

## HYBRID TECHNOLOGY–A NEW VISTA IN PIGEONPEA BREEDING

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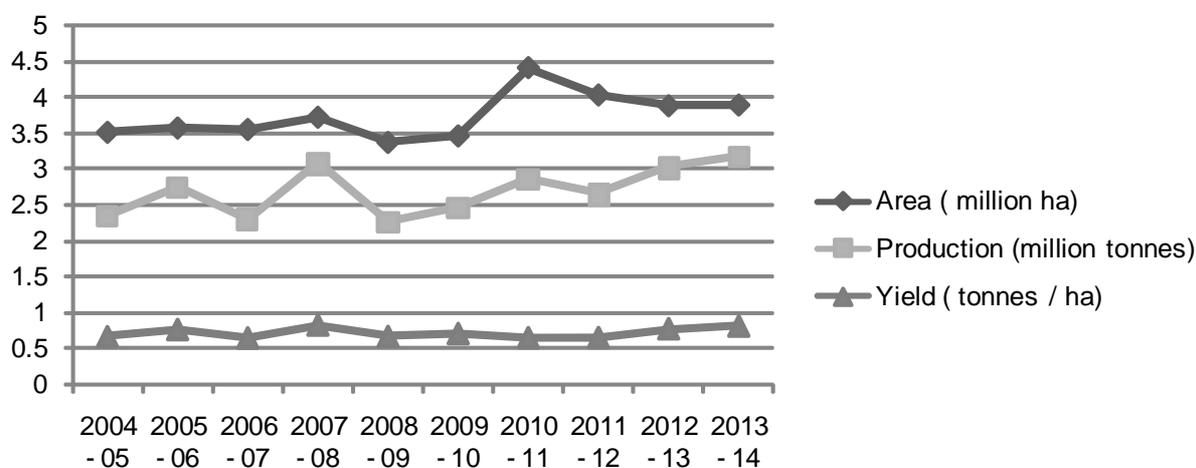
### 1. Introduction

Once designated as an 'orphan crop' to now being crowned as a mainstream 'commercial crop', pigeonpea has evolved over the decades as lifeline for millions of resource poor farmers in the semi-arid tropics, where it is cultivated for both subsistence and commercial purpose. Pigeonpea [*Cajanus cajan* (L.)] is the sixth most important legume crop, grown predominantly in the tropical and sub-tropical regions of Asia, Africa and Latin America. India is considered as the center of origin of pigeonpea (Van der Maesen, 1980) because of its natural genetic variability available in the local germplasm and the presence of its wild relatives in the country.

The global pigeonpea area, production and yield was approximately 6.23 m ha, 4.74 M T and 762.4 Kg ha<sup>-1</sup>, respectively (FAOSTAT, 2015). The major producers of pigeonpea are India (63.74% of global production), Myanmar (18.98%), Malawi (6.07%), Tanzania (4.42%) and Uganda (1.98%). In India pigeonpea was cultivated on 5.06 M ha with a total production of 3.29 M T and yield of 649.9 Kg ha<sup>-1</sup> during 2014 (FAOSTAT, 2015). The

leading states in pigeonpea production are Maharashtra (0.259 M T), Karnataka (0.51 M T), Madhya Pradesh (0.39 M T), Uttar Pradesh (0.259 M T), Gujarat (0.258 M T) and Jharkhand (0.19 M T). These six states account for 84% of the total production in India during 2014-15 (E-Pulse Data Book, 2016).

Pigeonpea is a hardy, widely adapted and drought tolerant crop. It has a range of maturity which helps in its adaption in a wide range of environments and cropping systems. It can be grown either as sole crop or intercrop with urd bean, mung bean, castor, sorghum, soybean, cotton, maize and groundnut. Pigeonpea is mostly consumed as dry split dal. It is an excellent source of protein (20-22%), supplementing energy rich cereal diets in a mainly vegetarian population. The per capita net availability of pulses in India has reduced from 51.1 g day<sup>-1</sup> (1971) to 41.9 g day<sup>-1</sup> (2013) as against WHO recommendation of 80 g day<sup>-1</sup>. According to Indian Institute of Pulses Research (IIPR) estimation, India's population is projected around 1.68 billion by 2030 pulse requirement around 32 million tones.



**Fig 1.** Area, Production and Yield of Pigeonpea over the last decade in India.

(Source: E- Pulse Data Book, IIPR Kanpur, 2016)

The wide difference between production and demand of pulses has resulted in larger imports in recent past (2012-13) reaching a record 4.0 million tons an increase of 500,000 tons over 2011-12 (India's Pulses scenario 2014). Pigeonpea has been in focus in recent times due to the continuous inflation in its price. Stagnant productivity coupled with declining availability has created substantial supply & demand gap forcing heavy import bill on the exchequer and affecting nutritional security of majority of the population for whom pulses are one of the cheapest source of protein. Thus, this review in the backdrop of 'International Year of Pulses' attempts to give a bird's eye view of the advent of hybrid pigeonpea technology, which played a pivotal role to in breaking the yield plateau and achieving the quantum leap in its production.

**2. Early Research:**

To promote pigeonpea production, genetic improvement of pigeonpea was emphasized by

researchers for more than five decades and a number of cultivars were developed from selection of land races (Singh *et al.*, 2005). However, the progress in the genetic improvement of yield potential was limited and the improved cultivars enhanced the productivity to some extent. Therefore, an alternative breeding approach i.e., hybrid technology, was attempted in pigeonpea to enhance the yield.

**2.1 Gene action and Heterosis**

In crops where high yielding commercial hybrids have been developed, the breeders have reported that additive and dominance gene actions are predominantly responsible for the expression of hybrid vigour for yield. Very limited research has been done on the genetic aspects of various agronomic traits in pigeonpea. Sharma and Green (1975) concluded that the important agronomic characters are controlled primarily by genes with additive effects. Dominance and non additive effects were also detected for yield, plant height and protein content.

**Table 1. Estimates of gene effects in pigeonpea for vegetative characters**

Character(s)	Genetic variance(s)	Reference (s)
Days to flowering	Additive	Dahiya and Barar, 1977.
Flower initiation	Additive	Venkateswarlu and Singh, 1982.
Plant height	Predominantly additive High GCA than SCA thus more additive Additive Single recessive (d) gene for dwarf height	Singh and Pandey, 1974. Sharma <i>et al.</i> , 1973b. Singh <i>et al.</i> , 1983b. Sen <i>et al.</i> , 1966.
Plant growth	Predominantly additive	Singh and Pandey, 1974.
Early maturity	Additive Partial dominance, A x D	Dahiya and Satija, 1978. Mohamed <i>et al.</i> , 1985.
Days to maturity	Additive Non additive	Sidhu and Sandhu, 1981. Patel <i>et al.</i> , 1987.
Leaf area Leaf mass Petiole length Petiole mass	Additive Additive Additive Additive	Sharma and Saxena, 1983.
Fruiting branches Raceme length	Additive Additive	Singh <i>et al.</i> , 1983b.

(Source: Singh and Oswalt, 1992.)

**Table 2. Estimates of gene effects in pigeonpea for productivity characters**

Character(s)	Genetic variance(s)	Reference (s)
Pods plant <sup>-1</sup>	Non-additive (over dominance) Both additive and non-additive Additive	Dahiya and Barar, 1977. Venkateswarlu and Singh, 1982. Singh <i>et al.</i> , 1983b.
100 seed mass	Non-additive (over dominance) Additive Both additive and non-additive AxA&DxD	Dahiya and Barar, 1977. Sidhu and Sandhu 1981. Venkateswarlu and Singh, 1982. Mohamed <i>et al.</i> , 1985.
Seed yield	Non-additive (over dominance) Predominantly non-additive Higher GCA than SCA thus additive Non-additive Both additive and non-additive	Dahiya and Barar, 1977. Singh and Pandey, 1974. Sharma <i>et al.</i> , 1973b. Dahiya and Satija, 1978. Venkateswarlu and Singh, 1982.
Seed size	Additive, partial dominance of small seed	Singh and Pandey, 1974.
Protein content	Non-additive Both additive and non-additive	Singh and Pandey, 1974. Sharma <i>et al.</i> , 1972.
Early maturity	Additive Partial dominance, A&D	Dahiya and Satija, 1978. Mohamed <i>et al.</i> , 1985.
Pod width	Additive	Sidhu and Sandhu, 1981.
Seeds pod <sup>-1</sup>	Both additive and non-additive Dominance, A x D & D x D	Venkateswarlu and Singh, 1982. Mohamed <i>et al.</i> , 1985.

(Source: Singh and Oswalt, 1992).

Heterosis is a natural phenomenon which makes hybrid offspring of genetically diverse individuals and display improved physical and functional characteristics relative to their parents (Coors and Pandey 1997; Shull 1948). Heterosis in crops can be perceived in the form of increased growth rate, total biomass, stress resistance, seed yield and population fitness (Kalloo *et al.*, 2006). Heterosis is largely universal and can result in quantum leap in yield by 15-50% depending on crop type. Many major cereal crops as well as commercial varieties of vegetables and flower crops are cultivated using hybrid seeds for increased agricultural performance (Duvick 1999; Birchler *et al.*, 2003).

Heterosis was first utilized in maize (*Zea mays*), followed by other crops (Melchinger and Gumber, 1998). Yield advantage in maize, rice, wheat

and grain sorghum owing to hybrid vigour were around 15% (Duvick 1999), 20-30% (Cheng *et al.*, 2007), 10-25% (Hoisington *et al.*, 1999) and 35-40% (Duvick, 1999), respectively in USA and Asian sub-continent. In pigeonpea, significant heterosis was also reported by Kumar and Srivastva (1998), Pandey and Singh (2002), Wankhade *et al.*, (2005), Baskaran and Muthiah (2006), Dheva *et al.*, (2009), Chandirakala *et al.*, (2010), Vaghela *et al.*, (2011), Pandey *et al.*, (2013) and Kumar *et al.*, (2015).

## 2.2 Mechanism of Natural out-crossing in Pigeonpea

The first report of natural out-crossing in pigeonpea was in 1919 (Howard *et al.*, 1919) wherein, they found 14% out crossing. Studies were conducted to understand the factors responsible for out crossing in this crop and frequent insect visits was the main

cause (Pathak 1970; Williams 1977; Onim 1981; Zeng-Hong *et al.*, 2011). These studies revealed that over two dozen insect species were found foraging on pigeonpea flowers, but out crossing was affected only by few. Williams (1977) reported that *Megachile bicolor* and *M. conjuncta* were the major pollinators, while Onim (1981) reported cross pollination in pigeonpea was mainly a function of foraging by *Apis mellifera* and *Megachile* species in Kenya. Lately, Zeng-Hong *et al.*, (2011) found that in Yuanmou (China) the insects belonging to *Megachile spp.*, *Xylocopa* and *Apineae* were most frequent visitors to pigeonpea fields and they were very active in collection and transportation of pollen grains from one plant to another, thereby, resulting in cross-fertilization.

Over the period, population of pollinating insect tend to fluctuate across the locations and time of the year resulting in natural out crossing (Saxena *et al.*, 1990). Moreover, some external factors are also known to affect the extent of out crossing like speed and direction of wind (Bhatia *et al.*, 1981), extended periods of stigma receptivity (Dalvi and Saxena 2009) and floral morphology of geno type (Byth *et al.*, 1982).

### 3. Dawn of heterosis breeding era in pigeonpea

The first pigeonpea variety was developed for wilt resistance by selection in land races (Shaw 1933). Subsequently, more than 100 pureline pigeonpea varieties were released for cultivation over the past seven decades (Singh *et al.*, 2005) resulting in substantial increase in area & production, but productivity showed little increment. This encouraged breeders to move towards heterosis breeding for harnessing the inherent heterotic potential for breaking the yield plateau, as no further horizontal increase in area under pigeonpea was possible. For economically viable hybrid seed production system, hand emasculation and pollination is not commercially feasible. Hence, development of stable male sterile line became imperative for utilization of available natural out crossing in pigeonpea.

### 4. Male Sterility Systems

Plants that fail to produce functional pollen grains are male sterile and such plants reproduce only when fertile pollen from other plants falls on their stigmatic surface through any mechanical means. The first report of male sterility came in 1763

(Kolreuter, 1763). The male sterility is controlled by specific genetic factors which are generally recessive in nature. Such genes are exposed during inbreeding and their maintenance is affected by fertilization with the pollen that carries corresponding dominant gene(s).

In plant system, the male sterility is generally caused by some specific bio-chemical events that hinder normal biological processes of pollen production. It is also noticed that all the male-sterility systems identified so far in different crops could not be used in hybrid breeding programmes because of non-availability of other complementary genetic systems required for restoring their male fertility. For efficient utilization of male sterility system in heterosis breeding, it is necessary that the expression of both male-sterility and male fertility restoration systems are stable over the years and locations. A perfect male sterility in the female parent in conjunction with natural out-crossing makes the hybrid seed production easy and affordable. For crops where seed is of economic value, either Genetic Male Sterility (GMS) or Cytoplasmic Genetic Male Sterility (CGMS) system can be used.

#### 4.1 Genetic Male Sterility (GMS) System

This system of sterility is the first break through in hybrid seed production of redgram and it arises when male fertility controlling dominant (Fr) nuclear gene mutates to its recessive form under the influence of some natural forces and with subsequent natural selfing of heterozygotes (Frfr) the male sterile genotypes (frfr) appear within the population. Such genotypes if not cross pollinated by fertile pollen are eliminated from its parental population. An elaborate search for male sterility system in pigeonpea was flagged off in 1970's and resulted in the discovery of GMS system. The first report on male sterility in pigeonpea was documented by Deshmukh (1959). Reddy *et al.*, (1977) made an extensive search on CGMS system in 7216 germplasm accessions sown in ICRISAT in 1974, but instead a source of GMS was identified in a field collection from Andhra Pradesh. This GMS line was medium duration (180 days maturity) and susceptible to fusarium wilt and sterility mosaic disease. The male sterility was controlled by single recessive gene 'ms<sub>1</sub>' which arose spontaneously (Reddy *et al.*, 1978). The male sterile lines derived from ms<sub>1</sub> source were extensively used

in breeding program at ICRISAT and ICAR institutes. The outcome of this effort was release of world's first pigeonpea hybrid ICPH8 in 1991 (Saxena *et al.*, 1992). It was found superior to national checks UPAS120 and Manak by 30.5% and 34.2%, respectively.

hybrids. Correns (1908) reported that cytoplasmic factors could influence occurrence of male sterility and the trait would be maternally inherited.

Nagur and Menon (1974) studied several of these sources and recognized four different classes based on fertility/ sterility responses in hybrids. These

**Table 3. List of Genetic Male Sterile hybrids of pigeonpea released in India**

Hybrid	Origin	Year	Adaptation zone	Maturity group	Standard heterosis (%)	Year of release
ICPH 8	ICRISAT	1991	Central	Early	35	1991
PPH 4	PAU, Ludhiana	1994	North west	Early	14	1993
CoH 1	TNAU, Coimbatore	1994	South	Early	21	1994
CoH 2	TNAU, Coimbatore	1997	South	Early	35	1997
AKPH 4104	PKV, Akola	1997	Central	Early	64	1997
AKPH 2022	PKV, Akola	1998	Central	Medium	30	1998

**4.2 Cytoplasmic Genetic Male Sterility (CGMS/ CMS) System**

Considering the shortcomings in large scale hybrid seed production in GMS hybrids, the development of cytoplasmic nuclear male sterility became imperative. CGMS is a physiological abnormality, resulting from a disharmonious interaction between the cytoplasmic factors and nuclear genetic factors, leading to the production of

were further studied by Reddy (1992) in an effort to classify them and to find minimum differential testers. These were designated as A<sub>1</sub> (CK60; origin-East Africa), A<sub>2</sub> (IS 12662C; origin-Ethiopia), A<sub>3</sub> (IS 1112C; origin-India) and A<sub>4</sub> (M35-1, VZM2 and G1; origin-India).

**5 (a). First CMS-based pigeonpea hybrid GTH-1**

The first CMS-based pigeonpea hybrid GTH-1 was developed at GAU, SK Nagar and released by

**Table 4. List of CMS systems in wild species of pigeonpea**

Wild species	CMS System	Remarks
<i>C. sericeus</i>	A1	CMS sensitive to temperature
<i>C. scarabaeoides</i>	A2	Fertility restoration unstable
<i>C. volubilis</i>	A3	Large variation in expression
<i>C. cajanifolius</i>	A4	Stable and used in hybrid program
<i>C. cajan</i>	A5	Uses cultivated pigeonpea cytoplasm
<i>C. lineatus</i>	A6	CMS system very stable
<i>C. platycarpus</i>	A7	A new CMS using tertiary gene pool
<i>C. reticulates</i>	A8	Searching for restoration in progress

degenerated or non-viable pollen grains or non-dehiscent anthers with or without functional pollen grains. Kolreuter (1763) first noticed cytoplasmic genetic male sterility in interspecific and intraspecific

ICAR in 2004 for cultivation in Gujarat. This hybrid was bred by crossing A<sub>2</sub> CMS line GT 288A with fertility restorer GTR-11. Based on multi-location yield trials conducted during 2000 to 2003, GTH-1 (average

yield 1830 Kg ha<sup>-1</sup>) gave 32% higher yield over the best local check (GT 100/110 with average of 1330 Kg ha<sup>-1</sup>). This hybrid is non-determinate type and early maturity (140 days). In frontline demonstrations conducted in three districts (2003), the hybrid exhibited 25.3% yield superiority over the popular check. After multi-location trials conducted by ICAR, the hybrid GTH-1 was released for cultivation in central zone.

in overcoming short spells of early season drought that is often encountered in July-sown rainfed crops. ICPH 2671 also exhibited high survival (88%) under water-logged conditions and this was found to be related to its ability to utilize stored assimilates through an aerobic metabolism (Sultana *et al.*, 2012). During 2005-2008, ICPH 2671 was tested in multi-location trials and its mean performance in different years varied from 2200 to 3183 kg ha<sup>-1</sup> and on an average, it gave 47% heterosis over national check Maruti.

**Table 5. Performance (yield Kg ha<sup>-1</sup>) of three hybrids in on-farm trials**

Hybrid	State	Farmers (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
	Madhya Pradesh	288	1460	864	69
	Total / Mean	360	1940	1326	46
		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

#### 5 (b). First commercial CMS-based pigeonpea hybrid-ICPH 2671

The first ever commercial hybrid of any grain legume is ICPH 2671. It was produced by crossing a male sterile line ICPA 2043 with restorer ICPR 2671. The plants of ICPH 2671 have semi-spreading habit, non-determinate type and profuse branching. It grows over two meter in height, matures between 164-184 days and contains 3.7-4.0 seeds pod<sup>-1</sup>. The purple coloured seeds weigh between 10.5 to 11.2 g 100<sup>-1</sup> seeds. ICPH 2671 has high resistance to both wilt and sterility mosaic diseases. In comparison to inbred cultivar 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

#### 5 (c). ICPH 3762

The Odisha University of Agriculture and Technology released ICPH 3762 in 2014, in Odisha state. This hybrid registered 20 to 67 % superiority over check in multi-location testing. In on farm demonstrations over 144 locations it out yielded local check by 124 % superiority. It is resistant to *Fusarium* wilt and Sterility mosaic disease with yield potential of 3.5 to 4 Tons ha<sup>-1</sup> and is suitable for all soil types of different states in India.

#### 5 (d). ICPH 2740

In 2015, this hybrid was released by PJTSAU (Professor Jayashankar Telangana State Agricultural University), Hyderabad, Telangana State.

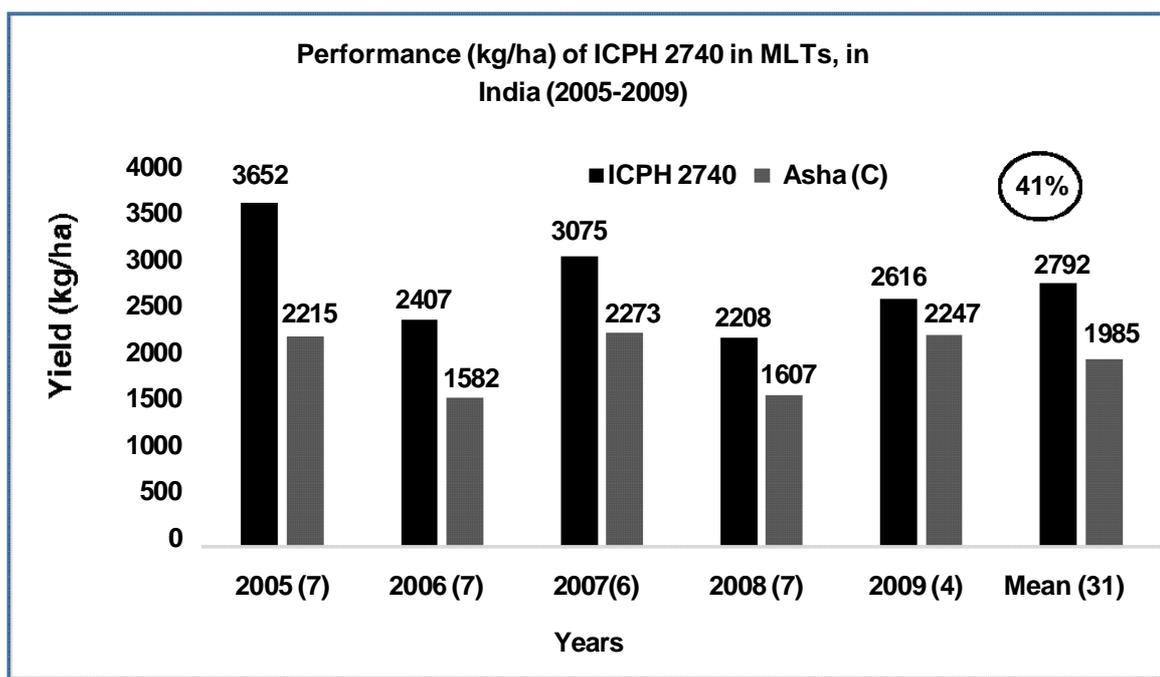


Fig 2: Performance of ICPH 2740 in multi location trials

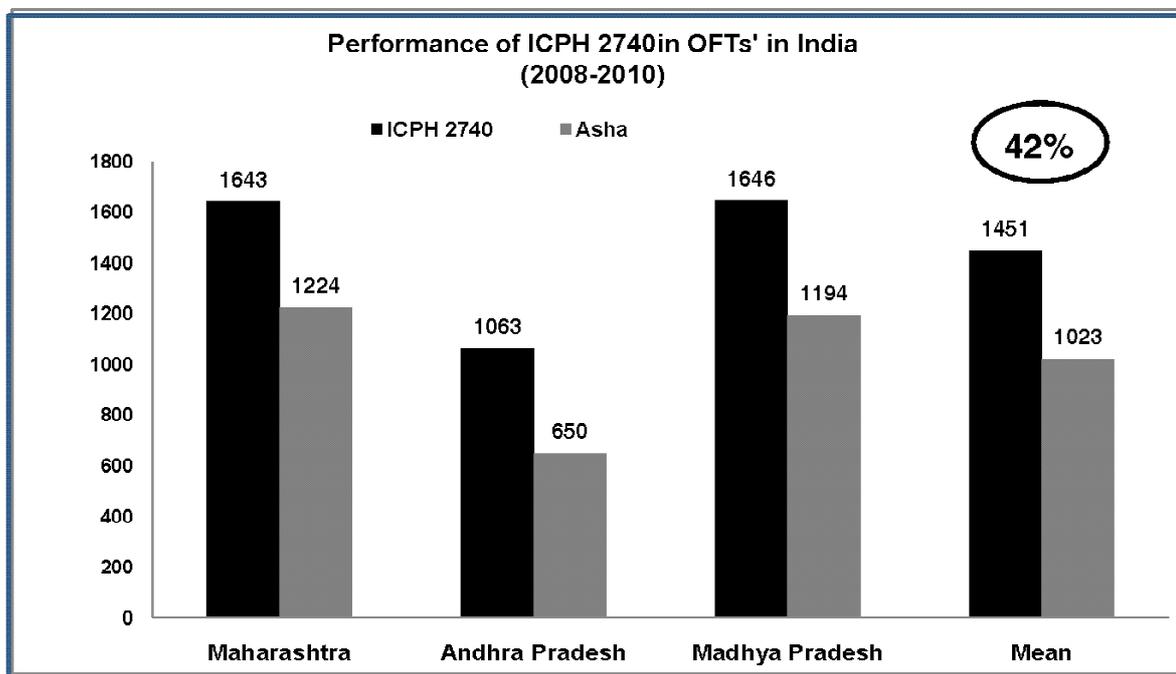


Fig 3. Performance of ICPH 2740 in on farm trials

## 6. Seed production technology

The benefit of hybrid technology can not be realized unless sufficient quantities of genetically pure hybrid seed is commercially produced and sold at affordable prices. The experiments conducted in various locations revealed that the extent of natural out-crossing (20-70%) in pigeonpea varies considerably (Saxena *et al.*, 1990). The hybrid seed set on the male sterile plants is mainly determined by the availability of bee population in the vicinity of seed production plot. The known prime pollinating agents in pigeonpea are *Megachileanata*, *Apisflorae* and *Apismellifera* (Pathak 1970). Onim (1981) reported that each insect visit lasts for 15-55 seconds when they trip the open floral buds, thereby introducing foreign pollen on the stigmatic surfaces to affect cross fertilization. To ensure a good seed set, hybrid seed production plot should be surrounded by bushes that harbour bee colonies and should be located near a water source to maintain high population of pollinating vectors.

### 6.1 Seed production of hybrid parents

Nucleus seed production of parental lines is an important component of hybrid seed production, since it determines their purity and quality.

**A-line:** For seed production of A-line, at initial stages both A and B lines are grown inside insect proof net. Single plants of A and B lines, conforming to the standards of the lines are selected and paired crossed. The crossed seed sets on the A-line plants and selfed seeds on the B-line plants are harvested separately. To produce breeder/ foundation seeds of A-line in large quantities, a field with appropriate isolation distance is selected, A and B-lines are grown with recommended agronomic practices. At ICRISAT, planting ratio of 4 female rows (A-line) and 1 male (B-line) row with staggered sowing at 15 days interval is found to be effective for the production of pure seed of A-line. In short duration A-line, the mature pods on the male sterile and male fertile plants can be harvested by pod picking or by cutting the top pod bearing branches.

**B-line and R-line:** The nucleus seeds of B and R-lines are produced by sowing pure seed lots of B and R- lines in separate isolations. Hundred plants are to be harvested from the central portion of the seed production plot and their progenies are grown in

subsequent seasons. After analyzing their purity aspects, the selected progenies should be bulked to serve as nucleus seed. For breeder and foundation seed production, the lines should be multiplied in separate isolations. Sufficient care should be taken to rouge out the off-types when identified in the field.

**Certified seed production:** The foundation seeds of A-line and R-line is the source for hybrid seed production. A and R- lines should be sown in 4:1 or 3:1 (depending on the bee population) in a staggered planting at 15 days interval in an isolated block. Additional rows of R-line can also be sown on each side of the plot. The pollinating insects visit the male and female flowers randomly and effects pollination and hybridization on the male sterile female lines. The row ratio of female and male lines should be varied according to the environment, insect activity and the time of sowing to ensure good seed set.

### 6.2 Breeding of hybrid parents with naked eye polymorphism (NEP)

For grow out test (GOT), it is necessary to have quick assessment system to identify pure hybrid seed. As pigeonpea is grown as an annual crop, days to flowering takes a long time and it is very difficult to conduct a quick GOT. Therefore, some phenotypic markers, which help in easy and efficient identification of pure hybrid seed within a short period of time, are needed. A new approach of using distinct phenotypic trait, that can be identified by naked eye and called as "naked eye polymorphic marker" was used to assess purity and 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 after sowing. The hybrids developed by crossing the parents involved normal and obcordate leaf types will always show 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.

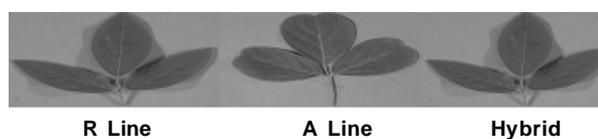


Fig 5. Assessment of parental line and hybrid purity using NEPs



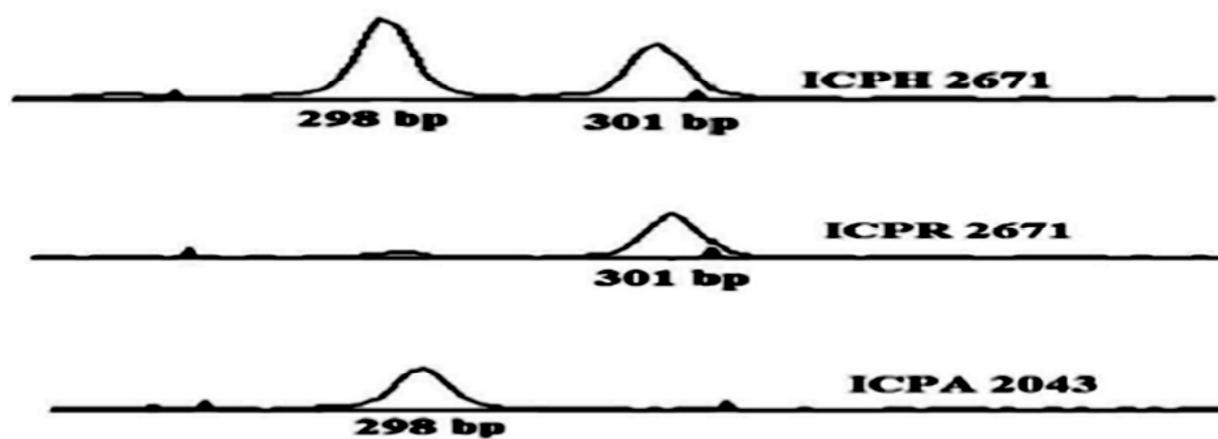


Fig 6. Seed purity assessment of hybrid ICPH 2671 with the CcM 0021 marker. The female line, ICPA 2043 (298bp) and male line ICPR 2671 (301 bp) show clear peaks using diagnostic SSR marker (CcM 0021, and the true hybrid (ICPH 2671) showed the presence of both the alleles (298 and 301 bp). Sameer Kumar (2015).

ICPH 2751). SSR markers of these kits amplify only one specific allele in their respective parents and both alleles in their true hybrids. For example, for the hybrid ICPH 2438, SSR marker CCB 4, amplifies 228 bp fragment in ICPA 2039 (CMS line or female parent) and 220 bp fragment in ICPR 2438 (male or restorer parent), while the true hybrid (F<sub>1</sub>) seeds show both alleles (228 bp and 220 bp). If some seeds of hybrids show only one allele or other allele than the parental genotypes, in such case those seeds are considered as impure hybrid seeds.

In addition to hybrid seed purity testing kit, marker for A4CMS (nad7a del) seed purity has been developed and validated in range of A4 derived CMS lines and large seed lots. This marker amplifies 150 bp fragment in A4CMS lines and 160 bp fragment in their cognate maintainer lines and can be visualized on 3.5% agarose gel. The developed CMS associated gene based marker is capable of detection of <2% of adulteration on low-cost agarose gel system.

#### SUMMARY

With the release of three pigeonpea hybrids viz., ICPH 2671, ICPH 3762 and ICPH 2740, the world's first set of commercial hybrids in any food legume, the hybrid technology has become successful. This climate-smart crop can be a boon to farmers as it requires less water, enriches soil, withstands weather variability and is packed with nutrients. In order to sustain the gains of research

and development of hybrid technology, its breeding require continuous attention with respect to developing new parental lines. These hybrids have demonstrated their significant superiority in farmers' fields for three consecutive years or more and are now available for general cultivation in India. To sustain the achievements of this breakthrough, it is essential that superior hybrids are made available from time-to-time to the farmers of different regions and to achieve this, development of potential parental lines is an important pre-requisite. Pigeonpea is a new crop as far as hybrid technology is concerned and therefore, development of potential hybrids with easy seed production techniques in this crop needs special attention. Exhaustive research in public-private-partnership mode in tandem with government support is essential for realizing the dream of self-sufficiency in pigeonpea.

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