and the quantity of nectar produced (Zeng-Hong *et al.*, 2011) also affect insect activity and degree of cross-pollination. Factors such as extended period of stigma receptivity (Dalvi and Saxena, 2009) and competitive advantage of foreign pollen in germination (Onim *et al.*, 1979), pollen tube growth (Dutta and Deb, 1970), and fertilization (Reddy and Mishra, 1981) have also been reported to encourage cross-pollination in this species. Bhatia *et al.* (1981) observed that the density of pollinating insects is the key factor for cross-pollination in a particular field.

Other factors influencing out-crossing were:

- (i) direction and velocity of wind;
- (ii) habitat of production plots;
- (iii) general weather conditions (dry or rainy days);
- (iv) general health of the crop.

Under such circumstances, it is logical to expect that these factors will not be uniform across locations and, hence, the outcome with respect to natural out-crossing will be site-specific.

In view of the potential dangers of out-crossing, the production of genetically pure seed needs special skills and planning in artificial means of selfing, or the use of adequate isolation is necessary. Small quantities of selfed seed are generally produced by enclosing branches (part or full) or whole plants before the flowers open, using muslin cloth bags of different sizes. For producing medium quantities (10–20 kg)

of selfed seed, small net houses (nylon nets attached to fixed metal frames) are used. The production of larger quantities of pure seed is done in field plots isolated by at least 500 m from other pigeonpea fields.

### III. CROP PRODUCTION

#### A. General Agronomy

Traditional pigeonpea cultivars are known to excel under subsistence agriculture. Under heavy soils or higher latitudes (27–30°N), long duration (>250 days) cultivars are adapted whereas, at lower latitudes (12–25°N), medium maturing (161–200 days) genotypes are grown. Interestingly, both of these types are cultivated with short-aged cereals as intercrop under rain-fed conditions, and require similar agronomy. In contrast, the early maturing group is always cultivated as a high-density sole crop. The plants of this group have small canopy and produce less biomass; hence, the crop is sown under high densities (up to 300,000 plants ha<sup>-1</sup>) to allow mechanical cultivation of pigeonpea and save labour. The late maturing cultivars are grown at densities of around 45,000 plants ha<sup>-1</sup>.

In general, pigeonpea is susceptible to waterlogging, so selection of well drained fields is essential. The crop does not respond to phosphate fertilizers, but an initial dose (20 kg ha<sup>-1</sup> N) of nitrogen helps by boosting its initial slow seedling growth. The plants are nodulated with a cowpea group of bacteria. All of the three groups are equally susceptible to insects, particularly *Helicoverpa armigera* and *Maruca testulalis* pod borers, so chemical control of insects is essential in order to minimize damage. D. Sharma (ICRISAT, person. commun.) demonstrated that the productivity of early, medium, and late maturity group genotypes is comparable and that, under optimum crop management practices and conducive environment, each group can produce about 2–3 t ha<sup>-1</sup> of seed yield.

#### B. Major Production Constraints

**1. Diseases.** Pigeonpea plants and seeds are known to encounter the invasion of over 100 pathogens, but only a few of them cause economic losses. Among these, some diseases, such as *Phytophthora*, *Alternaria* blight, bitches broom, phoma canker, and leaf spots are site- or environment-specific. Considering the global importance, Fusarium

wilt and sterility mosaic virus are widespread diseases, and each year cause huge losses. From a hybrid breeding point of view, only these two diseases have been considered for discussion. According to Kannaiyan *et al.* (1984), the estimated combined annual losses due to these diseases in India were worth US\$ 113 million while, in Africa, wilt losses were US\$ 5 million.

Wilt, caused by *Fusarium udum* Butler, is a soil-borne fungal disease found in most pigeonpea growing areas of Asia and Africa. Butler (1908) was the first to report this disease from India. The symptoms of the disease (wilting) usually appear during the flowering and podding stage of the plant, when carbohydrate depletion occurs in the roots and stem, and the affected plants die quickly without producing any seed. Yield losses due to this disease can be up to 100%. The fungus multiplies and remains viable in the soil for at least three years or more and, consequently, it appears year after year. Likewise, the cultivation of susceptible cultivars increases inoculum in the fields.

The chemical control of this disease is expensive and not very effective. Hence, the use of genetic resistance has been given high priority both in Asia and Africa. Studies on the genetics of resistance to *Fusarium* wilt have shown the presence of two genes: one dominant and another recessive. Both genes impart resistance to the disease and segregate independently. In the literature, perhaps for this reason, depending on the parents used in crosses, reports on multiple genes (Pal, 1934), two complementary genes (Shaw, 1936), single dominant gene (Joshi, 1957), and single recessive gene (Odeny *et al.*, 2009) controlling the resistance to *Fusarium* wilt are available. Saxena *et al.* (2012) confirmed the presence of both genes in a single study. Races of the pathogen for *Fusarium* wilt in pigeonpea remain unclear (Tiwari and Dhar, 2011).

The second most important disease of pigeonpea is sterility mosaic virus, which is transmitted by an eriophyid mite (*Aceria cajani*). Alam (1931) and Mitra (1931) were the first to document its occurrence in India. Its incidence in farmers' fields varies from 0 up to 100%. Its infection can occur at any stage of growth, but the tender leaves emerging from seedlings or regenerated plant growth are first infected. The disease spreads quickly, as the viruliferous mites can be airborne, and the direction of wind can spread the disease up to 2 km (Reddy *et al.*, 1990).

The genetics of resistance to sterility mosaic disease is complex. Its inheritance is affected by undefined interactions among the host plant, mite, and virus, and parental lines used in crosses.

These interactions affect the symptoms of the disease. Singh *et al.* (1983) reported that the resistance to sterility mosaic disease was controlled by four independent non-allelic genes. Of these, two each were dominant and recessive. They further mentioned that, for a plant to express resistance reaction, the presence of one dominant and one recessive gene is essential. Sharma *et al.* (1984) found that two alleles, one dominant and one recessive, controlled the immunity to disease; the tolerance reaction was attributed to the presence of a single recessive gene. Srinivas *et al.* (1997) found that the resistance was recessive in some crosses but dominant in others, and it was isolate-specific. Ganapathy *et al.* (2012) reported monogenic recessive resistance in one cross, and non-allelic digenic with complementary epistasis in another cross.

For sterility mosaic, three prevalent isolates have been described: Hyderabad, Bangalore, and Coimbatore. The presence of different isolates makes the understanding of the resistance more challenging.

**2. Insect Pests.** Among insects, pod borers (*Helicoverpa armigera* and *Maruca testulalis*) and pod fly (*Melanogromyza obtusa*) cause severe losses to the pigeonpea crop. However, due to the absence of any reliable source of genetic resistance, conventional breeding to improve insect resistance has not been undertaken, and the incorporating of *Bt* genes is being pursued by ICRISAT (Sharma *et al.*, 2008) and ICAR (Ramu *et al.*, 2011).

**3. Waterlogging.** Among the abiotic stresses that affect pigeonpea productivity, waterlogging is the second most important constraint after drought. There is no success with respect to breeding drought tolerant cultivars in pigeonpea but, with the development of an effective waterlogging screening technology (Chauhan *et al.*, 1997), research on resistance breeding was started. Sultana *et al.* (2013) screened in excess of 400 pigeonpea germplasm and identified a number of waterlogging tolerant genotypes. Saxena and Tickle (2015) reported that, among the tolerant genotypes, 100 were fertility restorers and 26 maintainers of  $A_4$  male sterility system. This finding facilitated the breeder's job.

Since the resistance to waterlogging is controlled by a single dominant gene (Perera *et al.*, 2001; Sarode *et al.*, 2007), its incorporation in the productive hybrid parents is relatively easy and resource-efficient. Some of the male sterile lines, such as ICPA 2092, ICPA 2078, and ICPA 2098, are productive, resistant to wilt and sterility mosaic disease, good combiners, and produce high yielding hybrids, but they are

highly susceptible to waterlogging. Potential A- lines can be crossed directly with waterlogging-tolerant restorers in order to develop waterlogging-tolerant pigeonpea hybrids. Considering the seriousness of this constraint and the need to breed high-yielding hybrids adapted to temporary waterlogging conditions, breeding of new tolerant hybrid parents (A, B, R) is essential for the long-term stability and wide adaptability of hybrids.

#### IV. EXTENT AND NATURE OF HETEROSIS IN PIGEONPEA

The literature on heterosis in pigeonpea is limited. The first report on this aspect was published by Solomon *et al.* (1957). They reported that a single cross hybrid recorded 24.5% heterosis over the better parent for seed yield. Recent reviews on this aspect by Sawargaonkar (2011), Kyu (2011), Wanjari and Rathod (2012) and Mudaraddi (2015) revealed that, in over 50 publications, the heterobeltiosis for seed yield was significant, but with a wide range.

The development of genetic (GMS) and cytoplasmic (CMS) male sterility systems triggered a change in the reporting of heterosis. Most researchers, keeping in view its practical application, used 'standard heterosis' (superiority over best cultivar used as check) as an indicator of hybrid vigour. Both the GMS- and CMS-based hybrids demonstrated the presence of significant heterosis that led to the release of hybrid (details in subsequent sections).

Saxena *et al.* (2005b) reported over 50% standard heterosis in the first set of CMS-based experimental hybrids, while Kandalkar (2007) recorded up to 156% standard heterosis for grain yield. Subsequently, Dheva *et al.* (2009), Kumar *et al.* (2009, 2012), Shoba and Balan (2010), Gupta *et al.* (2011), Mudaraddi and Saxena (2012), Gedam *et al.* (2013), Saxena *et al.* (2013a; 2014b; 2014c), Pandey *et al.* (2013), Patel and Tikka (2014), Yamanura *et al.* (2014), Patil *et al.* (2014), and Ajay *et al.* (2015) also recorded highly significant levels (>20%) of standard heterosis in CMS-based hybrids in pigeonpea.

Mhasal *et al.* (2015) reported 18% and 34% superiority over the most popular cultivars of central and south India, 'Tara' and 'Asha', respectively. From most studies on heterosis in pigeonpea, it was concluded that:

- (1) the range of reported standard heterosis was large (up to 156%);
- (2) genotype × environment interactions played an important role in the expression of hybrid vigour;



- (3) in some instances, genetic diversity was related to hybrid vigour, but it was not a rule;
- (4) heterosis started expressing from germination and continued thereafter;
- (5) in most cases, the heterosis for seed yield was associated with hybrid vigour for plant biomass, height, number of secondary branches, and number of pods plant<sup>-1</sup> and, in some cases, with seed size and seeds pod<sup>-1</sup>.

## V. GENETIC MALE STERILITY-BASED HYBRID TECHNOLOGY

Kolreuter (1763) is credited with having recorded the first ever naturally occurring male sterile plants with impaired anthers. About 175 years later, Stephens (1937) in sorghum, and Jones and Emsweller (1937) in onions, demonstrated the use of male sterility in hybrid seed production. Subsequently, plant breeders and geneticists began research to understand various aspects of male sterility such as its variants, processes of its origin and development, stability and uses in crop improvement in various plant species.

The origin of male sterility in plants is attributed to mutations that generally occur naturally, but it can also be induced through the application of different physical or chemical mutagens. Besides these, the male sterility system can also be bred through wide hybridization and selection. For its effective utilization in plant breeding, it is essential that the individuals with altered male sterility retain their female fertility intact. In pigeonpea, on the basis of their genetic control, the male sterility systems are classified into genetic (GMS), cytoplasmic-nuclear (CMS), or temperature-sensitive (TGMS). Of these, so far, only the CMS system has been explored to breed commercial hybrids (Saxena *et al.*, 2010c).

### A. Genetic Male Sterility Systems

Genetic male sterility (GMS) has been reported in over 150 plant species, both in the dicots and also the monocots (Kaul, 1988). In most cases, GMS is independent of any cytoplasmic or environment factors, and it is controlled by recessive nuclear gene(s) but, in odd cases, dominant genetic control is also reported. In nature, GMS arises due to mutation of the male fertility nuclear gene to its recessive form. In the self-pollinated crops, such mutants are invariably lost but, in out-crossed or partially out-crossed species, they are preserved in heterozygote form.

**Table 3.3.** Distinctive characteristics of genetic male sterility (GMS) systems reported over time in pigeonpea.

Breakdown stage	Distinctive information	Genotype	Reference
– <sup>z</sup>	Linked with female sterility	–	Deshmukh (1959)
–	Variable pollen sterility	–	Reddy <i>et al.</i> (1977)
Tetrad	Translucent anthers	$ms_1ms_1$	Reddy <i>et al.</i> (1978)
–	Linked with obcordate leaf	–	Venkateshwarlu <i>et al.</i> (1981)
Pre-meiotic	Sparse pollen production	–	Saxena <i>et al.</i> (1981)
Pre-meiotic	Photoperiod insensitive	–	Dundas <i>et al.</i> (1982)
Pre-meiotic	Arrow-head anthers	$ms_2ms_2$	Saxena <i>et al.</i> (1983)
–	Single recessive gene	–	Gupta and Faris (1983)
–	Linked to obcordate leaf	–	Pandey <i>et al.</i> (1994)
–	Single recessive gene	–	Verulkar and Singh (1997)
–	Single dominant gene	–	Wanjari <i>et al.</i> (2000)
–	Rudimentary anthers	$ms_3ms_3$	Saxena and Kumar (2001)

<sup>z</sup> Not reported

In pigeonpea, during the period extending from 1959–2001, a total of 12 GMS systems were reported (Table 3.3). With the exception of one (translucent anthers), the rest were chance selections.

Deshmukh (1959) reported the first spontaneous male sterile mutant in pigeonpea. This mutant also carried severe female sterility, and was lost in the same season. Reddy *et al.* (1977) made a deliberate search for male sterility in 7,216 germplasm accessions at ICRISAT genebank, and selected 75 male sterile plants from different accessions. Among these, six selections had fully developed translucent anthers and had no pollen grains. This male sterility was found to be controlled by a single recessive gene (Reddy *et al.*, 1978), which was later used in hybrid breeding. Subsequently, in Australia, two sources of male sterility were also reported: a natural photoperiod-insensitive mutant (Dundas *et al.*, 1982); and a GMS mutant detected in the breeding line B15B (Saxena *et al.*, 1983).

Gupta and Faris (1983) reported the identification of 11 male-sterile plants in a breeding population, while Venkateshwarlu *et al.* (1981) and Pandey *et al.* (1994) reported GMS systems that were linked to characteristic obcordate leaves. Verulkar and Singh (1997) reported another recessive male sterile mutant in a population of cultivar UPAS 120. Wanjari *et al.* (2000) recorded the first case of a

dominant gene controlling male sterility within an inter-specific progeny. Saxena and Kumar (2001) reported a GMS mutant that was selected from an inbred cultivar, ICPL 85010. This trait was found to be controlled by a single recessive gene that was non-allelic to the other reported cases of GMS. A case of sparse pollen production caused by partial collapse of microsporogenesis was also reported by Saxena *et al.* (1981).

Different studies related to microsporogenesis of GMS sources revealed that male sterility occurred due to pre- or post-meiotic breakdown of pollen mother cells (PMCs). In hybrid breeding, only the translucent anthers-type GMS system (Reddy *et al.*, 1978) was used, and the others remained of academic interest. This male sterility was found to be highly stable and was used in the early stages of hybrid breeding at ICRISAT and ICAR. Under field conditions, the male sterile plants produced a good number of pods through natural out-crossing, and this encouraged breeders to accelerate research efforts towards the development of hybrid technology in pigeonpea.

### **B. Heterosis in GMS-based Hybrids**

For the first five years, only 53 experimental hybrids were developed using the original GMS source (Reddy *et al.*, 1978). Of these, only ten hybrids exhibited standard heterosis (20–40%). By 1990, a few improved GMS lines were bred and, in the next eight years, 203 hybrid combinations were assessed, and all of these hybrids exhibited > 20% standard heterosis. Of these, 80 hybrids recorded above 40% superiority over the best control, and 46 hybrids exhibited more than 80% standard heterosis (Table 3.4). This information was useful and demonstrated that, in a pulse crop like pigeonpea, exploitable heterosis is available.

### **C. Release of the First GMS-based Pigeonpea Hybrid**

Since no commercial hybrid has ever been bred before in food legumes, the release of the world's first pigeonpea GMS hybrid, ICPH 8, in 1991 (Saxena *et al.*, 1992) was considered a major technological achievement. This hybrid was developed at ICRISAT by crossing a GMS line (MS Prabhat DT) with a fertile inbred line ICPL 161. Evaluation of this hybrid in 100 yield trials under different agro-ecological conditions showed that ICPH 8 was superior to the control cultivar UPAS 120 by 35%. In the on-farm trials conducted in two Indian states, ICPH 8

**Table 3.4.** Summary of standard heterosis<sup>z</sup> (%) reported in genetic male sterile-based experimental hybrids produced at ICRISAT in the early phases.

Years	GMS hybrid (no.)				Total
	Standard heterosis				
	<20%	20–40%	41–80%	81–>100%	
1977–1981	43	10	0	0	53
1990–1998	0	77	80	46	203
Total (no.)	43	87	80	46	256
Hybrids (%)	17	34	31	18	

<sup>z</sup> Superiority over the best cultivar

Source: ICRISAT.

**Table 3.5.** Standard heterosis of genetic male sterile pigeonpea hybrids belonging to early and medium maturity, released across India in the 1990s.

Name	Maturity	Year	Originating			Control	Heterosis <sup>z</sup> (%)
			centre	State	Zone		
ICPH 8	Early	1991	ICRISAT	Telangana	Central	UPAS 120	31
PPH 4	Early	1994	Ludhiana	Punjab	North West	UPAS 120	32
CoH 1	Early	1994	Coimbatore	Tamil Nadu	South	VBN1	32
CoH 2	Early	1997	Coimbatore	Tamil Nadu	South	Co 1	35
AKPH 4104	Early	1997	Akola	Maharashtra	Central	BDN 2	35
AKPH 2022	Medium	1998	Akola	Maharashtra	Central	BSMR 736	64

<sup>z</sup> In relation to the best local control

Source: IIPR, Kanpur, India.

demonstrated 20–30% superiority over the national control (Saxena *et al.*, 1992). Subsequently, five additional GMS-based hybrids were also released by the Indian National Agriculture Research System (Table 3.5). These included PPH 4 (32% heterosis), CoH 1 (32% heterosis), CoH 2 (35% heterosis), AKPH 4104 (35% heterosis), and AKPH 2022 (64% heterosis).

#### D. Hybrid Seed Production Technology

In order to develop effective and economical field plot techniques for large-scale seed production of GMS hybrid, various row (male : female) ratios and population densities were tested. A combination of one male and six female rows, and 60,000 plants ha<sup>-1</sup>, gave the best results with hybrid seed yields of 0.6 to 0.8 t ha<sup>-1</sup>. Roguing of male fertile plants

within the segregating female rows was the key factor in hybrid seed production. The first few floral buds that appeared on each plant were examined, and the male-fertile segregants were rogued out before their flowers opened. This was a time-bound roguing operation, and needed great attention. If the roguing were delayed for some reason, then the quality of the hybrid seed would be adversely affected, due to cross-pollination.

Since pigeonpea is perennial in nature, flowering of the male-sterile plants continues for relatively more time until optimum pod load on each plant is achieved (Sheldrake, 1979). To ensure pollen availability for an extended duration, and more hybrid yield, the flowers and young pods from the male parent were removed periodically, which was a labour-intensive and inefficient activity and economically unviable.

### **E. Assessment of GMS-based Hybrid Technology**

In field crops, where hybrid seed requirement is high, the GMS-based hybrid technology did not attract seed producers. The main problem was associated with the maintenance of genetic purity of female parent and hybrid seed; its implementation leads to high production cost. The manual roguing of 50% of the fertile plants within the female rows posed practical difficulties in large-scale seed production as it was a difficult, time-bound and labour-intensive field operation. The large-scale seed production of GMS hybrid seed was not grower-friendly. In spite of high seed demand, none of the GMS-based hybrid could reach farmers.

ICRISAT was aware of this potential constraint before launching the GMS-based hybrid breeding program, but continued with it to understand the degree of difficulty in seed production. The seeding rate (@ 5–10 kg ha<sup>-1</sup>) was not very high, and each male sterile plant could produce 350–500 g of hybrid seed. Another issue that needed answering in developing the hybrid technology was to know to what extent the partial natural out-crossing was sufficient to produce large quantities of hybrid seed under natural conditions. Besides this, even more important was the need to generate information on the nature and quantum of heterosis in this pulse. In fact, the information generated from working on GMS-based hybrids turned out to be useful when the CMS system was bred. The investment on GMS hybrid technology paid off handsomely because, in breeding CMS-based hybrids, the GMS system was replaced by the CMS system and the outputs came faster.

## VI. TEMPERATURE-SENSITIVE MALE STERILITY

The effect of different environmental factors on the expression of genes controlling male sterility or fertility has been well documented in various plant species (Kaul, 1988). Such specific effects, in terms of conversion of male sterility to fertility and *vice versa* were reported in both GMS and CMS systems. These natural events were considered to be only of academic interest until Yuan (1987), Sun *et al.* (1989) and Lu *et al.* (1994) demonstrated the utility of a temperature-sensitive male sterility system (TGMS) in commercial hybrid rice breeding in China. Levings *et al.* (1980) hypothesized that loss of some cytoplasmic, rather than nuclear, genetic factor is responsible for the reversion of male sterility to male fertility. Small *et al.* (1988) showed that no DNA loss was associated with the reversion of male sterility. Overall, the conversion of male sterility to fertility and its reversal is a complex genetic phenomenon, and more research is required at genomic and physiological levels to understand it better.

Recent success in breeding a TGMS (Saxena, 2014; Saxena and Mudaraddi, 2015) has opened up similar options to breed pigeonpea hybrids. In this system, when a given TGMS line is sown under low (<24°C) temperature, it will become fully fertile and produce self-pollinated seeds; hence, it will not require any maintainer (B-) line. In contrast, if the same A-line is sown under a high (>25°C) temperature regime, it will remain male sterile (Table 3.6), and it can be used to produce hybrid seed with assistance from insect pollinators.

The large-scale seed production involving TGMS lines will require two different sites, with distinct temperature requirements, during crop

**Table 3.6.** Number of sterile/fertile plants recorded in temperature-sensitive male sterile (TSMS) selections planted under an insect-proof net at ICRISAT, Patancheru (17°N), India. The plants were male-sterile at temperatures above 25°C (could be used to produce hybrids). The same plants became fully fertile at temperatures below 24°C (produced self-pollinated seed of the maternal line).

Selection name	September (>25 °C)		November (<24 °C)	
	Sterile	Fertile	Sterile	Fertile
EnvS S-1	37	0	0	37
EnvS S-2	32	0	0	32
EnvS S-3	27	0	0	27
EnvS S-5	23	0	0	22

Source: Saxena (Saxena, 2014). Reproduced with permission of ICRISAT.

growth. These sites should also satisfy the requirement of photoperiod, the other important determinant of floral induction in pigeonpea. Site #1 should have a temperature above 25°C, and all the plants will be male-sterile, which can be used for the production of hybrid seed. Site #2 should have temperatures below 24°C, which will induce male fertility in the plants, to produce self-pollinated seed. Ensuring temperatures above 25°C during the production of hybrid seed could be challenging, since there is a lack of control over the meteorological conditions. Identifying the right site(s) to produce hybrid seed, using appropriate controls and temperature monitoring, is essential to guarantee production of true hybrid seed.

An experiment at ICRISAT in India demonstrated that, in the early maturity group, it is possible to obtain hybrid seed and self-pollinated seed of the female parent from the same isolated field (Saxena, 2014). After harvesting hybrid seed from the male sterile rows, the male parent rows were uprooted. The remaining population of female rows was ratooned at about 30cm from top of the canopy, and allowed to grow during the winter season. The prevailing low temperatures converted the sterile plants to complete male fertile plants and, thus, produced a self-pollinated crop (Table 3.6).

To take full advantage of this system, as well as site selection, a suitable agronomy package needs to be developed. The two-parent hybrid breeding system in pigeonpea, when fully developed, will have several advantages, such as simplification of the technical demands and the number of lines involved in hybrid production. The TGMS system uses two lines instead of three (A, B and R in the CMS hybrid system), eliminating the need of having maintainers and fertility restorers. The deleterious effects of cytoplasm on hybrids will also be avoided, and incorporation of genetic variability can be achieved in a short time and rather easily.

## **VII. CYTOPLASMIC-NUCLEAR MALE STERILITY-BASED HYBRID TECHNOLOGY**

The cytoplasmic-nuclear male sterility system (CMS) has been extensively used in commercial hybrid breeding in a number of food crops. The hybrid-breeding program based on this system revolves around three distinct genotypes, namely, male sterile (A-), its maintainer (B-), and its fertility restorer (R-) line. In a plant system, CMS occurs due to interaction between its cytoplasmic and nuclear genomes. A cytoplasmic-nuclear male sterility (CMS) system in plants can arise either through

spontaneous mutation, intra-specific, inter-specific or inter-generic crosses. So far, the wide hybridization programs have been more successful in producing CMS systems in different crops (Kaul, 1988).

### **A. Early Efforts to Produce CMS System**

Reddy and Faris (1981) made the first attempt to breed a CMS line in pigeonpea, using a wild relative of pigeonpea. To start this program, they crossed a cultivated type (as female) with pollen from two wild species, *C. sericeus* and *C. scarabaeoides*. The maternally inherited male sterility was identified within some BC<sub>1</sub>F<sub>2</sub> progenies, but it was tightly linked to floral abnormalities such as petaloid anthers, free stamens or heterostyly. This male sterility could not be stabilized for use in breeding hybrids.

Ariyanayagam *et al.* (1993) attempted to develop CMS by treating seeds of a genetic male sterile line with chemical and physical mutagens. The partial male sterile genotypes selected from this exercise also failed to stabilize to establish a working CMS system. After these failures, the breeding efforts aimed at breeding CMS lines were shifted to combine the cytoplasm of wild relatives and nuclear genome of the cultivated type.

### **B. Breakthrough in Breeding Stable CMS Systems**

In order to breed a cytoplasmic nuclear male sterility (CMS) system, a targeted breeding program was launched at ICRISAT by placing the nuclear genome of the cultivated pigeonpea into the cytoplasm of its wild relatives. The initial success in breeding CMS was achieved by Ariyanayagam *et al.* (1993), by crossing *Cajanus sericeus* as a female with a cultivated genotype as male. This was followed by the development of a stable CMS line, using the cytoplasm of another crop wild relative, *Cajanus scarabaeoides* (L.) (Tikka *et al.*, 1997).

Subsequently, Saxena *et al.* (2005a) bred a CMS from a cross between *C. cajanifolius* and the pigeonpea line ICP 28. This CMS line had early maturity (140 days) but, considering that the main target group for breeding hybrids was medium maturity with resistance to diseases, this CMS trait was transferred into some medium duration selected maintainer genotypes by backcrossing, selection and screening in the disease nursery.



### C. Diversification of Cytoplasm

Keeping in view the long-term sustainability of the CMS-based hybrid technology, it was decided to infuse sufficient mitochondrial diversity into the breeding program. This will protect the program from any potential genetic threats (e.g. susceptibility to specific biotic or abiotic stress) associated with the use of a single cytoplasm, to which, when a disease outbreak occurs, most hybrids may succumb, causing severe productivity loss (Tatum, 1971). In rice, Virmani and Shinjyo (1988) listed several CMS inducing cytoplasm, but 95% of the Chinese hybrid rice represents a single (WA) cytoplasm (Brar *et al.*, 1998).

The efforts made in this direction in pigeonpea produced good results, and nine CMS-inducing cytoplasmic systems (Table 3.7) have been identified. This can help in developing a strong broad-based hybrid program. Of these, eight CMS systems represent different wild relatives of pigeonpea from secondary and tertiary gene pools, while one has cytoplasm from the cultivated type.

A brief account of these CMS sources and their cytoplasm donors is discussed chronologically below.

**Table 3.7.** Cytoplasmic male sterility (CMS) systems reported in pigeonpea, based on various cytoplasm donors.

<i>Cytoplasm donor spp.</i>	Gene pool	CMS		Remarks
		ID	Reference	
<i>C. sericeus</i>	Secondary	A <sub>1</sub>	Ariyanayagam <i>et al.</i> (1993)	Unstable CMS
<i>C. sericeus</i>	Secondary	A <sub>1</sub>	Saxena <i>et al.</i> (1996)	Used in breeding
<i>C. scarabaeoides</i>		A <sub>2</sub>	Tikka <i>et al.</i> (1997)	
<i>C. scarabaeoides</i>		A <sub>2</sub>	Saxena and Kumar (2003)	
<i>C. volubilis</i>	Tertiary	A <sub>3</sub>	Wanjari <i>et al.</i> (1999)	No restorer found
<i>C. cajanifolius</i>	Secondary	A <sub>4</sub>	Saxena <i>et al.</i> (2005a)	Used in breeding
<i>C. cajan</i>	Primary	A <sub>5</sub>	Mallikarjuna and Saxena (2002)	
<i>C. lineatus</i>	Secondary	A <sub>6</sub>	K.B. Saxena (unpubl.)	
<i>C. platycarpus</i>	Tertiary	A <sub>7</sub>	Mallikarjuna <i>et al.</i> (2006)	
<i>C. acutifolius</i>	Secondary	A <sub>8</sub>	Saxena (2013)	
<i>C. cajan</i>	Primary	A <sub>9</sub>	Srikanth <i>et al.</i> (2015)	

**1.  $A_1$  CMS System from *Cajanus sericeus* (Benth. ex Bak.) van der Maesen.** Ariyanayagam *et al.* (1993) crossed *Cajanus sericeus* as a female with a cultivated genotype. In the  $F_2$  generation, a few male sterile segregants were identified, but these could not be maintained, due to the serious problem of their reversion to male fertility. Additional attempts were made to stabilize the selections by using the approach of multiple genome transfer (Ariyanayagam *et al.*, 1995). Progenies derived from this effort produced a few male-sterile segregants that were maintained by other pigeonpea inbred lines. Saxena *et al.* (2010a) carried forward these selections through additional hybridization and selection, which led to the development of male-sterile lines, such as CMS 85010A, CMS 88034A and CMS 13091A. This cytoplasm was identified as  $A_1$ .

**2.  $A_2$  CMS System from *Cajanus scarabaeoides* (L.) Thou.** Tikka *et al.* (1997) and Saxena and Kumar (2003) reported the development of CMS lines by combining the cytoplasm of *Cajanus scarabaeoides* with the genome of a cultivated type. In an  $F_2$  population, male-sterile segregants were recovered by Tikka *et al.* (1997) and, subsequently, a perfect male-sterility maintainer line, ICPL 288, was also identified. This male-sterile source exhibited high stability across diverse environments and was subsequently used in developing hybrids in the Gujarat state of India. This cytoplasm was designated as  $A_2$ .

Initially, this CMS system appeared promising for hybrid breeding and, soon afterwards, a number of fertility restorers were identified (Chauhan *et al.*, 2004). One of the experimental hybrids, GTH 1, with more than 30% standard heterosis, reached up to the release stage. Unfortunately, during its large-scale on-farm promotion, its major weakness of unstable fertility restoration was exposed and, in several farmers' fields, the hybrid plants remained male-sterile and produced no pods. This disastrous situation led to the demise of the hybrid-breeding program based on  $A_2$  cytoplasm.

**3.  $A_3$  CMS System from *Cajanus volubilis* (Blanco) Blanco.** Wanjari *et al.* (1999) reported the identification of male-sterile segregants between a cross involving *Cajanus volubilis*, a member of the tertiary gene pool, and a cultivated type. These selections exhibited maternal inheritance for male sterility, but the breeders failed to identify any fertility restorer among cultivated types. Hence, it was discarded from the hybrid breeding program.

**4.  $A_4$  CMS System from *Cajanus cajanifolius* (Haines) Maesen.** Saxena *et al.* (2005a) used *C. cajanifolius*, a wild relative of pigeonpea, for developing a CMS system. Although this wild species belongs to the secondary gene pool, various serological and genomic studies placed *C. cajanifolius* as the closest wild relative to the cultivated type (Ratnaparkhe *et al.*, 1995), and the two species were reported to be separated by only 1–5 genes (Mallikarjuna *et al.*, 2012). Further, De (1974) and van der Maesen (1980) postulated that *C. cajanifolius*, which is endemic to the hilly forests of the east Indian coast, is the progenitor of the cultivated pigeonpea.

To get the cytoplasm of this wild species into hybrid plants, it was crossed as a female parent with over a dozen diverse pigeonpea inbred lines. Among the  $F_1$ s, the hybrid plants of the cross between *C. cajanifolius* × ICP 28 were completely male-sterile, with no pollen grains, perfect female fertility, and free from any morphological defect. This CMS system, designated as  $A_4$  (Saxena *et al.*, 2005a), proved to be a success. In this system, both the male sterility (Dalvi *et al.*, 2010; Chaudhari *et al.*, 2015; Sawargaonkar *et al.*, 2012a) and its fertility restoration (Sawargaonkar *et al.*, 2012b; Chand *et al.*, 2014) were stable across diverse environments, and it is now being used in breeding commercial hybrids.

**5.  $A_5$  CMS System from *Cajanus cajan* (L.) Millsp.** To breed a viable CMS system, Rathnaswamy *et al.* (1999) crossed a GMS line (carrying cytoplasm of cultivated species) with *Cajanus acutifolius* (F.V. Muell.) van der Maesen as male parent, but they failed to recover any male sterile genotype. Mallikarjuna and Saxena (2002) made a reciprocal cross using *C. acutifolius* as a female parent, and with pigeonpea accession ICP 1140 as male. Gibberellic acid (at 50 mgL<sup>-1</sup>) was used to enhance the pod set, but the hybrid seeds were immature and failed to germinate. To overcome this barrier, the developing embryos were rescued and successfully cultured in artificial media (Mallikarjuna and Moss, 1995), but it did not yield any CMS.

Encouraged by the success of embryo rescue technology, Mallikarjuna and Saxena (2005) crossed six pigeonpea cultivars as female parent with two accessions (ICPW 15613, ICPW 15605) of *C. acutifolius*, using the embryo rescue approach. The  $F_1$ s involving pigeonpea genotypes ICPL 85010, ICPL 85030, and ICPL 88014 exhibited male-sterility, and some of them had 100% pollen sterility. The anthers of these male-sterile plants were shrunken and pale yellow, and they maintained their sterility when crossed to their respective wild relative accessions.

Most of the cultivated accessions, when crossed to these male-sterile plants, restored the male fertility of the plants. An exception was HPL 24, where the  $F_1$  progeny produced both male-sterile and fertile plants (Saxena *et al.*, 2013b). This suggests the presence of both *rf* and *Rf* nuclear genes in this genotype. Further backcrossing with this line, and selection for pollen sterility, helped in stabilizing the cytoplasmic male-sterility. Interestingly, HPL 24 was bred from a cross involving *C. sericeus*, another wild relative of pigeonpea (Saxena, 2008). This suggested that, as well as *C. acutifolius*, the *Rf/rf* genes are also present in *C. sericeus*.

**6.  $A_6$  CMS System from *Cajanus lineatus* (W & A) van der Maesen.** In the open-pollinated population of *C. lineatus*, a naturally outcrossed partial male-sterile plant was observed towards the end of the 2002 rainy season (K.B. Saxena, unpubl.). This single plant was morphologically distinct from the rest of the population. The stem cuttings of this plant were raised in a glasshouse, and the plants were found to be completely male-sterile. They were crossed as a female parent with a pigeonpea line, ICPL 99044, with a normal pod set. The  $F_1$  plants were partially male-sterile. Back-crosses ( $BC_1F_1$ ) were made with ICPL 99044 and, out of 20 plants grown, five were partially male-sterile. In the  $BC_4F_1$  generation, 167 plants were examined for pollen sterility, and four plants, exhibiting 100% pollen sterility, were crossed to maintain the cytoplasmic nuclear male sterility with ICPL 99044.

**7.  $A_7$  CMS from *Cajanus platycarpus* (Benth.) van der Maesen.** *Cajanus platycarpus*, a wild species from the tertiary gene pool of pigeonpea, is cross-incompatible with cultivated types and, therefore, hormone-aided pollinations coupled with embryo rescue techniques were applied to obtain viable  $F_1$  and  $BC_1F_1$  progeny (Mallikarjuna *et al.*, 2006). In  $BC_2F_1$  generation, a progeny ( $BC_2-E$ ) with low pollen fertility was selected. Within this progeny, two plants with 100% pollen sterility were selected and crossed with a set of pigeonpea cultivars. Examination of their  $F_1$  progenies revealed that the hybrid involving cultivar ICPL 85010 maintained complete male-sterility, whereas cultivars ICPL 88014 and ICP 1444 restored the male fertility in the hybrids (Mallikarjuna *et al.*, 2011).

**8.  $A_8$  CMS System from *Cajanus reticulatus* (Aiton) F. Muell.** An outcrossed plant with distinct morphology was identified by ICRISAT within the population of *C. reticulatus* grown from an open-pollinated

seed lot. The pollen production in this plant was sparse, with partial male sterility. This plant was crossed to an inbred cultivar and, in  $BC_2 F_1$  generation, plants with complete male sterility were recovered. These plants had under-developed anthers and normal gynoecium. Six perfect maintainers have already been identified, and a search for fertility restorers is in progress (Saxena, 2013).

**9.  $A_9$  CMS System from *Cajanus cajan* (L.) Millsp.** This CMS is of recent origin, and based on cultivated cytoplasm. It is different from  $A_5$ , which used *C. acutifolius* as a male parent. In  $A_9$  CMS, on the contrary, *C. lanceolatus* (W. Fitzg) Maesen was used as a male parent (Shrikanth *et al.*, 2015). The CMS  $A_9$  appears to be better than  $A_5$ , since both the maintainers and restorers have already been identified.

Although the reported CMS systems ( $A_1$ – $A_9$ ) represent a wide spectrum of mitochondrial variability, so far only one ( $A_4$ ) is being used in commercial hybrid breeding. This situation necessitates more research in breeding diverse A-lines with both cytoplasmic and nuclear diversity. With the exception of the  $A_3$  system, the rest of the cytoplasmic resources are available at ICRISAT for further breeding and purification. In this endeavour, however, it should be noted that, while aiming for cytoplasmic diversity, the effect of a particular cytoplasm on yield, disease resistance, and other related traits should also be monitored.

In this direction, so far only one study has been carried out (see next section), with limited nuclear variability. In fact, this exercise should be an inseparable part of hybrid parent breeding programs. CMS lines derived from cultivated genotypes as female will benefit the hybrid parent breeding program, since linkage drag will be minimum, and most hybrid combinations will be free from morphological and genetic disorders.

#### **D. Effect of Pigeonpea Cytoplasm on Yield**

While advocating cytoplasmic diversity in hybrid breeding, it is also important to know if a particular cytoplasm has any adverse effect on seed yield or on any other important agronomic, disease resistance or market-preferred trait. In pigeonpea, so far, only one study of this kind has been done, and it involved  $A_4$  (*C. cajanifolius*) and cultivated (*C. cajan*) cytoplasm (Saxena *et al.*, 2015). For this study, two iso-nuclear lines with  $A_4$  and cultivated cytoplasm were developed. Pusa Ageti-(F) had cultivated pigeonpea cytoplasm, while Pusa Ageti- $A_4$  CMS

carried the cytoplasm of a wild species, namely *C. cajanifolius*. These lines were crossed with seven known fertility restorers. The data showed that, for seed yield, the hybrids with cultivated cytoplasm performed marginally better than those carrying *C. cajanifolius* cytoplasm. However, the extent of the superiority of such hybrids was inconsistent. The greatest yield penalty of 19%, due to the cytoplasm of the wild species, was recorded in a cross involving restorer line R-2364. For other traits, such as days to maturity, seed size, seeds pod<sup>-1</sup> and plant height, there was no definite trend in favour of any specific cytoplasm. These studies also showed that the cytoplasmic effects on the performance of hybrids were influenced by the genetic constitution of the restorers.

### E. Fertility Restoration of A<sub>4</sub> CMS System

Cytoplasmic nuclear male sterility is the consequence of certain defects arising in the mitochondrial genome, which are repaired by certain specific nuclear genes (*Rf*) and make the hybrid plants fully male fertile. Such genotypes are identified as 'fertility restorers', and form a vital component of hybrid-breeding programs. To breed hybrids with stable performance, it is essential that reliable information is available about the inheritance and stability of fertility of *Rf* genes. Normally, *Rf* genes are distributed within the primary gene pool but, if need arises, these could also be mined from the wild species that was used in breeding the particular CMS system.

The inheritance of fertility restoration of A<sub>4</sub> cytoplasm was studied by Dalvi *et al.* (2008), Kyu and Saxena (2011), Saxena *et al.* (2011a) and Sawargaonkar *et al.* (2012b). In all these cases, two dominant genes were found to control fertility restoration of hybrids. However, the reported mode of gene expression varied, and included duplicate dominant, complementary, and recessive epistasis. Saxena *et al.* (2011a) reported that two independent dominant *Rf* genes controlled fertility restoration of A<sub>4</sub> CMS system, and that the restoration was stable across environments only when both the genes were present in a single individual. If the hybrids carried either of the *Rf* genes, then the fertility restoration varied a lot across diverse environments. Similar situations were also reported in maize, where the perfect fertility restoration was under the control of four *Rf* genes. Of these, two were major genes, and the remaining two only resulted in partial restoration of male sterility (Wise *et al.*, 1999).

Selection of fertility-restoring pigeonpea genotypes with both the dominant genes together within segregating populations under field

conditions is difficult. To assist breeders in this endeavour, Saxena (unpublished) developed an easy field-oriented 'pollen load' methodology with > 75% success rate. In this method, four or five fully grown but unopened floral buds of  $F_1$  hybrid plants were examined for pollen load between 11 am and 3 pm on a clear-sky day. It was further observed that the genotypes with high pollen load had good pod set and perhaps carried both the *Rf* genes, while plants with sparse pollen resulted in very poor pod set on selfing, and likely had a single *Rf* gene.

## VIII. BREEDING NEW HYBRID PARENTS

### A. Fixing Priorities

In order to set up breeding criteria to select parental lines and hybrids, it is necessary to take into consideration the needs of local farmers in specific target regions, and to identify and give relative weight to their priorities. In India, there are several agro-ecological zones and multiple interests related with pigeonpea (variation for maturity, plant type, use, etc.). A parallel analysis applies to other pigeonpea growing areas in the world. Hence, it is important to perform a situation analysis about the target environment, considering the preferred or prevalent production systems (crop rotations, crop windows), how the seed is managed, soil type, moisture availability during the cropping season, weather (temperature, precipitation, evapotranspiration), geographical coordinates (latitude, longitude, altitude), photoperiod, length of the season, prevalent biotic and abiotic stresses, preferred growth habit, plant type, maturity class, available germplasm base, seed market class (vegetable vs. dry seed), use (food, feed, fodder, fuel), preferred seed morphology (size, colour) and quality, as it relates to the use of pigeonpea for the fresh or processing markets.

This will be a huge exercise, requiring considerable resources. The immediate objective in hybrid breeding is to significantly enhance the productivity in a stable fashion, in combination with tolerance or resistance to biotic and abiotic stresses, in the context of the local needs and priorities. These efforts would contribute to increase crop productivity, better health and nutrition, crop diversification, environmental protection and economic growth.

In a dynamic hybrid breeding program, the development of elite inbred lines (parents) at regular intervals is essential to produce new hybrids with greater yield and adaptation. Besides high *per se* performance, the parents should also have high combining ability, stability

across environments, and key market-driven traits. Such lines can either be bred or selected from the available germplasm and genetic stock. The popular methods used for breeding inbred lines in the self-pollinated crops are generally also followed, to develop new parental lines with emphasis on diversity at nuclear level.

The logical steps involved in this program are selection of parents, development and screening of segregating populations and, finally, selection and evaluation of inbred offspring with desirable traits. In pigeonpea, the selection efficiency is always threatened by partial natural out-crossing in the preceding generation. Therefore, breeders should take precautions to minimize the incidence of out-crossing in the breeding plots. In the following sections, we discuss some strategies to diversify the nuclear base of the fertility restorers.

## **B. Selection of Hybrid Parents from Germplasm and Breeding Populations**

The primary gene pool of pigeonpea contains over 20,000 accessions in the genebanks of ICRISAT and ICAR. These resources harbour tremendous genetic variability that can be used for mining the traits of interest. Keeping in mind the limitations of physical and financial resources, the diversification efforts in the breeding program should be implemented in a step-by-step manner. To start this activity, it is essential to choose stable A-lines, such as ICPA 2039, ICPA 2043 and ICPA 2092, accompanied by their high *per se* performance, high general combining ability, dominant gene for wilt resistance, desirable seed traits (size, shape and colour), and adaptation to the target locations.

These A-lines should be crossed with about 100 testers. The selection of testers should also be done scientifically, considering the objectives, target cropping system and environment, heterotic grouping, and genetic diversity. In addition to germplasm collections, the list of testers can also involve, among other bred-germplasm sources, old or new cultivars and advanced breeding lines. The latter should be given priority over the unexplored germplasm, due to their high yield potential and adaptation.

Saxena *et al.* (2014a) launched a broad-based hybrid parent-breeding program at ICRISAT by crossing 503 testers with different A<sub>4</sub> CMS lines. The testers included advanced breeding (F<sub>5</sub> onwards) lines, released cultivars and germplasm, representing diverse pedigree and origin. The evaluation of the resultant hybrids for their fertility restoration revealed that, in this lot, there were 26 male sterility maintainers and



179 fertility restorers, which represented good variability with respect to key traits. The remaining 296 hybrids were segregated for different proportions for fertile and sterile plants.

In another attempt to breed new maintainers and restorers, a targeted breeding program involving 35 inbred testers and three A-lines were identified. The criteria for selection were growth habit and plant type (non-determinate and semi-spreading), maturity (early and medium), seed size (8–14 g 100 seeds<sup>-1</sup>), seed colour (white or brown), seed shape (round or oval), resistance to wilt and sterility mosaic diseases, and their origin. The parental lines also included eight known restorers. The crosses were made in a line × tester mating design, and 105 hybrid combinations were produced. Based on the F<sub>1</sub> phenotype, the hybrid progenies were classified into fertile/restorers (33), sterile/maintainers (4) and segregating types (68). Interestingly, the frequency of restorers and maintainers was similar to that recorded earlier with 503 testers.

For developing new A-lines, the F<sub>1</sub> hybrids showing male sterility should be selected and backcrossed to the same parent. At the same time the pollinator line needs to be maintained by selfing. It has been observed at ICRISAT that, compared with bulk pollinations, if the backcrosses are made on a plant-to-plant basis, then the male sterility stabilizes rapidly. Similarly, for identifying new fertility restorers, the F<sub>1</sub> hybrids exhibiting full pollen fertility and good pollen load should be identified, their pollen parent should be selected, and selfed seed should be produced for reconfirmation. Such male parents should be maintained in the purest possible form.

The information generated at ICRISAT indicated that in pigeonpea germplasm the alleles responsible for male fertility/sterility are distributed randomly. Further, it was also noted that a greater proportion of germplasm suffered from intra-accession variability for male sterility/fertility. Hence, the prospect of using A<sub>4</sub> cytoplasm in hybrid breeding is promising. A perusal of the fertility restoring and male sterility maintaining lines showed a significant variation for important yield contributing traits, such as, flowering, maturity, seed size, seed colour, plant height and disease resistance (Table 3.8). This provides ample opportunities for selecting hybrid parents of choice.

Saxena and Tikle (2015) listed hybrid parents which will likely produce hybrids with tolerance against stresses like waterlogging and host plant resistance to pathogens, besides various agronomic and market-driven traits. They identified six male sterility maintainers and 27 fertility restorers that were found tolerant to waterlogging and were highly resistant to both wilt and sterility mosaic diseases.

**Table 3.8.** Phenotypic variation observed for important traits among pigeonpea fertility restorers (R lines) and maintainers (B lines) used to produce cytoplasmic male sterile (CMS) hybrids.

Trait	Restorers ( $n=210$ )	Maintainers ( $n=30$ )
Days to 50% flowering	50–158	53–167
Days to 75% maturity	100–241	98–287
Plant height (cm)	70–288	70–290
100-seed weight (g)	6–18	5–19
Fusarium wilt (%)	0–100	0–100
Sterility mosaic (%)	0–100	0–100

Source: Saxena (Saxena, 2014). Reproduced with permission of ICRISAT.

These parental lines can be used to breed pigeonpea hybrids with stable performance for the areas prone to waterlogging and these diseases.

### C. Isolation of Fertility-Restoring Inbred Lines from Heterotic Hybrids

Additive and non-additive genetic variation affect grain yield in pigeonpea (Mudaraddi, 2015; Sawargaonkar, 2011; Sharma and Dwivedi, 1995; Saxena and Sharma, 1990). Theoretically, part of this variation can be fixed in some inbreds, by accumulating desirable alleles with additive effects through pedigree selection. For this exercise, the best possible heterotic hybrid combinations should be selected for subsequent pedigree selection, to obtain new inbred lines. Such improved inbreds can be used directly as inbred cultivars, or can form good parental materials for the development of new hybrids.

At ICRISAT, a similar exercise was carried out in a GMS-based pigeonpea hybrid ICPH 8, and some of the derivative inbred lines achieved 70–75% of seed yield produced by the hybrid (Saxena *et al.*, 1992). In another attempt, the improved inbred lines selected from hybrid IPH 487 were used as new hybrid parents (KB Saxena (person. commun.)), and these produced the high-yielding hybrid ICPH 3762 that was released for cultivation. Selection of inbred lines from heterotic hybrids requires the elimination of male sterile plants within early generation segregating populations, so a large  $F_2$  population is required to enable good segregation and selection. Also, in each cycle, special care should be taken to protect the selected individuals from unwanted cross-pollination by selfing one branch of the selected plants with a muslin cloth bag.

#### **D. Breeding Dwarf Parental Lines**

In pigeonpea, popular cultivars achieve a height of over 250 cm at flowering, and the hybrids grow even taller by a margin of 20–30%, which increases the difficulty of managing pod borer insects and harvesting. To find a genetic solution to this problem, a search for genetic dwarf types was made (Saxena and Sharma, 1995). Two dwarfing sources ( $D_1$  and  $D_6$ ), with condensed primary branches and growing up to only 100–120 cm, were identified. This trait, controlled by a single recessive gene, is being transferred into good hybrid parents.

So far, the breeding efforts to develop productive dwarf male sterile genotypes have been unsuccessful. Once such A-lines are available, their insect management will be easier and production of seed will be simplified. In this type of genetic materials, the maintenance of purity will also be easier, because any off-type among the dwarf types will be tall, and can be rogued easily before flowering.

#### **E. Breeding Determinate/Non-determinate Parental Lines**

There are two recognized growth habits in pigeonpea: determinate (caused by a single recessive gene); and non-determinate (dominant gene). In the determinate type, the branches and main stem terminate in a reproductive bud, and this restricts plant growth after the flowering is induced. Consequently, the plants remain compact, short, produce fewer pods, and less yield per plant, and high plant population per unit area is essential for optimizing productivity. These types are also suitable for mechanized high input cropping systems.

The non-determinate types, in contrast, are tall, spreading and have numerous secondary and tertiary branches. On an individual plant basis, the non-determinate plants produce a large number of pods and give more grain yield. These types are best suited for subsistence agriculture and, in all the pigeonpea growing countries, most of the cultivars are of non-determinate growth habit. In these types, flowering is non-synchronous, and partial recovery from insect damage is possible. At present, all of the three released medium duration CMS pigeonpea hybrids are non-determinate in growth habit.

In the early maturing group, both determinate and non-determinate hybrids have been bred, with similar degrees of hybrid vigour. Some elite hybrids have demonstrated 30–80% standard heterosis (Saxena *et al.*, 2014c). A range of parental lines is available in both the plant types (Saxena *et al.*, 2014a), and this provides ample opportunities to

the national programs to breed hybrids for their areas of interest. In the medium and long maturity groups, only non-determinate types are cultivated. In this group, many parental lines, with different trait combinations and high yielding hybrids, such as ICPH 2671, 2740 and 3762, are available. Hence, it will always remain a high-priority group of materials.

## F. Disease-resistant Parental Lines

Since both *Fusarium* wilt and sterility mosaic virus are major yield reducers in most pigeonpea growing areas, the strategy has been to breed hybrids carrying resistance to both diseases. To start this program, all the known fertility restorers and maintainers of A<sub>4</sub> CMS system were screened for host plant resistance to both diseases simultaneously, and this program was implemented using the screening technology developed by Nene *et al.* (1981).

In this field-oriented screening, high levels of both the inoculums were maintained. *Fusarium udum* inoculum was sustained at  $5 \times 10^6$  spores m<sup>-2</sup> in soil by ploughing chopped wilted plants every year for over decades. For sterility mosaic virus screening, the spreading-row technique was used. Every test and susceptible control seedling growing in the nursery was inoculated by stapling a heavily mite- and virus-loaded leaf. This allowed quick migration of mites that carried sterility mosaic virus. This method provided no chance of escape from the two diseases. Test material was sown for screening, along with a highly susceptible control (one each for the two diseases) at regular intervals, to monitor the effectiveness of inoculum.

Host plant resistance to wilt and sterility mosaic is often controlled by recessive genes (Saxena and Sharma, 1990). Restorers and maintainers exhibiting resistance to both the diseases were selected for breeding hybrids, especially in the medium maturity group (Reddy *et al.*, 1990; Saxena *et al.*, 2014a; Saxena and Tikle, 2015). Recently, Saxena *et al.* (2012) reported the presence of a dominant gene for resistance to *Fusarium* wilt, and this has eased the process of breeding wilt-resistant hybrids. This gene has now been transferred to some A-/B- lines; and it will now allow the production of wilt-resistant hybrids from cross-combinations involving both resistant A- x resistant R- and resistant A- x susceptible R- lines.

A number of fertility restorer and maintainer lines have been reported to carry the dual resistance (Saxena *et al.*, 2014c), and these are being used to produce new high-yielding, disease-resistant hybrids. Pyramiding

of disease resistant genes should be beneficial for the long-term maintenance of disease resistance, despite possible changes in the pathogens. Additional new sources of resistance to *Fusarium* wilt and sterility mosaic disease found in the pigeonpea mini-core collection (Sharma *et al.*, 2012) should be useful to the pigeonpea hybrid breeding program.

### **G. Use of a Naked-Eye Polymorphic Marker in Hybrid Breeding**

‘Obcordate leaf’ is a distinctive morphological marker, and rare in occurrence. It is easy to identify by the naked eye, and is thus also known as a ‘naked-eye polymorphic marker’. This marker is controlled by a single recessive gene (Saxena *et al.*, 2011b). This trait has been found quite stable across environments, and expresses within 3–4 weeks from sowing. It has been shown to be a great tool to ensure purity of parental lines and hybrids, by testing true identity with minimum resources.

At ICRISAT, this marker has already been incorporated into A-/B-lines through backcrossing. In the obcordate A-lines, any out-crossed ‘off-type’ plant with dominant normal (lanceolate) leaves can be rogued out easily at seedling stage, and the genetic purity of the female parent can be maintained easily and economically. Likewise, when a restorer line (normal leaves) is crossed to an A-line having obcordate leaves, all the true hybrid plants will have normal leaves, and any plant within the hybrid population with obcordate leaves will be due to selfing (from pollen shedder) in the preceding generation. Such plants can be detected easily, to assist in determining seed quality of the hybrid. The limitation of this approach is that any outcrossed plant in the hybrid population, arising due to pollination from any other line with normal leaves, cannot be detected. Thus, the seed production of hybrids should be done with appropriate isolation distance.

The newly developed A-/B- lines with obcordate leaves have recently been used in developing new hybrid combinations at ICRISAT. Some of the lines, such as ICPA 2203, 2204 and 2208, have high combining ability (Patil *et al.*, 2014). These hybrids yielded 35–60% standard heterosis, and two of them were free from wilt and sterility mosaic diseases (Table 3.9). All the hybrids had normal lanceolate leaves and few contaminated plants (<1%), arising due to the fact that sibbing of the female parent in the preceding generation was visually detected by their obcordate leaves. Promotion of such hybrids will ensure greater genetic purity of female parents and hybrids.

**Table 3.9.** Performance of medium maturity pigeonpea hybrids involving A-lines with obcordate leaf shape.

Cross ICPA × ICPL	Yield (kg ha <sup>-1</sup> )	Standard heterosis (%)	SCA <sup>z</sup>	Disease <sup>y</sup> (Wilt + SM)	Leaf type ratio <sup>x</sup> normal : obcordate
2203 × 20116	1,384	35	382*	Resistant	397: 2 (0.50%)
2204 × 20093	1,543	50	48**	Resistant	388: 3 (0.77%)
2208 × 20108	1,650	60	448**	Resistant	411: 8 (0.02%)
SE ±	214	–	–	–	–

<sup>z</sup> – Specific combining ability

\*\* – significant at 0.05 and 0.01, respectively

<sup>y</sup> Wilt – Fusarium wilt

SM – sterility mosaic disease.

<sup>x</sup> – S.A. Patil (pers. commun.)

Source: Patil, <http://www.academicjournals.org/journal/JPBCS/article-full-text-pdf/68DEDFB46306>.

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## H. Formation of Heterotic Groups

Following the classic work of Shull (1908) in the early part of the 20th century on hybrid vigour and inbreeding depression in maize, Richey (1922) demonstrated the importance of geographic (= genetic) divergence in the expression of hybrid vigour. Subsequently, a popular concept of general and specific combining ability was launched by Sprague and Tatum (1942) to discriminate the parental lines with respect to their ability to combine with other genotypes and produce more vigour.

Even though the genetic mechanisms that explain heterosis are not fully understood, the value of genetically distinct parents in hybrid breeding has been well established (Stuber, 1994; Hallauer, 1999). Various concepts and processes of selecting elite parental lines, proposed from time to time, eventually matured into a concept of ‘heterotic groups’. This involved clustering and subsequent selection of parental lines on the basis of their combining ability, origin, or genetic diversity. In recent times, the availability of improved statistical tools and application of genomics have made the formation of heterotic groups more refined and realistic.

In pigeonpea, this exercise has just begun. A number of diverse A-lines and a range of fertility restorers are now available, and the formation of heterotic groups appears to be the right step forward as a long-term hybrid breeding strategy. Saxena and Sawargaonkar (2014) formulated seven heterotic groups based on multi-location specific combining ability (SCA) data, and reported that heterosis for seed yield was much greater when the parental lines represented two diverse

**Table 3.10.** Distribution of CMS A lines and restorer lines in different heterotic groups based on diversity using SSR markers.

Heterotic Group	CMS (A-lines)	Restorers (R-lines)
I	ICPA 2128, 2156, 2078, 2043, 2170, 2042, 2089	MN 5, JBP 36, Sarita, Pusa Ageti,
II	ICPA 2202, 2051, 2047, 2048, 2050, 2189, 2207, 2208, 2092, 2098	ICPL 99009, 99010, 99015, 94062, 20130, 20137, 151, 20139, 20098, 96061, 20094, 20205, 20203, 20129, 20123, 88039, 87091, ICP 149, UPAS 120ICPX 060137, 060148, ICP 7035, ICPL 161, 131, 20107, 87053, 22097, 20127, 20177, 99055, 20237, 20100, 5063, 20214, 20125, 20115, 20117, 99051, 99050, 20241, 20112, 20106, 20120, 20186, 20202, 20099, 20243, 99055, 96053, 20346.
III	–	ICPL 20344, 87119, 20342, 20349

Source: Mudaraddi (Mudaraddi, 2015). Reproduced with permission of Indian Journal of Genetics.

heterotic groups. Pandey *et al.* (2015) used multivariate analysis to develop heterotic groups in long duration pigeonpea.

Mudaraddi and Saxena (2015) used simple sequence repeats (SSR) to classify 20 male sterile lines and 132 fertility restorers into different heterotic groups. In this study, the male sterile lines were distributed in two heterotic groups (Table 3.10), while the fertility restorers exhibited relatively more variability and formed three heterotic groups. They also demonstrated that a few inter-specific derivatives formed a distinct and diverse group on the basis of SSR, but the hybrids involving these lines were unproductive, perhaps due to unwanted linkage drag. Aguiar *et al.* (2008) also reported that use of SSR markers eliminated environmental and genotype  $\times$  environment effects, and the results were not in full agreement with phenotypic data.

### I. Inbreeding Depression

The expression of heterosis is a consequence of numerous additive and non-additive interactions. The non-additive genetic variance declines in each selfing generation, due to gradual reduction of heterozygosity. This is referred to as ‘inbreeding depression’. In pigeonpea, since not many heterotic hybrids are available, the information related to inbreeding depression is insufficient to draw useful conclusions regarding the nature of this genetic phenomenon. However, there are some reports where significant inbreeding

depression has been recorded with respect to yield and other traits, although this is cross-specific.

For example, Ananthraju and Muthiah (2008), Kumar *et al.* (2012), Kyu and Saxena (2011) and Mudaraddi (2015) have recorded significant inbreeding depression only in some crosses for seed yield, plant height, number of primary branches, number of pods plant<sup>-1</sup>, seeds pod<sup>-1</sup>, and 100-seed weight, but there was no consistency among these results. Mudaraddi (2015) also concluded that, in crosses where inbreeding depression for yield was significant, it was found to be associated with inbreeding depression in the major yield components, such as pods plant<sup>-1</sup> or number of branches, among others. Limited research showed that, in general, inbreeding depression in pigeonpea was not high, and it appears that grain yield is predominantly under the control of additive gene action. These observations need confirmation using diverse hybrids and multi-environment evaluations.

## IX. APPLICATION OF GENOMICS IN BREEDING HYBRIDS

For large-scale adoption of hybrid seed technology, it is imperative that the steps involved in breeding and seed production should be simple, rapid and cost-effective. Although considerable progress has been made in developing hybrid pigeonpea technology, it still suffers from some inherent limitations, such as long generation turnover time, difficulty in assessing disease resistance, seed quality and true genetic identity. Plans have been made to apply genomic approaches to help breeders to enhance the efficiency of hybrid technology.

Pigeonpea is a diploid species with 11 pairs of chromosomes ( $2n = 2x = 22$ ) and a genome size equal to 833.07 Mb. For a long time, it was considered as one of the 'orphan legumes', because very few genomic resources were available. However, recent efforts have strengthened the genomic resources in this crop. Successes in genome sequencing of pigeonpea (Varshney *et al.*, 2012; Singh *et al.*, 2012), construction of various inter- and intra-specific genetic maps using various molecular markers (Yang *et al.*, 2006; Bohra *et al.*, 2012) and sequencing of the mitochondrial genome (Tuteja *et al.*, 2013) will help in enhancing the pace and quality of hybrid pigeonpea breeding programs. Applied genomic efforts pursued at ICRISAT, in relation to hybrid pigeonpea research and development are briefly described below.



### A. Understanding the Molecular Genetics Basis of the A<sub>4</sub> CMS System

Hanson and Bentolila (2004) reported that male sterility may be associated with alterations in promoter regions and portions of coding regions of mitochondrial ATP synthase, and this impairs its normal functioning. Recent research showed that male sterility is associated with chimeric mitochondrial open reading frames (ORFs). Wang and Zhou (2006) demonstrated that ORF encodes a cyto-toxin peptide that determines the expression of male sterility. Iwabuchi *et al.* (1993) showed that an abnormal copy of a mitochondrial gene produced aberrant mRNA transcripts containing an additional ORF.

In order to understand the molecular basis of the A<sub>4</sub> CMS system in pigeonpea, the mitochondrial genomes of lines containing *C. cajan* (cultivated) and *C. cajanifolius* (A<sub>4</sub>) cytoplasm were sequenced, which yielded 51 genes. This research identified 13 ORFs that, perhaps, triggered the expression of male sterility in ICPA 2039 (Tuteja *et al.*, 2013). Of these, five ORFs carried parts of other mitochondrial genes, and eight were located in proximity to the mitochondrial genes. These researchers concluded that certain rearrangements in the mitochondrial genome resulted in novel ORFs that generated modified proteins associated with the expression of male sterility. In another study, Sinha *et al.* (2015) reported a possible association of sequence modifications in *nad4L* and *nad7* genes in the expression of male sterility in A<sub>4</sub> CMS. Studies are in progress to validate the function of these genes using transformation technology.

### B. Tagging Fertility-restoring Genes

The development and commercialization of high-performing improved new hybrids to farmers at regular intervals is key for a successful hybrid breeding program. This can be achieved through breeding new parental lines with traits in demand. Cytoplasmic male sterility in the A-line, caused by unusual mitochondrial ORFs (previous section), is restored by fertility restorer genes (*Rf*) present in the restorer, R line; thus, hybrid seed (A × R) is successfully produced. In this context, screening germplasm for the presence of fertility restoring (*Rf*) genes and breeding of productive restorer lines requires good understanding of the inheritance of the *Rf* genes.

A number of genetic studies have confirmed that the fertility restoration of A<sub>4</sub> CMS is controlled by two independent dominant genes (Saxena *et al.*, 2011a). The presence of both genes together

maximized fertility restoration. While breeding new fertility restorers, the selection of genotypes carrying *Rf* genes, especially within the populations derived from crosses involving restorers and non-restorers or new germplasm, is difficult and time-consuming. This is because it will require progeny testing data to confirm the presence of the *Rf* genes in every individual selection, which is considered a resource-consuming activity.

However, recent advances in genomics, particularly marker-assisted selection, can hasten this process in a cost-effective manner. Significant progress has been made at ICRISAT by constructing a genetic map of pigeonpea, using various intra-specific mapping populations segregating for *Rf* genes. With the help of linkage mapping and QTL analysis, Bohra *et al.* (2012) identified ten markers associated with QTLs for fertility restoration in pigeonpea. Their usefulness is, however, limited, due to the low marker density of map and high intervals between the QTL regions.

### C. Assessment of Genetic Purity

The value of CMS pigeonpea hybrids in producing high grain yields sustainably and stability depends on the ability of the seed producers to maintain high levels of hybridity. Undesired genetic or physical impurities and changes in parental lines can be caused by out-crossing, mixtures, non-stability of male sterility, damage by pests, diseases, abiotic stresses, *de novo* variation (mutations, epigenetics), and unintended selection.

Many agronomic and management precautions should be taken in order to successfully produce true CMS hybrids. This is possible only when the highest level of genetic purity of the parental lines is maintained, and appropriate purity assessment technology is available to test the purity of parental lines and to confirm hybridity. In most crops, the purity testing of representative seed samples is carried out using a 'grow-out test'. In pigeonpea, this approach cannot be applied, due to its strong photoperiod-sensitive reaction and long generation turnover. The application of a molecular marker-based technology was considered the best option to overcome this constraint. This approach has already been developed for rice (Sundaram *et al.*, 2008), cotton (Asif *et al.*, 2009; Ali *et al.*, 2008) and safflower (Naresh *et al.*, 2009).

The genetic and physical purity of parental lines should be ensured by: starting with a trusted seed source; testing genetic purity; testing physical purity (seed quality, seed health, germination); selecting good

planting locations (isolated fields, net houses, cages or bags); maximizing the presence of pollinators; roguing morphological off types; using recommended spacing (plant to plant and row to row); using recommended female : male ratios (A : B and A : R); mapping the fields and labelling genotypes properly; selecting optimum planting time; using sequential planting to maximize pollen availability (off-season planting could also be an option to prevent contamination); harvesting sequentially to avoid mixtures; using appropriate crop management practices (weeding, fertilization, irrigation, disease/pest control); and using proper storage (segregating materials, preventing pests and the preservation of a high germination rate).

In pigeonpea, molecular purity assessments started in 2010 (R.K. (Saxena *et al.*, 2010)), by identifying two diagnostic nuclear SSR markers for the short-duration hybrid ICPH 2438. This was followed by the identification of a set of 42 SSR markers by Bohra *et al.* (2015) for purity assessment of hybrids ICPH 2671 and ICPH 2438. Out of these, four common markers (CcM0257, CcM1559, CcM1825, and CcM1895) were used for multiplexing purity assays of those hybrids at the nuclear level. Another set of seven SSR that differentiate A, B and hybrids was also recently identified (Bohra *et al.*, 2015).

In addition, A- and B- lines can also be assessed for purity at the cytoplasmic level. A gene-based marker, designated as *nad7a\_del* (derived from the mitochondrial gene *nad7*) can easily differentiate between the male sterile line and its maintainer (Sinha *et al.*, 2015).

#### **D. Potential Role in Breeding Two-line Hybrids**

The use of temperature-sensitive male sterility (TGMS) (see section VI) could simplify the technology, steps and cost associated with pigeonpea hybrid production. A two-parent hybrid scheme would be used to produce hybrids, instead of the three-line system (A, B, R) currently used for CMS hybrids. The identification of putative candidate gene(s) or the loci controlling fertility reversal (male sterile to male fertile) in TGMS lines and the underlying molecular mechanisms will play an important role in breeding two-line hybrids. Transcript profiling and proteomics analysis will help in understanding the molecular basis underlying the fertility transition in the TGMS lines. The identification, cloning and transferring of major sterility gene(s) to other parental lines will benefit tremendously the two-line breeding efforts. Genetic analysis and fine mapping of TGMS trait are currently being undertaken by ICRISAT (R.K. (Saxena *et al.*, 2015a).

## X. COMMERCIALIZATION OF HYBRID PIGEONPEA TECHNOLOGY

### A. Standard Heterosis

Soon after the successful breeding of  $A_2$  and  $A_4$  CMS systems in pigeonpea, and establishing their stability, the process of breeding new A-lines and testing of experimental hybrids began. The CMS hybrids were compared with the best available cultivars in the early, medium, and late maturity groups. As expected, the range of standard heterosis (superiority of hybrid over control cultivar) within each maturity group was high. Hybrids recording above 40% standard heterosis in station trials were considered for multi-location and advanced level testing. In this review, no attempt has been made to summarize the performance of over 3,000 hybrids that were synthesized and evaluated over years in different experiment stations. However, the productivity recorded in some representative experimental hybrids is briefly discussed.

**1. Early-maturing Hybrids.** Since the early maturity group (121–150 days) has limited adaptation, it received relatively less importance while breeding hybrids. The original sources of earliness arose through natural mutational events. Cultivars such as Prabhat, Pusa Ageti, UPAS 120, Pant A 8 and so on were subsequently released and used to breed new cultivars, including ICPL 87, ICPL 151, ICPL 88039, Manak, Pusa 9 and so on (Singh *et al.*, 2005). The genetic variability in this maturity group is rather limited (Mudaraddi and Saxena, 2015), and relatively fewer heterotic hybrids were bred.

GTH 1 was the first early maturing CMS hybrid with  $A_2$  cytoplasm. This hybrid was bred at Gujarat Agricultural University (Gujarat, India), and it performed well on station, multi-location and on-farm trials. In multi-location trials, conducted from 2000–2003, GTH 1 recorded more than 50% yield advantage over the best control AKPH 4101 ( $1.2 \text{ t ha}^{-1}$ ). In the following year, GTH 1 recorded 25.3% standard heterosis in on-farm demonstrations and was subsequently released for cultivation. During its promotional mini-kit trials in some regions, the hybrid plants did not produce any pollen and remained male sterile, resulting in huge losses to farmers. This disaster, caused by instability of fertility restoration, led to the withdrawal of hybrid GTH 1 by the state government.

At ICRISAT, the first set of early maturing hybrids carrying  $A_4$  cytoplasm was evaluated in multi-location trials for four consecutive years (Table 3.11). Based on the mean performance, hybrids ICPH 2433, ICPH 2438, and ICPH 2383 were found promising, with 54%, 42%, and 36%

**Table 3.11.** Yield and standard heterosis of CMS pigeonpea hybrids, representing three major maturity groups, recorded in multi-location trials across India.

Maturity group	Hybrid number	Locations	Mean yield (kg ha <sup>-1</sup> )	Standard <sup>z</sup> heterosis (%)
Early	ICPH 2433	25	2,306**	54
	ICPH 2438	25	2,127**	42
	ICPH 2363	25	2,048**	36
Medium	ICPH 3491	18	2,919**	57
	ICPH 3497	18	2,686**	44
	ICPH 3481	18	2,637**	41
Late	ICPH 2307	05	2,855**	53
	ICPH 2306	05	2,600**	39
	ICPH 2896	05	2,579**	38

<sup>z</sup> – Superiority over the best control cultivar in the trial

\*\* – significantly different from the corresponding control variety at  $p < 0.01\%$

Source: Courtesy of Dr. K. B. Saxena.

superiority, respectively, over the early maturity popular cultivar UPAS 120. The highest mean yield of 2.3 t ha<sup>-1</sup> was recorded by hybrid ICPH 2433 (Table 3.11, Figure 3.1). The estimates of unit productivity (yield ha<sup>-1</sup>day<sup>-1</sup>) also showed that the hybrids (17.1–22.2 kg ha<sup>-1</sup>day<sup>-1</sup>) were far superior to the control (12.52 kg ha<sup>-1</sup> day<sup>-1</sup>; data not shown). This information suggested that the hybrids were more efficient in dry matter production and accumulation in the grains.

Recently, some promising genotypes that mature below 90 days (designated as ‘super-early’ types) have been bred at ICRISAT (Vales *et al.*, 2012). These lines exhibit relatively less sensitivity to photo-periodic changes, and can be grown successfully at places located at high altitudes and latitudes, or at places where the days are longer, thus offering potential for the expansion of pigeonpea cultivation to new niches. Currently, efforts are under way to breed hybrid parents in the super-early maturity group. Super-early hybrids would allow the exploitation of heterosis for greater and stable yields during a short season (around 90–100 days). The incorporation of super-early pigeonpea open pollinated cultivars or hybrids in crop rotations with cereals, in northern latitudes in India and elsewhere, could be beneficial to improve soil fertility and structure, human nutrition and economy.

**2. Medium- and Late-maturing Hybrids.** The largest area under pigeonpea cultivation is represented by the medium maturity group (161–200 days). It received priority in breeding new hybrids, and at



**Figure 3.1.** Short duration non-determinate pigeonpea CMS hybrid ICPH 2433 at the flowering stage. ICRISAT, Telangana, India. Source: Courtesy of M.I. Vales.

present this group has the largest number of A- and R- lines (Saxena *et al.*, 2014a), which has allowed the development of over 3,000 hybrid combinations. As expected, the hybrids demonstrated a large variation for standard heterosis but, interestingly, about 10% of them exhibited in excess of 30% heterosis. It is believed that some of the hybrids, such as ICPH 3491 (57% heterosis), ICPH 3497 (44% heterosis), and ICPH 3481 (41% heterosis), which performed consistently well in diverse environments (Table 3.11), can benefit farming communities.

Traditional long-duration (>250 days to maturity) pigeonpea types have a strict short day photoperiod requirement for the induction of flowering. This restricts their adaptation to areas where the day length is about ten hours. The adoption of this group is limited to deep soils with high moisture holding capacity, and occupies a large area. In this group, the potential of hybrids is also high, but not much research has been carried out with respect to the exploitation of heterosis.

Some hybrids, such as ICPH 2307 (2.9 t ha<sup>-1</sup>, 53% heterosis), ICPH 2306 (2.6 t ha<sup>-1</sup>, 39% heterosis), and ICPH 2896 (2.6 t ha<sup>-1</sup>, 38% heterosis), hold promise (Table 3.11).

### B. Release of the World's First Commercial Legume Hybrid

The first commercial CMS-based pigeonpea hybrid, ICPH 2671, was produced by crossing a restorer line, ICPL 87119, designated as 'ICPR 2671', with a male sterile line, ICPL 2043. The hybrid has non-determinate growth, is medium maturing (164–184 days), is tall (210–226 cm) and has profuse branching. It is highly resistant to wilt and sterility mosaic diseases. ICPH 2671, by virtue of its greater root mass and depth, also recovers easily from short spells of drought. The hybrid has also demonstrated high survival (88%) under waterlogging. In multi-location trials conducted from 2005–2008, the mean yield of ICPH 2671 ranged from 2–2.7 t ha<sup>-1</sup> and, on average, it recorded 35% superiority over the check cultivar Maruti (2 t ha<sup>-1</sup>) (Table 3.12) in the 'All India Coordinated Trials'.

In 1,829 pre-release on-farm trials, conducted by ICRISAT and ICAR in five provinces and using farmers' cultural practices, the hybrid ICPH 2671 (1.4 t ha<sup>-1</sup>) produced 52% more than the local check (954 kg ha<sup>-1</sup>). In the state of Maharashtra, the largest number (782) of trials were conducted, and ICPH 2671 produced 35% more yield than the check cultivar Maruti (Table 3.13). Considering its overall performance, the hybrid ICPH 2671 was released for general cultivation in the state of Madhya Pradesh in 2010 (Saxena *et al.*, 2013a). This hybrid matched well with the popular cultivar 'Asha' in various seed quality, de-hulling, and organoleptic parameters (Sawargaonkar, 2011).

**Table 3.12.** Yield and standard heterosis of the cytoplasmic male sterile medium maturity pigeonpea hybrid ICPH 2671 in comparison with cultivar Maruti, planted across India in multi-location trials from 2005–2008.

Year	Locations (no.)	Yield (kg ha <sup>-1</sup> )		Standard heterosis (%)
		ICPH 2671	Control	
2005	5	3,138**	1,855	69
2006	5	2,694**	2,066	30
2007	11	2,702*	2,140	26
2008	22	2,022*	1,746	16
Mean		2,639	1,952	35

\*\*\*- significantly different from the control variety at  $p < 0.05$  and  $p < 0.01\%$ , respectively

**Table 3.13.** Yield of medium maturity cytoplasmic male sterile pigeonpea hybrid ICPH 2671 and popular cultivar Maruti recorded from on-farm trials spread over four states in India in 2008 and 2009.

State	Farmers (no.)	Range for yield (kg ha <sup>-1</sup> )		Mean yield (kg ha <sup>-1</sup> )		Standard heterosis (%)
		Hybrid	Control	Hybrid	Control	
Maharashtra	782	760–4,000	660–2,900	969	717	35
Andhra Pradesh	399	701–2,900	458–2,100	1,411	907	56
Jharkhand	288	934–2,850	784–2,222	1,460	864	69
Madhya Pradesh	360	1,111–3,358	890–3,000	1,940	1,326	46
Mean				1,445	954	52

Source: NGOs and ICRISAT.



**Figure 3.2.** Medium duration non-determinate pigeonpea CMS hybrid ICPH 2740 at podding stage. Jalgaon, Maharashtra, India.



After the release of ICPH 2671, two more pigeonpea hybrids ICPH 3762 and ICPH 2740 (Figure 3.2) were also released in India. Their yield advantage over popular cultivars was above 40% in most farmers' fields (Tables 3.14 and 3.15). According to recent estimates, in 2015, CMS-based pigeonpea hybrids were grown on over 150,000 hectares in central and southern India. With a conservative estimate of 25% hybrid yield advantage, the replacement of inbred cultivars with hybrids will add about 30,000 tons of additional grain to the national pigeonpea production. The productivity levels recorded by the three hybrids were extremely encouraging, and it is expected that large-scale adoption could lead to a breakthrough in the national production and productivity of pigeonpea in India. Expansion of pigeonpea hybrids to other areas is also encouraging (i.e. Myanmar (Kyū *et al.*, 2011)).

**Table 3.14.** Yield and standard heterosis of medium maturity cytoplasmic male sterile pigeonpea hybrid ICPH 3762 and popular cultivar Asha obtained from on-farm trials conducted in four districts of Odisha, India, in 2013.

District (in Odisha)	Farmers (no.)	Yield (kg ha <sup>-1</sup> )		Standard heterosis (%)
		Hybrid	Control	
Kalahandi	72	2,000	803	149
Rayagarh	28	2,290	695	229
Naupada	21	1,734	1,230	41
Boudh	12	803	662	21
Bolangir	11	1,804	676	167
Mean		1,726	813	112

Source: Courtesy of Dr. K. B. Saxena.

**Table 3.15.** Yield and standard heterosis of medium maturity cytoplasmic male sterile pigeonpea hybrid ICPH 2740 and popular cultivar Asha obtained from on-farm trials conducted in four states in India from 2009 to 2011.

State	Farmers (no.)	Yield (kg ha <sup>-1</sup> )		Standard heterosis (%)
		Hybrid	Control	
Maharashtra	230	1,525	975	56
Andhra Pradesh	47	1,999	1,439	39
Gujarat	40	1,633	1,209	35
Madhya Pradesh	13	1,874	1,217	54
Mean		1,758	1,210	45

Source: Saxena (2016). Reproduced with permission of *Indian Journal of Genetics*.

### C. Hybrid Seed Production Technology

Standard recommended field isolation distances for seed production of pigeonpea CMS hybrids have not yet been established. Even for inbred cultivars, recommendation for certified seed production have not been standardized, and several options have been suggested. These include, 100 m (Tunwar and Singh, 1988), 180 to 360 m (Ariyanayagam, 1976), 200 m (Agarwal, 1980), and 300 m (Faris, 1985). Based on the information from different research stations in India, ICRISAT recommended an isolation distance of 500 m for the production of both certified as well as breeder seed of pigeonpea hybrids (Saxena, 2006) and, so far, the results are encouraging.

Another important consideration in selecting isolation plots is their natural habitat. It has been observed that seed production plots located closer to wild bushes, fruit or other flowering trees, and small natural or artificial water bodies give the best pod setting on the male sterile plants. It is believed that such conditions are conducive for harbouring and survival of the insects responsible for cross-pollination.

In addition to site selection, the adoption of efficient field plot techniques is also important for optimizing hybrid yields. The main focus in designing the field layout should be to make fresh pollen available for as long as possible. This will ensure more visits of the pollinating insects, to enhance pod setting on male sterile plants. A row ratio of four females : one male is recommended (Saxena, 2006) for the seed production of A-lines, as well as hybrids.

The two released hybrids ICPH 2671 and ICPH 2470 were chosen for on-farm seed production (A × R) in four states and, on average, the yields ranged between 1,000–1,500 kg ha<sup>-1</sup>. The seed production program organized in the state of Madhya Pradesh (Table 3.16) showed that this state has excellent ecology, conducive for hybrid pigeonpea

**Table 3.16.** Main areas in India suited for cytoplasmic male sterile pigeonpea hybrid seed production (A × R) based on accomplished yields in farmers' fields, 2008–09.

State	Locations	Mean yield (kg ha <sup>-1</sup> )	Main recommended areas	Yield in recommended areas (kg ha <sup>-1</sup> )
Madhya Pradesh	6	2,055	Tikamgarh, Seoni, Indore	2,602
Andhra Pradesh	5	1,255	Nizamabad, Medak, Medchal	1,404
Gujarat	3	1,558	Dhagandra, Vadali, Halvad	1,558
Mean		1,623		1,855

seed production, and it has the potential to become a hub for hybrid seed production in pigeonpea.

Seed storage is also critical, and needs fair attention to avoid losses caused by bruchid (*Callosobruchus maculatus*). Bruchid infestation often leads to seed quality deterioration, including physical damage and germination. Vales *et al.* (2014) reported that the use of *Purdue Improved Cowpea Storage* bags significantly reduced bruchid damage, and also preserved germination of pigeonpea seeds.

In pigeonpea, raising two crops in a year for field oriented grow-out quality testing is not possible, due to long generation turnover time. Alternatively, a genomics approach based on SSR was developed and implemented at ICRISAT to carry out quality tests (RK (Saxena *et al.*, 2010)). This technology has so far been established for three pigeonpea hybrids, ICPH 2671, ICPH 2740 and ICPH 2438. These assays can be used for reliable assessment of hybrid seed purity within the commercial seed lots of the hybrids. Since commercial application of this technology includes a large number of seeds samples, an alternative cost-effective approach, involving single nucleotide polymorphism (SNP), has also been developed.

#### **D. Economics of Hybrid Seed Production**

M.K. Saxena *et al.* (2011) estimated that the total production cost of hybrid ICPH 2671 seed on one hectare plot was Rs 26,395 (US\$ 1 = Rs 66 on 2015.12.24), excluding the rental value of land. From this plot, a total of 1,440 kg hybrid seed was produced, which yielded net profits of Rs 70,000 ha<sup>-1</sup>. Using these estimates, the hybrid seed cost at farm gate was Rs.18.85 (= US\$ 0.29) kg<sup>-1</sup>, which is 20–25% higher (due to more labour and seed cost) than that of inbred cultivars. The production statistics of hybrid pigeonpea are comparable with other hybrid field crops (Singhal, 2013), and this will raise the confidence of seed producers in opting for hybrid pigeonpea seed business.

In farmers' fields, a grain yield of 2–3 t ha<sup>-1</sup> by cultivating a hybrid crop is not uncommon, with estimated net production advantage of 1–1.2 t ha<sup>-1</sup> over the local cultivars. With this level of productivity from the cultivation of hybrids, farmers will fetch an additional 30–50% profit (Rs 40,000–75,000 (= US\$ 605–1,135) ha<sup>-1</sup>). It has been also observed that many farmers have opted for modern production technologies, and consider agriculture as a challenging but potentially profitable business. The attractive market prices and high demand have encouraged them to invest and reap more profits from pigeonpea. During the on-farm promotion of hybrids during the past three years, a number of such farmers recorded exceptionally high yields (up to 4.5 t ha<sup>-1</sup>) from

hybrids, with 40–50% superiority over the control, leading to additional profits. These yields were obtained under well-drained fields with irrigation and good fertilization, weed and insect management.

To take advantage of this technology on a large scale, the availability of sufficient quantities of quality hybrid seed is the number one prerequisite. A seed-to-seed ratio of 1 : 200/300 for pigeonpea hybrids means that hybrid seed can be obtained with a little effort, and a productive seed chain can be established to address seed requirements. Overall, pigeonpea CMS hybrid technology has reached a mature stage, and can be compared with other crops as far as their levels of realized standard heterosis, hybrid ( $A \times R$ ) seed yields and profitability are concerned. To meet the current domestic demand, India annually imports about 500,000 t of pigeonpea (DES, 2015), and the authors believe that with 30–40% yield advantage, this deficit can be reduced gradually with increased adoption of hybrids.

## XI. OUTLOOK

Hybrid seed technology was conceptualized and flourished in the USA in the early part of the 20th century, and the first crop to benefit from this breakthrough was maize. Gradually, this technology reached farmers' fields, as its large-scale and economically viable hybrid seed production technology evolved. Initially, de-tasseling (physical removal of male reproductive parts from female rows) and wind pollination were used for producing hybrid seed. Subsequently, male sterility systems were incorporated into the female parents, and this made hybrid seed production much easier and economical.

The impact of hybrid technology in combating global hunger has been immense. The six-fold increase in maize yields in the USA (Troyer, 1991) can easily be attributed to breeding and adoption of high yielding hybrids. Similarly, in China, the adoption of rice hybrids has enhanced the mean crop productivity by three folds. Significant yield gains associated with the exploitation of hybrid vigour have also been recorded in other crops, such as sorghum, pearl millet, cotton, sunflower, safflower, castor, and various vegetables and fruits. The benefits of hybrid technology, however, have eluded legume crops. Male sterility systems exist in various food legumes (Table 3.17).

The principal aspects precluding the production of hybrids in legumes are: low pollen movement from the male to female (low out-crossing rates), due mainly to flower morphology and/or low participation of insect pollinators; unstable male-sterility systems; and scarcity of good maintainers and restorers. These aspects have limited the

**Table 3.17.** Better parent heterosis for yield, male sterility systems, and out-crossing rates recorded in several food legumes.

Crop common name	Scientific name	Better parent heterosis for yield <sup>z</sup> (%)	Male sterility system <sup>y</sup>	Out-crossing <sup>x</sup> (%)
Black gram	<i>Vigna mungo</i> L.	<121	GMS	NA
Chickpea	<i>Cicer arietinum</i> L.	<153	GMS	<2 <sup>w</sup>
Common bean	<i>Phaseolus vulgaris</i> L.	<114	CMS	<10
Cowpea	<i>Vigna unguiculata</i> L.	<89	GMS, CMS	<15
Faba beans	<i>Vicia faba</i> L.	<130	CMS	<84
Lentil	<i>Lens culinaris</i> Medik.	<145	GMS	<6
Mung bean	<i>Vigna radiata</i> L.	<121	GMS	<13
Pea	<i>Pisum sativum</i> L.	<162	GMS	<2
Pigeonpea	<i>Cajanus cajan</i> (L.) Millsp.	<200	GMS, CMS, TGMS	<70
Soybean	<i>Glycine max</i> L.	<77	CMS	<3

<sup>z</sup> Better parent heterosis for yield ranged from negative to positive values. The highest values found in the literature are shown for illustration purposes.

<sup>y</sup> CMS: cytoplasmic nuclear/genetic male sterility; GMS: genetic male sterility; TGMS: temperature-sensitive male sterility.

<sup>x</sup> Highest values found in the literature.

<sup>w</sup> Open flower mutants could reach close to 6% out-crossing.

Source: Adapted from Palmer (2011).

exploitation of heterosis in legume breeding, and make hybrid development and large-scale hybrid seed production in most food legumes not very efficient/easy, nor economically viable.

Faba beans, soybean, and pigeonpea have the most attractive features for hybrid production. Faba beans have high heterosis for yield and enough out-crossing, the main bottlenecks to hybrid production being related to the instability of the male sterility system and shortage of good restorers and maintainers (Bishnoi *et al.*, 2012). Soybean has high heterosis for yield, stable CMS, good maintainers and restorers (Palmer *et al.*, 2001). Soybean CMS hybrids have been developed, but low pollen movement from the male to the female makes large-scale hybrid seed production challenging (Palmer *et al.*, 2011). Pigeonpea has high heterosis for yield, sufficient natural cross-fertilization, a stable male-sterility system (CMS), good maintainers and restorers, and cost-effective and efficient hybrid seed production. All this has facilitated the successful development and commercialization of CMS hybrids, and has opened a new chapter in legume breeding.

A perusal of global pigeonpea production statistics shows that there has been a serious productivity stagnation in the last six decades. The recent success in breeding commercial CMS hybrids has shown the way forward to combating the persisting yield plateau. This was

achieved by ICRISAT through the development of a stable CMS system, and then breeding and releasing the world's first commercial legume hybrid, ICPH 2671, in 2010, with full support from ICAR. This hybrid registered an on-farm mean yield advantage of 52% over the best cultivar in four states. This was followed by the release of two more pigeonpea hybrids, ICPH 3762 and ICPH 2740, in India in 2014 and 2015, respectively.

In fact, the heterotic effects in pigeonpea hybrids are visible right from the early stages, with quicker germination and faster seedling growth. This ability enhances the early establishment of seedlings, and enables hybrids to compete well with both intercrops and weeds. Pigeonpea hybrids have shown better environmental buffering and an extra degree of resilience to tolerate certain biotic and abiotic stresses, to provide much-needed stability to production.

More studies are needed to understand the nature of genotype  $\times$  environment interaction (Saxena and Raina, 2001), the physiological aspects of yield determination (Lawn and Troedson, 1990) and their relationships with parents. To achieve this, data on dry matter production, its partitioning and harvest index needs to be generated, and the role of grain yield-contributing traits has to be better understood. The availability of substantial levels of additive and non-additive genetic variances for grain yield, and vast resources of primary and secondary gene pools, provide tremendous scope for commercial exploitation of hybrid vigour in pigeonpea.

The three released CMS pigeonpea hybrids have demonstrated huge on-farm yield advantages over the best local checks, under diverse environments and cropping systems. This hybrid advantage is comparable with those recorded in crops like rice or cotton, where hybrid seed technology has already created a big impact. Besides seed yield, the hybrid technology is also capable of addressing issues such as nutrition, drought, stability, soil health, and so on. In this context, maize, rice, pearl millet, and sorghum are good examples where breeding of new hybrids involve a balance between productivity and nutrition. This will require a robust hybrid parent breeding program in pigeonpea, with sufficient nuclear and cytoplasmic diversity and full support from genomics, physiologists, and agronomists.

The profitability from hybrids both in seed business and commercial cultivation is high enough to attract seed producers and cultivators, although the key to success in such an endeavour lies in harnessing the complementary skills of various research partners, specializing in different disciplines. At present, the hybrid pigeonpea program is well knit with various ICAR institutions, state universities, and public and

private seed sectors. The main activities include sharing of technology, knowledge, training of technical/scientific staff, and organizing field days and formal/informal discussions. The partnership of ICRISAT with the private seed sector has been fruitful in sharing breeding materials and resource mobilization under the umbrella of 'Hybrid Parents Research Consortium'. These partnerships will be strengthened further, to take the hybrid pigeonpea technology to the doorsteps of the farmers.

Most of the commercial pigeonpea hybrid efforts have been done in India in the medium maturity class intended for the processing market (dry split peas), using the A<sub>4</sub> cytoplasm. Additional efforts are required to diversify the cytoplasm type, to improve tolerance to biotic and abiotic stresses of parental lines, and to extend commercial production to other maturity groups and market uses (e.g. vegetable pigeonpea). Internationally, expansion of pigeonpea using less photoperiod-sensitive lines will contribute to improving the nutrition and health of the growing human and animal populations, as well as diversifying food options and aiding in environmental preservation and improvement.

The success of the pigeonpea CMS system depends, in great part, on the use of pure parental lines, maximization of heterosis, professional implementation of the CMS hybrid technology, constant training, monitoring and evaluation, efficient formal and informal seed production systems and promotion/marketing efforts.

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