



Review article

From concept to field: evolution of hybrid pigeonpea technology in India

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Abstract

The role of hybrid vigour in the enhancement of productivity is well recognized. Its application, however, has been restricted to a few crops and legume breeders could never take its advantage due to various seed production issues. Recently, plant breeders have succeeded in creating the world's first commercial hybrid in a food legume, popularly known as red gram or pigeonpea [*Cajanus cajan* (L.) Millsp.]. This was possible due to success in breeding a stable cytoplasmic nuclear male sterility (CMS) system and exploitation of its partial natural out-crossing. This development has provided golden opportunities to break the decades-old yield plateau in this crop. Among the hybrid combinations evaluated, ICPH 2671 and GTH 1 were found most promising. In the on-farm trials ICPH 2671 recorded 46.5% superiority over the best available cultivar Maruti; while GTH 1 was 33.9% superior to the control, and it was released in Gujarat state in 2004; but for some reasons associated with its stability of fertility restoration it failed to reach the level of commercialization. The outstanding performance of hybrid ICPH 2671 led to its release by both, a private seed company as 'Pushkal' and by the state Government of Madhya Pradesh as 'RV ICPH 2671' in 2010. The journey of the evolution of hybrid pigeonpea technology was fascinating and challenging. It took a long time of over 39-years from its conceptualization to finally reaching Indian farmers. The impact of this breakthrough was visible when two more hybrids ICPH 2740 and ICPH 3762 were recently released in India. Since it is a path breaking research among food legumes, an attempt has been made here to archive the milestones of this historic plant breeding event.

Key words: *Cajanus cajan*, cytoplasmic nuclear male sterility, hybrid, out-crossing, pigeonpea

Introduction

Commercial exploitation of two genetic phenomena - the dwarfing genes in rice and wheat and hybrid vigour

in maize have saved the world from malnutrition and hunger. The maize yields in the USA increased five folds from a meager 25 bushels/acre in 1930 to 140 bushels/acre in 1998 (Trayer, 1991; Crow, 1998). This, beyond doubt, happened due to untiring and continuous efforts of scientists in developing improved hybrids and their production technology. The process of recombination and selection that contribute to yield and stability is still continuing and contributing towards further enhancement of yield and adaptation of maize hybrids in different parts of the world. The phenomenal success of hybrid maize led breeders to use this technology in other crops like cotton, sorghum, pearl millet, sunflower, brassica, safflower and various horticultural crops. At present efforts are also being made to bring together the genes responsible for dwarfing and hybrid vigour in wheat and rice with a target of harvesting more grains per unit of land area. After the adoption of hybrid technology in various crop species, now it is the turn of grain legumes. This endeavour started with research in faba bean in the west (Bond et al. 1966) and pigeonpea in India (Saxena, 2008). Efforts are also being made to develop hybrid breeding technology in soybean (Palmer et al. 2010). The commercial success in this direction, however, has come only from pigeonpea or red gram [*Cajanus cajan* (L.) Millsp.], a popular protein-rich pulse crop of Asia, Southern and Eastern Africa, and the Caribbean islands. The recent release of three commercial hybrids in India has established the hybrid technology in pigeonpea also. This paper briefly reviews the important research and developmental initiatives undertaken by International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) and Indian Council

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of Agricultural Research (ICAR) in the last 39 years that led to the successful development and adoption of hybrid pigeonpea technology.

Background information

Pigeonpea is a drought tolerant short-lived perennial pulse, cultivated on over 4 m ha (IIPR, 2013) at subsistence level in India, mainly as an intercrop. Its decorticated dry splits, whole grains, and fresh peas are consumed with cereals as a protein supplement. Besides fixing atmospheric nitrogen, it is also recognized for its ability to release soil-bound phosphorus, adding organic matter, and recycling of valuable nutrients in the soil (Saxena, 2008).

Over a half-century of productivity stagnation in pigeonpea

In India the formal genetic improvement of pigeonpea started in 1931 with pure line selection within landraces for disease resistance, plant type and seed yield (Ramanujam and Singh; 1981). Green et al. (1979, 1981), while reviewing the accomplishments and constraints of the global pigeonpea improvement programmes, concluded that almost all the breeding methods traditionally recommended for self-pollinated crops have been tried for the genetic enhancement of pigeonpea with limited success. Khan (1973), Sharma and Green (1977) and Onim (1981), however, had other ideas and decided to use the inherent partial

natural out-crossing of the crop for population improvement; and in spite of about 2% yield gain per cycle of selection in Kenya (Onim, 1981), this approach did not take-off as a standard breeding method in pigeonpea. Consequently, pure line breeding remained a major instrument for the genetic improvement of this pulse and in the past 4-5 decades over 100 pure line cultivars were released (Singh et al. 2005). These cultivars although exhibited significant genetic advances for traits such as earliness, plant type, disease resistance and market-preferred traits such as seed size and colour; but without any marked improvement in the productivity that remained unchanged over the past half century at around 700-800 kg/ha (Fig. 1).

Yield stagnation in pigeonpea has been a matter of concern, particularly in view of increasing population and decreasing per capita protein availability in the country, which is about 25% short of the prescribed standard of 42 g protein/head/day (NIN, 2010). According to Shalendra et al. (2013) the pulse production has registered an impressive growth of 3.47% during 1990-2009; but it was insufficient to take care of the national requirements. Further, high population growth and low crop productivity were considered the two key constraints in meeting the challenges of the national nutritional security. In this context it is reasonable to assume that a quantum jump in productivity is the only viable solution and

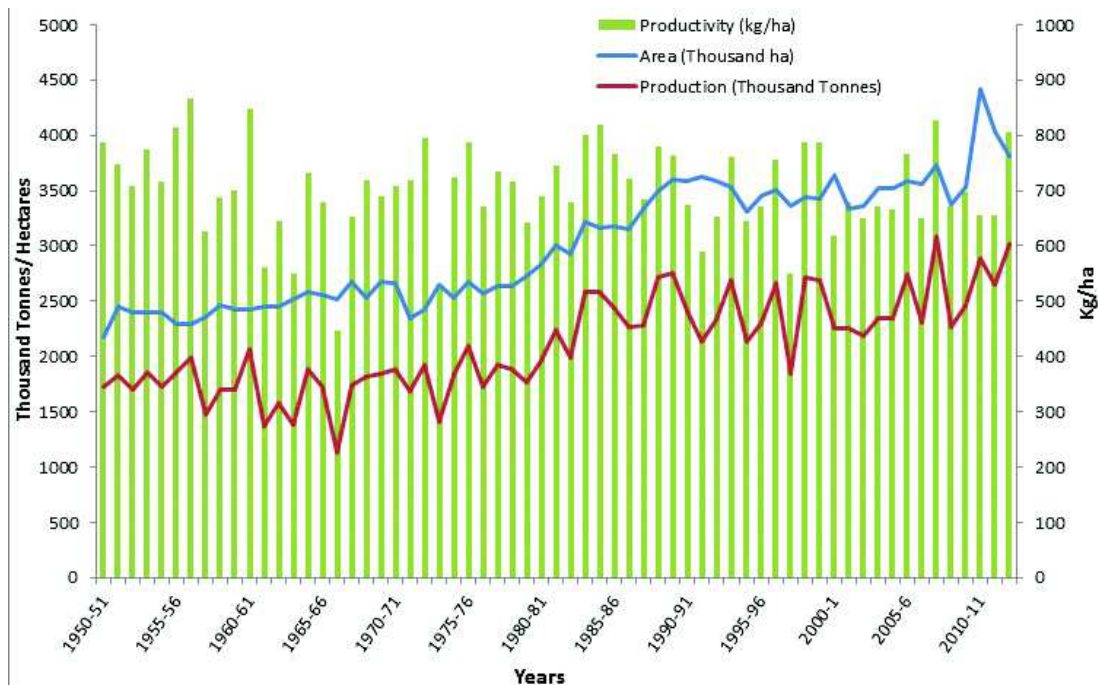


Fig. 1. Area, production and yield (kg/ha) of pigeonpea in India

hybrid technology offers great promise. The recent releases of three commercial hybrids have generated a lot of optimism among pigeonpea scientists and policy makers towards breaking the decades-old productivity barrier in this crop.

Significance of maturity groups in pigeonpea

Perhaps pigeonpea is the only pulse crop which is credited to have the largest genetic diversity for maturity among cultivated types with almost a continuous variation from 75 to >250 days. Broadly, it has five maturity groups – super early (<90 days), extra early (110-120 days), early (140-160 days), medium (180-200 days), and late (> 250 days). Of these, the super early group is of recent origin (Vales et al. 2012) while the rest being there for a long time. Each maturity group has its own significance in relation to cropping system. This classification is so strong that one group cannot easily replace the other, as far as its adaptation is concerned. The early maturing pigeonpea has a number of production niches (Saxena et al. 2014a), while the medium and long duration types are adapted to subsistence agriculture in intercropping systems. The hybrid breeding programmes, therefore, should be targeted towards specific cropping system and maturity group. To reduce genotype x environment interaction and enhance selection efficiency Byth et al. (1981) recommended that pigeonpea breeding activities should be carried out under the cropping system for which the end product (cultivar) is targeted.

Photo-period sensitivity and ratooning

Pigeonpea is known to be a quantitative short day plant. Its perennial growth habit, deep (3-4 m) and strong tap root system help in overcoming short spells of abiotic stresses by regenerating the plant growth, popularly called as 'ratoon growth', by utilizing its food reserves. By nature, pigeonpea plants of all the maturity groups are capable of ratooning, but the long duration types ratoon better than early types. The perennial growth habit of pigeonpea is also helpful in hybridizing the diverse (early with late) maturity types. This is possible due to emergence of second and third flushes of flowers within the same crop season (Saxena et al. 1976). In pigeonpea so far there is no report of the existence of any true photo-insensitive genotype. Wallis et al. (1981), however, reported that the earliest flowering types showed least sensitivity to photo-period. Like other crops in pigeonpea also, the influence of day/night temperature in the manifestation of photo-period effects is quite strong

and low temperature has been found to offset the effect of photo-period to some extent (Turnbull et al. 1981). An important aspect of photo-period sensitivity is induction of early flowering in the sensitive materials, when sown under inductive (short day) conditions. This not only has telescopic effect on flowering time but also reduces plant height and biomass (Spence and Williams 1972). This helps in the cultivation of pigeonpea as a *rabi* (post-rainy) season crop for generation advance and seed multiplication of elite lines.

Natural out-crossing

Most pulses are known for their cleistogamous flowers and self-pollination. It is, however, not entirely true for pigeonpea; and the first report on its partial natural cross-pollination was published by Howard et al. (1919). Subsequently, a number of reports have appeared in literature (see review by Saxena et al. 1990). Some efforts have also been made to understand the factors responsible for out-crossing in this crop; and frequent insect visits were recognized as the prime cause (Pathak 1970; Williams 1977; Onim 1981; Zeng-Hong et al. 2011). In these studies over two dozen insect species were found foraging on pigeonpea flowers, but out-crossing was affected by only a few of them. Williams (1977) reported that in Patancheru (India) *Megachile bicolor* and *M. conjuneta* were the major pollinators; while Onim (1981) reported that cross-pollination in pigeonpea was primarily a function of foraging by *Apis mellifera* and *Megachile* species in Kenya. Recently, Zeng-Hong et al. (2011) reported that in Yuanmou (China) the insects belonging to *Megachile* spp., *Xylocopa* and *Apinea* spp. were most frequent visitors to pigeonpea fields (Table 1) and they were very active in the collection and transportation of pollen grains from one plant to another, and thereby,

Table 1. Proportions of four important insect species recorded in pigeonpea fields and their activity on flowers in Yuanmou (China)

Pollinator insect species	Proportion of insects present (%)	Flowers visited/10 min	Stay on each flower (sec)
<i>Megachile</i> spp.	49.04	4.5	6.6
<i>Xylocopa</i> spp.	11.06	8.9	2.4
<i>Apinae</i> spp.	10.10	2.5	14.8
<i>Catopsilia</i> spp.	7.21	4.0	12.4
Others (15 spp.)	22.59	-	-

Source: Zheng-Hong et al. (2011)

affecting cross-fertilization. They observed that the pollinating insects were more frequent on male fertile plants with a mean of 4.8 visits/10 minutes as compared to 2.8 visits/10 minutes on the male sterile plants. They attributed this behaviour of the insects to the presence of (i) chemicals such as flavone and flavonol, (ii) nectar and (iii) specific scent that is produced by mature pollen grains in the fertile plants. They further reported that even with 50% less insect visits, the male sterile plants produced cross-pollinated seed yield (384 g/plant) similar to that of more frequently visited fertile plants (357 g/plant). These observations indicated that very high insect activity may not be essential to produce reasonably good quantities of hybrid seed. Since there is no wind pollination in pigeonpea (Kumar and Saxena 2010), the entire cross-pollination can be attributed to insect activity. In this situation it is logical to assume that the population of pollinating insects cannot be the same across the locations and time of the year. Therefore, a large variation (0-70%) in natural out-crossing has been recorded (Saxena et al. 1990). Besides this, some external factors are also known to affect the extent of natural out-crossing. These include speed and direction of wind (Bhatia et al. 1981), extended periods of stigma receptivity (Dalvi and Saxena 2009), floral morphology of genotype (Byth et al. 1982), and immediate surroundings of pigeonpea plots (R. V. Kumar, personal communication).

Evolution of hybrid pigeonpea technology

Hybrid technology flourished in the 20th century through open-pollinated crops such as maize, pearl millet etc. and benefitted millions of farmers; but for pulse breeders it remained a challenge. At ICRISAT and ICAR, the hybrid pigeonpea breeding programme started from a scratch and it passed through many cycles of successes and failures. Finally, the success in this endeavour was achieved, when pigeonpea hybrids GTH 1 (in Gujarat) and ICPH 2671 (in Madhya Pradesh) were released for cultivation.

In this article some major events of research and developmental activities that led to the development of hybrid technology are highlighted. The author, who was associated with this programme from the take-off point, has divided this review into four broad phases for the sake of clarity and has covered almost entire work of four decades. The Phase I summarize the initial research activities and covers the period up to the development/release of the first GMS-based pigeonpea hybrid ICPH 8. The second

phase deals with the process of achieving the breakthrough by breeding two viable CMS systems. The information about breeding and release of CMS-based hybrids and their seed production technology has been incorporated in Phase III. In the fourth Phase, the research and development plans for the production of second generation hybrids with potentially greater yields have been discussed. The important events of each phase along with some pertinent research data are illustrated in the following text.

Phase I (1974-1990): a giant step towards improving pigeonpea productivity

At ICRISAT a broad-based pigeonpea improvement programme was launched in 1974. The breeding team, jointly led by Dr. D. Sharma and Late Dr. John M. Green, decided that besides using traditional methods for breeding high yielding pure line cultivars to meet global needs, some non-conventional breeding approaches should be tried to overcome the constraint of persistent yield plateau. In this context, a decision was made to convert the constraint of partial natural out-crossing into an opportunity through heterosis breeding. Since this technology required a mechanism for mass hybrid seed production, as a first step, a programme was launched to look for a stable CMS source in germplasm that was sown at ICRISAT farm for a routine assessment and characterization exercise.

The first genetic male sterility (GMS) discovered

A thorough search of over 7000 germplasm accessions failed to meet the expectations of finding a cytoplasmic nuclear male sterility (CMS) for commencing a hybrid breeding programme, but instead, a source of genetic male sterility (GMS) was identified in a field collection from Andhra Pradesh. This GMS line was of medium (about 180 days) maturity, susceptible to both fusarium wilt and sterility mosaic diseases. The anthers were translucent and the male sterility was controlled by single recessive gene '*ms₁*' and most likely it evolved through spontaneous mutation (Reddy et al. 1978); and the recessive allele was maintained in the population through natural out-crossing. After the search of this GMS source, a few more GMS sources were identified at ICRISAT and ICAR centres at Akola and Pantnagar (see review by Saxena et al. 2010) but none of them was used in breeding hybrids.

Release of the first GMS-based hybrid ICPH 8

The breeding team knew that GMS was not a perfect platform for breeding commercial hybrids; but decided

to continue research in order to find answers to basic questions such as quantum of hybrid vigour available in the crop and secondly, if the limited natural out-crossing would be enough to produce large quantities of hybrid seed economically. Therefore, a programme was launched to transfer the male sterility gene to diverse elite lines.

By 1979, the first converted early maturing GMS line 'MS Prabhat (DT)' was bred using off-season facility and made ready for use in making the first set of experimental hybrids. At this time the male sterile line was shared with various ICAR centres, Agricultural Universities, and Maharashtra Hybrid Seed Company (Mahyco) to develop locally adapted hybrid technology. The entire hybrid breeding programme was coordinated jointly by ICAR and ICRISAT. Among the experimental hybrids tested, a short duration hybrid ICPH 8, synthesized by crossing MS Prabhat (DT) and an advanced breeding line ICPL 161, was found to be the most promising in station trials conducted from 1981 to 1983. Simultaneously, an exercise began to use this hybrid combination for developing its large-scale seed production technology.

In 1984, ICPH 8 was evaluated along with national check UPAS 120 in All India Co-ordinated Trials organized by ICAR. Seed yield of this hybrid over 94 trials, conducted over four years in three climatic zones, was 34.55% more than the control UPAS 120 (Saxena et al. 1992). This demonstrated both greater yield and stability of ICPH 8 over the inbred cultivars. With the out-standing performance in the Co-ordinated Trials, the hybrid was promoted by ICAR to pre-release on-farm testing in central zone. In this endeavour, the hybrid demonstrated >25% yield advantage in different districts of Maharashtra, Andhra Pradesh, and Gujarat states. These performances led to the release of ICPH 8 by ICAR in 1991 (Saxena et al. 1992). This endeavour clearly demonstrated the presence of commercially exploitable hybrid vigour in pigeonpea. In the seed production exercise also, it was observed that good hybrid yields of the order of 800-1000 kg/ha can be obtained through partial natural out-crossing.

Release of other GMS-based hybrids by ICAR

The primary GMS source (ms_1) was also simultaneously used in other breeding programmes of ICAR. This exercise resulted in the release of five more GMS-based hybrids. In 1993, Punjab Agricultural University, Ludhiana, developed pigeonpea hybrid PPH 4 (Verma and Sidhu 1995). In the state level multi-location trials conducted for two years, PPH 4 out-

yielded the control T 21 by a margin of 47.4%. In the All India Coordinated Trials also this hybrid exhibited 14% superiority over the best national check UPAS 120. In 1994, another pigeonpea hybrid CoH 1 was released by Tamil Nadu Agricultural University, Coimbatore. In 17 on-farm trials CoH 1 recorded 21% higher yield over control VBN-1 (Murugarajendran et al. 1995). Subsequently, two more pigeonpea hybrids, AKPH 4104 (in 1997) and AKPH 2022 (in 1998) were released for central India by PKV, Akola. These hybrids respectively recorded 30% and 64% superiority over the control cultivar (Wanjari et al. 1999). Interestingly, all the hybrids (non-determinate types) demonstrated large yield advantages in different zones (Table 2), but none of them could reach the level of commercialization, primarily due to the big issue of rouging fertile segregants within the female rows.

Table 2. List of GMS- based hybrids released in India

Hybrid	Origin	Year	Adaptation zone	Maturity group	Standard heterosis(%)
ICPH 8	ICRISAT	1991	Central	Early	35
PPH 4	Ludhiana	1994	North west	Early	14
CoH 1	Coimbatore	1994	South	Early	21
CoH 2	Coimbatore	1997	South	Early	35
AKPH 4104	Akola	1997	Central	Early	64
AKPH 2022	Akola	1998	Central	Medium	30

To assess the impact of GMS-based hybrid technology, ICRISAT appointed an 'impact assessment group' to record the views of seed companies, seed producing farmers, and hybrid cultivators in Maharashtra and Andhra Pradesh. The group concluded that "though the hybrid advantage in pigeonpea is salable and its cost of seed production is also within affordable limits, but the technology itself suffers from a major bottleneck, when it comes to large scale seed production" Niranjana et al. (1998). The hybrid advantage recorded in the GMS-based hybrids was encouraging and only seed production issue needed to be addressed. This was only possible if a stable cytoplasmic nuclear male sterility (CGMS or CMS), its maintainers and restorers were discovered.

Phase II (1991-2003): the major breakthrough in breeding CMS systems

In plants the CMS system is primarily conditioned by cytoplasm containing aberrant mitochondrial DNA,

commonly designated as 'sterile' (S) cytoplasm. In this case the extra nuclear genome of a genotype interacts with its nuclear genome; and in the presence of recessive non-restoring nuclear alleles (*frfr*), a male sterile phenotype is expressed. This form of male sterility can arise spontaneously or bred through wide hybridization. The complete CMS-based hybrid system involves three distinct genotypes - the male sterile female (A-) line with 'S' cytoplasm and recessive fertility (*frfr*) nuclear alleles; the maintainer (B-) line carrying fertile normal 'N' cytoplasm and the same recessive (*frfr*) nuclear alleles. The third parent, designated as restore (R-) line, contains dominant (*FrFr*) fertility restoring nuclear alleles and it is responsible for inducing male fertility in hybrid (A x R) plants. Further, depending on the number and type of fertility restoring nuclear genes and specificity of their interaction with mitochondrial genes, the expression of male fertility/sterility in hybrid plants could be total or partial. Sometimes such expressions are also affected by prevailing environmental conditions such as photo-period, temperature, or both.

Since extensive search in the pigeonpea germplasm did not yield any CMS system, elaborate plans were made to breed this system by integrating nuclear genome of the cultivated type with cytoplasmic genome of a wild relatives of pigeonpea. The first unsuccessful attempt in this direction was made by Reddy and Faris (1981) when they crossed *C. scarabaeoides* and *C. sericeus*, the wild relatives of pigeonpea, as female parent with a *C. cajan* line as male parent. Although in BC₁F₂ generation, some segregants exhibited male sterility that was inherited maternally, but unfortunately, these plants suffered with female sterility and abnormal (petloid) flowers. Hence, all the selections were discarded. Ariyanayagam et al. (1995) launched a targeted breeding programme to develop CMS using mutagenesis and wide hybridization approaches. Progenies of a cross between *C. sericeus* and a cultivar exhibited maternal inheritance for male sterility, but for some reasons, this trait could not be stabilized for use in hybrid breeding.

Success in breeding the first (A₂) CMS system

The first workable CMS system in pigeonpea was produced at Gujarat Agricultural University, SK Nagar by Tikka et al. (1997). It was derived from a naturally cross-pollinated (an off-type) plant observed in the

population of *Cajanus scarabaeoides* (L.) Thou., a wild relative of pigeonpea. This plant was partially male sterile and in its F₂ generation a number of segregants with complete male sterility were recovered. This male sterility was maternally inherited and perfectly maintained by a cultivar ICPL 288. It was designated as A₂ cytoplasm source. Subsequently, Saxena and Kumar (2003) also bred CMS lines by crossing *C. scarabaeoides* (as female) with cultivar ICPL 88039 (as male).

Breeding of another (A₄) CMS system

Another stable source of CMS was bred at ICRISAT by crossing *C. cajanifolius* with cultivar ICP 28 (Saxena et al. 2005a). In this cross all the hybrid plants were male sterile and it was maintained by backcrossing the hybrid plants with ICP 28. This CMS-inducing cytoplasm, designated as A₄ system, is being used in breeding hybrids at ICRISAT, ICAR and some public and private seed companies.

Fertility restoration

In search of good fertility restorers of A₄ CMS system, over 3000 testers were examined at ICRISAT and various ICAR centres. From the results it was concluded that i) plenty of fertility restorers were available in the germplasm, ii) the fertility restoring genes were distributed randomly with greater frequency in the medium maturing germplasm, and iii) in early maturity the fertility restoring genes were relatively less frequent (Dalvi et al. 2008; Saxena et al. 2014 a,b). Some early maturing restorers, identified during the study, could not produce stable hybrids with respect to their fertility restoration. A perusal of published reports on the genetics of fertility restoration of A₄ CMS system showed that either one or two dominant genes controlled the fertility restoration in F₁ generation with variable gene action. In a significant study Saxena et al. (2011) reported that the fertility restoration was stable across environments only when two dominant genes were present together in a single hybrid; and the hybrid combinations carrying either of the dominant genes were also fertile but not in all the environments.

Phase III (2003-2013): process of releasing the world's first commercial pigeonpea hybrid

Release of the first CMS-based pigeonpea hybrid GTH 1 in Gujarat

The first CMS-based pigeonpea hybrid GTH-1 was

developed at GAU, SK Nagar, and released by ICAR in 2004 for cultivation in Gujarat state. This hybrid was bred by crossing A₂ CMS line GT 288A with fertility restorer GTR-11. Based on yield trials conducted at multi-locations during 2000 to 2003, GTH 1 (mean yield 1830 kg/ha) recorded 32% yield superiority over the best local check (GT 100/101) (mean yield 1330 kg/ha). This hybrid is non-determinate in growth habit and early in maturity (140 days). Its flowers are yellow and seeds are large and white. In front-line demonstrations conducted in three districts in 2003, the hybrid recorded 25.3% yield advantage over popular control (Table 3). After multi-locational evaluation by ICAR, the hybrid GTH-1 was released for cultivation in Central Zone. Unfortunately, in spite of its high performance in farmers' fields, this hybrid could not be commercialized due to its inability to produce uniformly male fertile flowers and record good yields in different locations/environments.

Release of the first commercial hybrid in pigeonpea

Hybrid ICPH 2671 is the first ever commercial hybrid of any grain legume. It was produced by crossing a male sterile line ICPA 2043 with restorer ICPR 2671. The plants of ICPH 2671 are semi-spreading, non-determinate with profuse branching. It grows over two meter in height, matures between 164-184 days and contains 3.7-4.0 seeds/pod. The purple coloured seeds weigh between 10.5 to 11.2 g/100 seeds. ICPH 2671 has high level of resistance to both wilt and sterility mosaic diseases. In comparison to inbred cultivars the hybrid, by virtue of its greater root mass and depth, possesses greater ability to draw moisture from deeper soil profiles. Its fast root growth also helps in overcoming short spells of early season drought that is often encountered in July-sown rainfed crops. ICPH 2671 also recorded have high survival (88%) under water-logged conditions; and this was found to be related to its ability to utilize stored assimilates through anaerobic metabolism (Sultana et al. 2013).

During 2005-2008, ICPH 2671 was tested in multi-location trials and its mean performance in different years ranged from 2200 to 3183 kg/ha; and on average, it recorded 47% superiority over national check Maruti. In All India Advanced Hybrid Trials conducted at six locations in 2007, ICPH 2671 recorded 35% yield advantage over the control cultivar. In All India Coordinated Trials, the hybrid (2564 kg/ha) recorded 31% superiority over the control variety Maruti (1996 kg/ha). In 2009 and 2010, the hybrid was evaluated in on-farm locations in four provinces (Table

Table 3. Performance (yield kg/ha) of three hybrids in the on-farm trials

Hybrid	State	Farmers Hybrid (no.)	Hybrid yield	Control yield	Standard heterosis (%)
GTH-1	Gujarat	04	2673	1996	25
ICPH 2671	Maharashtra	782	969	717	35
	Andhra Pradesh	399	1411	907	56
	Jharkhand	288	1460	864	69
	Madhya Pradesh	360	1940	1326	46
	Total/mean	1829	1445	954	51
ICPH 2740	Madhya Pradesh	13	1814	1217	49
	Andhra Pradesh	47	1999	1439	39
	Gujarat	40	1633	1209	35
	Total/mean	100	1825	1288	41

Source: various reports and publications of ICRISAT and ICAR

3). In Maharashtra state, a total of 782 on-farm trials were conducted in seven districts and the hybrid produced 35% more grain yield (969 kg/ha) than the control. In Andhra Pradesh (399 trials), the hybrid exhibited 56% superiority over the control. Similarly in Madhya Pradesh (360 trials), the hybrid out-yielded the control cultivar by 46%; while in Jharkhand (288 trials) ICPH 2671 demonstrated 69% superiority over the control cultivar Bahar. Over all the four states (1829 trials), ICPH 2671 produced 1445 kg/ha average yield and it was 51% more than the local checks (954 kg/ha). ICPH 2671 was released for general cultivation in 2010 by the state Government of Madhya Pradesh and later notified for cultivation in the entire country (Saxena et al. 2013). After this release, two more hybrids ICPH 2740 in Andhra Pradesh (Saxena et al. 2014c) and ICPH 3762 in Odisha (Saxena et al. 2014d) were also released for cultivation.

Large-scale hybrid seed production of ICPH 2671

For hybrid pigeonpea seed production so far there are no officially prescribed guidelines, but according to the information generated by ICRISAT and various ICAR centres, an isolation distance of at least 500 m is necessary. Since the extent of pollen transfer is determined by the population of pollinating insects, the row ratio of female to male may vary from 3:1 to 4:1. The rouging operation is recommended at seedling, flowering, and pre-harvesting stages. It is also advised that for optimizing hybrid yields, the adoption of proper agronomy is essential (Mula et al. 2010 a, b). Our experience has shown that the hybrid seed can be produced easily by growers, if the pollinating vectors

are present in sufficient number. For other details regarding hybrid pigeonpea seed production technology, readers are advised to refer Saxena (2006). In order to demonstrate the feasibility of large-scale hybrid seed production, 94 professional seed producers were selected in six states and the production programme was undertaken under the direct supervision of a team of experts. On average, 1019 kg/ha of hybrid seed was harvested and the highest yield (1674 kg/ha) was recorded in Madhya Pradesh. It is estimated that with a recommended seeding rate of 5 kg/ha, an encouraging seed-to-seed ratio of 1:200 to 1:300 can be achieved.

Seed quality control

In order to ensure consistency in performance of hybrids in farmers' fields, it is important that a high level of genetic purity is maintained year-after-year. In most field crops it is achieved by assessing the quality of freshly harvested seed of hybrids and their parents is determined by standard grow-out tests but in pigeonpea this approach is not feasible due to its long generation turn-over time that may extend from 6 to 9 months. To overcome this constraint two approaches, briefly described here, have been launched by ICRISAT.

The first approach is based on genomics and it is simple, rapid, and cost effective. A set of 148 simple sequence repeat (SSR) markers was used for polymorphism survey of 159 hybrid parents and of these, 41 markers were found polymorphic (Saxena et al. 2010). Subsequently, 3072 SSR markers were also screened and a set of 42 SSR diagnostic markers was identified for assessing the hybridity of ICPH 2671 (Bohra et al. 2012). With the help of these markers (Fig. 2) reliable detection of off-type hybrid seeds in the commercial lots can be undertaken (Saxena et al. 2010; Bohra et al. 2012). Since at commercial level analysis of a large number of seed samples from different locations will be required, an alternative cost effective genomics-based quality testing approach will be an asset. To achieve this objective, several single nucleotide polymorphism (SNPs) have also been discovered for genotyping using Kbio Sciences Alleles Specific (KASPar) assays. This would allow assessing genetic purity of large number of seed samples with minimum number of loci.

The second approach involves breeding of hybrid parents with a naked-eye polymorphic marker: In this approach a distinct phenotypic trait that can be

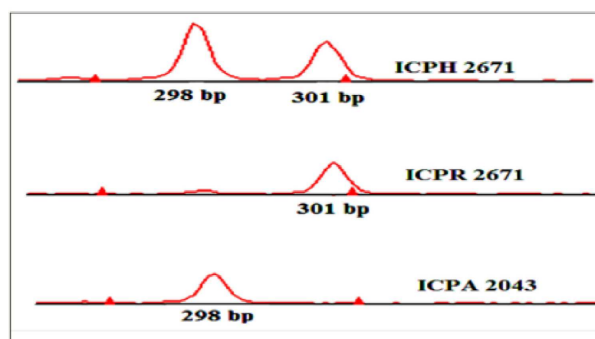


Fig. 2. Demonstration of seed purity assessment of hybrid ICPH 2671 with the CcM 0021 marker. The female lines, ICPA 2043 (298bp) and male line ICPR 2671 (301 bp) show clear peaks using a diagnostic SSR marker (CcM 0021, and the true hybrid (ICPH 2671) shows the presence of both the alleles (298 and 301 bp). Source : ICRISAT

identified by naked eye and called as “naked eye polymorphic marker” is used to assess purity. Saxena et al. (2011) identified ‘obcordate leaf’ as a polymorphic marker and incorporated it in to A- and B-lines. This marker, controlled by a single recessive gene, can be easily recognized within a month from sowing. The hybrids developed by crossing the parents involving normal and obcordate leaf types will always have normal leaves and the unwanted sibs will have obcordate leaves. Such off-types can be detected within a month from sowing. This approach of hybrid breeding should be promoted to help in maintaining seed quality of female parents and hybrids.

Phase IV (2013-to date): pathways for producing the next generation hybrids

Breeding hybrids with specific adaptation

In modern agriculture the importance of specific adaptation of cultivars to a given environment cannot be ignored. Many farmers have now moved above the subsistence level and regard agriculture as a challenging business. The prices of quality agricultural commodities are also high enough to attract greater investment for reaping more profits. The pigeonpea hybrids released so far have demonstrated high (2000-3000 kg/ha) yields and wide adaptation in farmers' field conditions. Besides this fact, it was also observed that some farmers harvested exceptionally high yields from the same hybrid in fairly large plots. For example, under good crop management a group of progressive farmers in Maharashtra state harvested 4000-4500 kg/

ha or more grains with >50% superiority over the control. These observations emphasize the importance of specific adaptation in breeding pigeonpea hybrids. Saxena and Raina (2001) analyzed data from 11 diverse environments (10° - 29° N) and reported significant effects of environments on the productivity of hybrids. They further concluded that greater yields can be obtained by breeding locally adapted hybrids. Chauhan et al. (1993) also advocated specificity of adaptation in early maturing cultivars. In this context, it is not unfair to believe that soon the hybrid pigeonpea breeding programmes will grow in size and more specifically adapted hybrids will be available to provide more returns to farmers.

Diversification of hybrid parents

Success of a dynamic hybrid research and development programme, primarily depends on the rate at which new parental lines are bred having high combining ability and key market-driven traits. Such parental lines can either be bred using both traditional and new technologies. In pigeonpea, the selection efficiency is adversely affected by out-crossing in the preceding generation. Hence, care should be taken to minimize the incidence of natural hybridization in breeding plots. In the following text some strategies for diversifying the hybrid parents are discussed.

Diversification of A-lines

There is a need to diversify A-lines with respect to both nuclear as well as extra-nuclear genomes. The available A-lines carrying A_2 or A_4 cytoplasm have perfect male-sterility system with high stability across diverse environments. To diversify the genetic backgrounds of these lines for use in hybrid breeding programmes, the male sterility was transferred into a number of genotypes of diverse origin. In early group, the A_4 male sterility has been transferred in 11 extra early and early maturing inbred lines through backcrossing. The A_2 CMS system was also transferred to 19 diverse early maturing genotypes (Parmar et al. 2008). The converted lines exhibited a wide range for flowering (60-81 days), maturity (105-128 days), plant height (71-157 cm), and seed size (8.1-12.5 g/100 seeds). In medium and long duration group, >50 A-lines were bred at ICRISAT and various ICAR centres and many of them have resistance to both wilt and sterility mosaic diseases. In this context, the incorporation of dominant gene conferring resistance to *fusarium* wilt (Saxena et al. 2012) into A-lines should be given a serious attention because this will allow development of wilt resistant hybrids

through both resistant x resistant and resistant x susceptible crosses.

Cytoplasmic diversification of A-lines

The historical outbreak of southern corn leaf blight disease in the USA (Tatum, 1971) gave a strong natural lesson to breeders about the significance of cytoplasmic diversity in hybrid breeding programmes. This happened because at one stage all the commercial corn hybrids in the USA were based on 'T cytoplasm' that carried genes for the susceptibility to blight disease; and when its outbreak occurred, most hybrids succumbed and resulted in severe yield losses. In this context, in pigeonpea a good beginning has already made and at present eight CMS-inducing cytoplasm are known (Saxena et al. 2010; Saxena 2013). Although these CMS systems represent a wide genetic variability for mitochondrial genome, so far only two systems (A_2 and A_4) have been used in hybrid breeding. This situation necessitates breeding of more number of CMS lines with greater cytoplasmic diversity. It should also be noted that while breeding for cytoplasmic diversity, the effect of cytoplasm on yield and other traits should also be studied. Recently, Saxena et al. (unpublished) reported some adverse effects of *C. cajanifolius* (A_4) cytoplasm on seed yield; but it was found to be cross-specific with the maximum cytoplasmic-induced yield penalty of 19.5%.

Nuclear diversification of R-lines

Perfect fertility restoration and its stability are important aspects in hybrid breeding programmes. In early maturity group during the past few years, a total of 46 fertility restorers have been identified among advanced breeding lines and germplasm (Table 4). These restorers exhibited >90% pollen fertility in hybrid combinations (data not reported) and have a wide range for flowering (50-85 days), maturity (101-141 days), and seed size (6.2-12.1 g/100 seeds). None of the restorers had resistance to *fusarium* wilt, while ICPL 149, ICPL 150 and ICPL 151 were resistant to sterility mosaic disease. For A_2 system also, a number of fertility restoring lines were identified (Chauhan et al. 2004). The number of restorers in the early maturity group is limited and more diversity would be required for a fruitful hybrid breeding programme. In medium maturity group, 113 fertility restorers were identified with a considerable range for maturity, seed size and most importantly disease resistance (Table 4). This variability can sustain hybrid breeding for a few years; but breeders should look towards breeding more

Table 4. Variation for important traits among fertility restorers of early, medium, and late maturity groups recorded at Patancheru

Trait	Early (n= 46)	Medium (n= 113)	Late (n= 31)
Days to flower	50-85	90-130	131-158
Days to mature	101-141	138-200	186-241
Plant height (cm)	70-165	90-228	135-260
100-seed wt (g)	6.2-12.1	6.8-17.3	7.7-18.1
Wilt (%)	52-100	0-100	0-100
Sterility mosaic (%)	3-67	0-100	0-100

Source: ICRISAT

diverse restorers. For late maturing group also, there is a need to search more restorers with diverse genetic backgrounds. Breeding new fertility restorers should be a continuous process in a dynamic hybrid breeding programme. To achieve this, besides normal pedigree breeding, there are other approaches such as selection from heterotic crosses and screening new germplasm which can produce productive fertility restorers.

Greater productivity of hybrids is conditioned by various types of gene actions. The beneficial effects of genetic variation arising due to dominance, over dominance and epistatic components will be lost in the subsequent self-generation; but its additive genetic fraction can be fixed through appropriate pedigree selection. Saxena and Sharma (1990) and Sharma and Dwivedi (1995), while reviewing the subject, concluded that in pigeonpea additive genetic variation plays an important role in the formation of seed yield. This means that at least in some inbred lines the desirable alleles, present in the two parents, can be brought together to realize high yields. These inbreds can form good parental base for producing the new generation high yielding hybrids. A similar exercise carried in hybrid ICPH 8 revealed that some inbred lines achieved 70-75% yield potential of the hybrid (Saxena et al. 1992). Use of such inbreds in hybrid breeding is expected to yield hybrids with productivity greater than the available hybrid combinations.

The primary gene pool of pigeonpea with >15000 collection maintained at ICRISAT and ICAR is a rich source of genetic diversity for almost all the important quantitative and qualitative traits. This genetic wealth can be exploited in identifying new maintainers and fertility restorers for use in hybrid breeding programmes. To start this activity at ICRISAT, two

most stable A-lines (ICPA 2039 and ICPA 2092) with high general combining ability, were crossed with 502 testers from the germplasm (Saxena et al. 2014a) and among F₁ progenies, 179 were fully fertile, 26 maintained male sterility, and the remaining 297 segregated for fertility and sterility. This study suggested that the frequency of fertility restoring genes in the germplasm is quite high and this resource can be exploited in hybrid breeding programmes. Overall, this is the best short cut method for the diversification of hybrid parents.

Formation and use of heterotic pools is another proven approach for selecting hybrid parents, which in high probability, produce high yielding hybrids. Shull (1908) and Richey (1922) observed that the maize hybrids involving diverse/dissimilar parents produced plants with greater vigour and yield. Subsequently, for discriminating the hybrid parents for their ability to produce high yielding hybrids, Sprague and Tatum (1942) evolved the popular concept of 'combining ability'. All these ideas of identifying good hybrid parents gradually sunk into the concept of 'heterotic groups'. This involves clustering of parental lines on the basis of their performance in F₁ generation, combining ability, origin, phenotypic or genetic diversity. In pigeonpea, the first such information was published by Saxena and Sawargaonkar (2014) and they constructed seven heterotic groups using specific combining ability effects from multi-location hybrid trials. According to this concept, crosses between the lines originating from the same cluster (group) are not likely to produce heterotic hybrids; while the crosses between the lines representing two diverse groups will have high probability to yield hybrids with high performance.

Search for new heterotic hybrids

All the four released hybrids have recorded a satisfactory (25-50%) level of standard heterosis in farmers' fields. Considering the vast area and variable agro-ecological conditions, there appears a need for breeding many more hybrids for the country. Efforts in this direction are being made both at ICRISAT and ICAR. In this context, several early maturing experimental hybrids were evaluated for their fertility restoration and productivity (Saxena et al. 2005b); and eight promising combinations were advanced to multi-location testing (Table 5). From this material, two hybrids ICPH 2433 and ICPH 2438 were found promising with respectively, 54% and 42% superiority over the control UPAS 120. Recently ICPH 2438 has

been identified for on-farm trials in Madhya Pradesh (AK Tikle, personal communication). The performance data demonstrated that the level of hybrid vigour in early maturity pigeonpea group is high enough for commercialization. A perusal of productivity data also showed that in comparison to inbred cultivars (12.5 kg/ha/day), the hybrids were more efficient in dry matter production and/or its partitioning in to grains (22 kg/ha/day). Further studies are needed to understand the physiological aspects of yield determination in hybrids, their inbred parents, and control cultivars. Information on the role of yield contributing traits needs to be generated for understanding the reasons for relatively greater productivity of the hybrids.

In the last few years over 1500 medium duration hybrids representing maturity groups V and VI were developed; and based on yield and disease resistance eight combinations were selected for on-station testing. These hybrids exhibited 37-62% standard heterosis and high levels of resistance to wilt and sterility mosaic diseases (Table 6). Pandey et al. (2013) also recorded over 20% standard heterosis in four long duration pigeonpea hybrids.

Exploring temperature-sensitive male sterility in hybrid breeding

Some environmental factors such as temperature and photo-period are known to play a key role by inducing male sterility and fertility in various crops (Kaul 1988). However, the credit of using such temperature and photo-period induced male sterility in breeding hybrid rice goes to Chinese scientists (Shi 1981; Zhou et al. 1988; Sun et al. 1989). This approach, popularly known as 'two parent hybrid breeding' has various advantages

over the well-known 'three line' system. These include i) multiplication of A- line without using the maintainer line and pollinating agents, ii) elimination of fertility restorers from hybrid system, iii) elimination of deleterious cytoplasmic effects, iv) utilization of greater genetic variability in hybrid breeding, and v) development of a large number of hybrids in a short time. In pigeonpea, the temperature sensitive male sterility (TGMS) is of recent origin (Saxena, 2014) and it was derived from an inter-specific cross involving *C. sericeus* and *C. cajan*. Plants carrying the sensitive gene are completely male sterile at the temperatures

Table 6. Some promising medium duration pigeonpea hybrids developed at ICRISAT

Hybrid (ICPH)	Yield (kg/ha)	Standard heterosis (%)	100-seed weight (g)	*Wilt (%)	*Sterility mosaic (%)
3371	3013	62	11.50	0	0
3491	2919	57	13.40	0	0
3497	2686	44	10.90	0	15
3481	2637	41	11.60	0	0
3494	2586	39	12.40	0	9
2740	2900	57	12.30	0	0
3762	3000	62	11.90	0	0
2671	2509	37	12.20	0	0
Check	1864	-	11.10	0	0
SEm	±205.7	-	± 0.33	-	-
Mean	2448.1	-	11.7	-	-
CV (%)	11.9	-	3.98	-	-

*Data from disease nursery

Table 5. Performance (yield kg/ha) of early maturing hybrids (A₄) in multi-location trials

Hybrid (ICPH)	Maturity (days)	2007 (n=7)	2008 (n=4)	2009 (n=8)	2010 (6)	Mean (n=25)	% gain	Prod. (kg/ha/d)
2433	114	2538	1864	2331	2489	2306	54	22.22
2438	115	2722	1570	2238	1979	2127	42	18.50
2363	115	2292	1763	2131	2005	2048	36	17.81
2429	114	1825	1907	2015	2037	1946	30	17.07
2431	117	2186	1400	1925	2165	1919	28	16.40
2447	114	1959	1456	2045	1782	1811	21	15.89
2364	114	1909	1294	2018	1883	1776	18	15.58
3310	106	1540	1344	1731	1546	1540	3	14.53
Check	120	1502	1204	1545	1758	1502	-	12.52

Source: ICRISAT

$\geq 25^{\circ}\text{C}$, while at $\leq 24^{\circ}\text{C}$ the same plants become fully male fertile. Field evaluation of this material at Patancheru (17°N) showed that in June sown crop, the plants were male sterile in the month of September (high temperature). The same plants turned fully male fertile in the month of November (low temperature); and when the temperatures rose in the month of February, they again reverted back to male sterility. To make full use of this system in pigeonpea 'critical fertility point (CFP)' and 'critical sterility point (CSP)' that determine the conversion of male sterility/fertility need to be worked out. In India the two-parent hybrid system can be adopted easily as the sites with desired temperature requirements can be identified for seed production using different seasons, altitudes, and latitudes. This system will require two sites each with specific threshold temperature regime to allow full expression of the TGMS gene(s). For multiplication of female parent, the maximum safe mean temperature during reproductive phase at the production sites should not exceed 20°C ; while the hybrid seed production can be taken at most places in rainy season when the temperatures are well over 26°C . This trait can be transferred easily to any inbred line (Saxena and Bharathi, 2015) provided the screening facilities with controlled temperature are available.

Integration of genomics science in hybrid breeding

Besides seed quality testing (Fig. 2), the genomics can also be used to identify fertility restorers without going into the cumbersome process of making hybrids and testing their progenies for the presence of genes responsible for restoring their pollen fertility. The marker assisted breeding may help in the identification and selection of lines carrying the *Fr* genes within segregating populations and germplasm at a faster pace and economically. This is facilitated by constructing a consensus genetic map using the populations segregating for the fertility restoring genes. The first pigeonpea consensus map has already been constructed (Bohra et al. 2012) and it contains 339 simple sequence repeat (SSR) loci spanning a distance of 1,059 cM. In three mapping populations a total of four major quantitative trait loci (QTLs) for fertility restoration have been identified. This technology has a great potential and research on its refinement is underway in the genomic laboratories at ICRISAT and ICAR. The genomics science can also be used to generate accurate information on genetic diversity of hybrid parents. This will eliminate environmental effect which may mask the true genetic variability in the traits of interest. This information can also be used to

develop heterotic groups and for the selection of potential genotypes for breeding high yielding hybrids.

Conclusion

The decades-old productivity stagnation in pigeonpea has now become a serious concern at national level, particularly in view of the national nutritional security. To overcome this GMS-based technology GMS-based technology constraint partly, a CMS-based hybrid pigeonpea breeding technology has now been evolved and the hybrids have demonstrated clear advantages over pure line cultivars for yield and stability. In this context, the release of three commercial pigeonpea hybrids is considered a major breakthrough in pigeonpea breeding. Extensive on-farm testing of the hybrids in seven Indian states has shown that the magnitude of standard heterosis in pigeonpea is comparable with other field crops, where commercial hybrids are already in market. The cultivation of hybrids has also given positive signals to farmers about the drought tolerance and high productivity of hybrids.

The hybrid seed production technology in pigeonpea is easy and high seed-to-seed ratio (1: 200 to 1: 300) ensures its economical production. However, the selection of production sites and development of a seed chain are essential for sustainable adoption of hybrid pigeonpea technology. Further, for the promotion of hybrids, use of expert hands and minds will bring a big difference in the pace and quality of technology transfer. In this context, training of seed producers will be a step in the right direction. At present there are no prescribed seed standards for hybrids in pigeonpea. Such issues must be addressed at national level and the information be made available to all the seed producers. Further, for large-scale adoption of hybrids, it is also necessary to convince both public and private seed companies about the benefits and profitability of hybrid pigeonpea business.

The hybrid technology has now provided a new instrument for realizing quantum jumps in the productivity of pigeonpea; and with a conservative view, it is estimated that with 25-30% on-farm yield advantage and about 10% replacement of pigeonpea area with hybrids, India can produce enough pigeonpea to meet its domestic needs.

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