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Review Paper

Pigeonpea hybrid breeding in India

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ABSTRACT

The constantly increasing gap in the demand to supply for pigeon pea has been a matter of concern to pigeon pea researchers in India to increase productivity and production. The entomophily abet cross-pollination behavior of pigeon pea is a desirable crop to develop and establish the hybrid system to exploit the commercial heterosis. Keeping this in view, pigeon pea research was directed towards a new initiative on hybrid pigeon pea breeding at ICRISAT, Hyderabad immediately after the identification of a male sterile line in 1974. Which in turn led to the development of a new GMS hybrid called ICPH 8 in 1991 for cultivation in the central zone. Then five GMS hybrids (PPH 4, CoPH 1, CoPH 2, AKPH 4101, and AKPH 2022) in the early maturing group were released by the state and central varietal release committee. Nevertheless, the GMS-based hybrids did not yield much success due to difficulty in the production of commercial F_1 seed. Trifurcation of All India Coordinated Pulses Improvement (AICPIP) and further strengthening of AICPRP on pigeon pea led to the development of national level strategic and basic research vis-à-vis provided testing platform for varieties and hybrids. The advertent GMS system led to the development of a stable and economically viable CGMS system in pigeon pea after 26 years of its first GMS initiative. The first CGMS line GT 288A and its maintainer B line was registered by Pulse Research Station, SDAU, GAU, SK Nagar, Gujarat in 2000. Consequently, in 2006 the first CMS hybrid GTH 1 was developed by SDAU, Gujarat, and released by CVRC for cultivation in the central zone. Thirty-nine CGMS lines have been registered with ICAR-NBPGR and four CMS-based hybrids (ICPH 2671, ICPH 2740, IPH 15-03, and IPH 09-5) are released for cultivation. It is to endorse that the joint efforts of the ICAR-NARS and ICRISAT led to the establishment of the hybrid system in pigeon pea by sharing the materials and technology. Nonetheless, supplying quality hybrid seed is a mammoth task to reap the true potential of hybrid technology in pigeon pea.

Key words**:** *Cajanus cajan*, CMS, Fertility restoration, GMS

INTRODUCTION

Pigeonpea (*Cajanus cajan* (L.) Millsp.) is also known as red gram, tur, or arhar. It is an annual to perennial shrub that is traditionally cultivated as an annual crop in the Indian sub-continent in association with different cropping systems. Pigeonpea is considered to be an excellent and affordable source of plant-based protein, essential amino, and fatty acids, fibers, minerals, and vitamins. India is the largest producer (4.22 Million Tonnes) and consumer of pigeonpea (Source: http://agricoop.gov.in) sharing the world's 81.26% acreage and 79.22% production (FAOSTAT 2022). The major pigeon pea-producing states in India are Maharashtra, Karnataka, Madhya Pradesh, Uttar Pradesh, Gujarat, and Andhra Pradesh all together contributing around 78 to 80% of total production. However, in productivity, few states which cultivate long-duration pigeonpea such as Bihar (1591 kg/ha) and West Bengal (1428 kg/ha) have high productivity compared to early and mid-early duration cultivating states viz., Haryana (1088 kg/ha), Gujarat (1324 kg/ha), Punjab (1222 kg/ha) and Uttar Pradesh (1084 kg/ha) (PC pigeonpea report, 2021).

Production of pigeonpea increased gradually from the 1970s to till date mainly due to an increase in area, particularly the less fertile, non-traditional coverage in southern and central zones at the cost of traditionally fertile alluvial soil of north Indian states. Even though sincere research efforts were dedicated to improving the productivity of

pigeonpea, which eventually led to the development and release of nearly 184 high-yielding varieties in different maturity groups, a considerable gap still exists between the potential and realized yield coupled with the increasingly soaring prices of pigeon pea have become a matter of concern today in the country.

Pigeonpea owing to its often cross-pollinated nature offers an excellent system among pulse crops to exploit the hybrid vigor for enhancement and stability of crop yield. Pigeonpea is unique among legumes as its floral morphology allows both self– as well as insect-aided cross-pollination and their extents vary from one place to another (Saxena *et al*. 2010). This exhibited large variation (20–70%) in natural outcrossing, so pigeonpea can be considered an often cross-pollinated crop (Saxena *et al.,* 1990). However, this considerable amount of natural out-crossing creates hurdles in maintaining the genetic purity of varieties. The existence of both additive and non-additive gene actions in pigeonpea for yield and yield components in turn brightens the scope for hybrid breeding. Besides, a substantially higher level of heterosis has been reported in pigeonpea. In this context, the first report of hybrid vigor in pigeonpea was published by Solomon *et al*. (1957), in which the authors reported nearly 25% heterosis over the better parent for grain yield. Subsequently, several reports have been published on hybrid vigor for yield and yield components in pigeonpea (Saxena and Sharma 1990). However, the cost of hybrid seeds poses a major limiting factor in achieving effective and rapid deployment of hybrid technology. Manual emasculation increases the cost of production, so the use of various cost-effective and time-saving mechanisms remains the need of the hour. The phenomenon of male sterility is one such event that raises special interest among the breeding community to harness the genetic gain in pigeonpea.

By 1974, male sterility systems are better known to play a greater role in enhancing the productivity of many crops by enabling the exploitation of heterosis/hybrid vigor. For effective utilization of a male-sterility system in hybrid breeding, the expression of both the male-sterility and its fertility restoration must be stable over years and locations. The availability of genetically pure seeds of improved pigeonpea cultivars is crucial for realizing their productivity and adoption in different agroecological niches. To this end, a definitive hybrid breeding technology could facilitate efficient seed production that provides an adequate quantity of quality seeds at economically viable costs. The immense potential of new hybrids cannot be fully realized until sufficient quantities of genetically pure and healthy seeds are commercially produced and made available at affordable prices to the farmers.

Therefore, hybrid technology offers a greater opportunity for increasing the yield potential of pigeonpea. However, the four pre-requisite, which are essentially important for the commercial development of pigeonpea hybrids, are i) the availability of a perfect male-sterility system, ii) identification of efficient fertility restorers, iii) substantially higher and exploitable levels of heterosis iv) the large-scale seed production of hybrids for commercialization.

PREFERENCE OF PIGEONPEA HYBRIDS OVER VARIETIES

Pigeonpea hybrids offer many advantages over varieties. The notable remarks are given below.

- ***Increased grain yield:*** Hybrid pigeonpea produces greater biomass by utilizing inputs like sunlight, water, and nutrients more efficiently while maintaining their partitioning at least at par with the varieties leading to higher grain yield.
- ***Increased seedling vigor:*** Hybrid pigeonpea has greater plant vigor compared to the pure line cultivars. This makes hybrids more competitive than the weeds under inter-cropping situations during the initial crop growth stages.
- ***Reduced seed rate:*** Pigeonpea hybrids produce more primary and secondary branches with wider canopy. This translates to a reduced seed rate of 40-50% compared to the traditional varieties.
- ***Drought tolerance:*** Hybrid pigeonpea by virtue of their greater root mass and deep root system compared to the varieties, have a greater ability to draw water from deeper soil profiles during drought conditions.
- *D**isease resistance:*** Hybrids may acquire an extra degree of genotypic plasticity compared to varieties, which helps them to tolerate and produce higher yields under biotic stress is such as fusarium wilt and sterility mosaic disease.

MALE STERILITY SYSTEMS USED IN PIGEONPEA HYBRID BREEDING

Genetic Male Sterility: The GMS systems reported so far in pigeonpea have emerged from spontaneous mutations. The occurrence of GMS even might be credited to a mutation in the male-fertility controlling dominant (*Fr*) nuclear gene that alters it to recessive form under the influence of some natural forces. Subsequently, the natural selfing of heterozygotes (Frfr) leads to the appearance of the male-sterile genotypes (frfr) within the population. These genotypes, if not cross-pollinated by fertile pollen, are gradually eliminated from their parental population. Therefore, such elimination processes depend on the rate of natural out-crossing in a given population. The male-sterile mutants have also been reported in some mutagen-induced populations. In most cases, such mutants could not be maintained either due to their tight association with female sterility or reproductive abnormalities such as chromosome addition or deletion. The most of spontaneous male-sterile mutants that have been detected so far are recessive. Relatively high occurrence of non-allelic recessive male-sterility genes suggests that the frequency of such natural mutations is quite high and their deletion from the parental populations is rather slow. In comparison with recessive genes, the frequency of dominant male-sterile genes in nature is very low (Kaul 1988). According to Kaul (1988) male sterility in legumes that is controlled by recessive genes was reported in broad bean [*Vicia faba* (L.)], grass pea [*Lathyrus sativus* (L.)], groundnut [*Arachis hypogea* (L.)], sun hemp [*Crotalaria juncea* (L.)], soybean [*Glycine max* (L.) Merr.], pea [*Pisum sativum* (L.)] white clover [*Trifolium repens* (L.)], common bean [*Phaseolus vulgaris* (L.)], alfalfa [*Medicago sativa* (L.) spp. sativa] etc.; while dominant genetic control of male sterility was reported in *Trifolium repens*. There are many reports for genetic male-sterility systems in pigeonpea (Table 1).

Cytoplasmic-nuclear male sterility (CGMS) systems: This is the reproductive abnormality leading to male sterility due to the complex interplay between the specific genetic elements residing in the nucleus and cytoplasm (Bohra *et al.* 2016). The phenomenon of CMS results from a competition in the cytoplasmic and nuclear genes that differ in their inheritance i.e. paternal and maternal. The sterility is induced by the factors contained in the cytoplasm (Bohra *et al.* 2016). On the other hand, the genes that counteract the effect of sterility, thus enabling CMS to restore fertility reside in the nucleus. Isogenic lines (B-lines) are required to retain the CMS trait that permits the production of non-functional pollen grains. Further, restoration of fertility in the hybrids is facilitated through a fertile parent that carries fertility-restoring genes. Involvement of three different parents this system is also known as the A, B, R system or three-line breeding. The CMS trait may result from spontaneous mutation, intra-specific crosses, or wide hybridization (inter-specific/inter-generic crosses). A literature survey led to the conclusion that CMS events stemming from inter-specific are mostly seen in the case of dicots, while inter-generic hybrids appear as the CMS sources in monocots (Kaul 1988). Unlike CMS, only a single nuclear mutation can lead to the development of male sterility in GMS. However, in contrast to GMS-inducing genes, the genetic elements involved in the manifestation of CMS are more prone to environmental changes. This in turn renders the CMS and restoration highly unstable.

***CMS systems in pigeopea*:** So far in pigeonpea eight sterilizing cytoplasms (Table 2) have been reported (Bohra *et al.* 2010, 2016). Of these, seven have

Table 1. Summary of genetic male sterility system reported in pigeonpea (modified from Saxena *et al*. 2010)

S. No.	Reported by	Gene symbol	Remarks
1	Deshmukh (1959)	-	Male sterility was associated with female sterility
2	Reddy *et al.* (1977)	-	Seven types of floral variants with varying degrees of male sterility
3	Reddy *et al.* (1978)	ms_1	Transparent male sterile anthers
4	Dundas *et al.* (1982)	-	Photo insensitive male sterile mutant
5	Saxena *et al.* (1983)	ms_2	Brown, arrowhead shape anthers; non-allelic to ms_1
6	Verulkar and Singh (1997)	-	Single recessive gene control
7	Wanjari *et al.* (2000)	-	Single dominant gene control
8	Saxena and Kumar (2001)	ms_3	Underdeveloped anthers non allelic to ms_1 & ms_2
9	Venkateshwarlu *et al.* (1981)	-	Male sterility linked to abcordate leaf type
10	Pandy *et al*. (1994)	-	Male sterility linked to abcordate leaf type
11	Saxena *et al.* (1981)	-	Partial male sterility with sparse pollen production
12	Gupta *et al.* (1983)	-	Recessive gene control

been developed utilizing wild relatives from the secondary gene pool of pigeonpea, while one CMS carries *C. cajan* cytoplasm (Mallikarjuna and Saxena 2005). The A_7 CMS involves *C. platycarpus*, a wild relative from the tertiary gene pool of pigeon pea (Saxena *et al.* 2010, Mallikarjuna *et al.* 2011).

Table 2. List of eight CMS systems developed in pigeonpea (Saxena *et al.* 2015)

CMS type	Cytoplasm donor species	Recipient species
A_1	*Cajanus sereceous*	*Cajanus cajan*
A_2	*Cajanus scarabaeoides*	*Cajanus cajan*
A_3	*Cajanus volubilis*	*Cajanus cajan*
A_4	*Cajanus cajanifolius*	*Cajanus cajan*
A_5	*Cajanus cajan*	*Cajanus acutifolius*
A_6	*Cajanus lineatus*	*Cajanus cajan*
A_7	*Cajanus platycarpus*	*Cajanus cajan*
A_8	*Cajanus reticulatus*	*Cajanus cajan*

(i) *A_1-CMS:* The CMS lines are derived from *Cajanus sericeus* (Saxena *et al.* 1997). However, the CMS trait becomes highly unstable at low temperatures (< 10°C), in some instances fertile revertants are also reported (Saxena 2005). The problem of CMS instability raises more concerns in the case of early duration lines. Further, the lack of robust resources to maintain A-lines is also one of the major drawbacks limiting the success of this cytoplasm.

(ii) *A_2-CMS:* The cytoplasm of this CMS is derived from *C. scarabaeoides* and was reported to be very stable (Saxena and Kumar, 2003; Tikka *et al.*, 1997). The majority of the hybrids developed at IIPR are based on A_2-CMS (IIPR 2007).

(iii) *A_3-CMS*: Interspecific hybridization between the *C. volubilis* and *C. cajan* (var. ICPL 83024) led to the generation of A3-CMS (Wanjari et al. 2001). However, CMS restoration has been a greater challenge for the deployment of A_3-CMS in regular breeding.

(iv) *A_4-CMS:* The most probable progenitor of cultivated pigeonpea i.e. *C. cajanifolius* is the cytoplasm donor of A4-CMS (Saxena *et al.* 2005). Several stable restorers and maintainers have been reported in A_4-CMS, thus acting as a testament to the practical applicability of this CMS.

(v) *A_5-CMS:* According to Mallikarjuna and Saxena (2005), the CMS event occurred when *C. cajan* cytoplasm was placed into the nuclear background of *C. acutifolius*. However, only the wild relative can maintain A_5-CMS.

(vi) *A_6-CMS:* Male sterility was reported in the open-pollinated population of *C. lineatus* (Saxena *et al.* 2010). Backcrossing this plant with ICPL 99044 provided male sterile plants. It is interesting to note that no pod setting was seen in the sterile plants even though all pollen grains stained red in acetocarmine stains, thereby warranting the exmanination of this CMS to a greater depth.

(vii) *A_7-CMS:* This CMS arises as an outcome of the cross (*C. platycarpus* x *C. cajan*). As potent crossability barriers exist between the primary and tertiary gene pool, the hybridization scheme required supplementation through hormone-aided pollination and embryo rescue

(viii) *A_8-CMS:* As reported by Saxena (2013) inter-specific crosses involving *C. reticulatus* (ICPW 176) as seed parent and *C. cajan* (ICP 28, ICPL 87119 and ICPL 20176) as pollen parent are known to induce CMS in pigeonpea. This is designated as A_8-CMS.

PIGEONPEA HYBRIDS DEVELOPED AND RELEASED IN INDIA

GMS-based pigeonpea hybrids: ICRISAT developed first GMS-based pigeonpea hybrid ICPH 8 and released for cultivation in India (Saxena *et al.* 1992). In farmers fields, this hybrid recorded a 31-40% yield advantage over the best control. This was followed by the release of five more GMS base hybrids that exhibited high levels of standard heterosis (Table 3). But, none of these could reach farmers fields at the commercial level. The male-sterility is controlled by a pair of recessive genes (*msms*) and it can only be maintained by crossing it to heterozygous (Msms) plants. The progeny of this cross (msms × Msms) will segregate into 50% male-fertile (Msms) and 50% male-sterile (msms) plants. Therefore, identification of the fertile segregants within the female population was a primary requirement of large-scale seed production and it was not found commercially viable.

CMS-based pigeonpea hybrids: CMS results from an inherent disharmony between cytoplasmic (mitochondrial) and nuclear genomes. A range of mitochondrial genes have been reported in different crops that confer CMS traits. The fertility in the CMS system is rescued because of fertility-restoring elements located in the nucleus. The first CGMS line GT 288A and its maintainer B line was registered

Table 3. Pigeonpea hybrids released in India (AICRP on pigeonpea report 201, Saxena *et al.* 2010)

Name of the hybrid	Zone	Released	Days to maturity	Superiority over check (%)
GMS-based hybrids				
1. ICPH 8	Central	1991	125	30-40
2. PPH 4	Punjab	1994	137	14
3. CoH 1	Tamil Nadu	1994	117	19-22
4. CoH 2	Tamil Nadu	1997	125	35
5. AKPH 4104	Central	1997	135	64
6. AKPH 2022	Maharashtra	1998	190	25-35
CMS-based hybrids				
1. GTH-1	Gujarat	2006	140	32
2. ICPH 2671	Madhya Pradesh	2014	175-180	31-34
3. ICPH 2740	Telangana	2015	180	34
4. ICPH 3762	Orissa	-	180-190	36
5. IPH 15-03	NWPZ	2019	150-155	28-55
6. IPH 09-5	NWPZ	2020	145-150	30

by Pulse Research Station, SDAU, GAU, SK Nagar, Gujarat in 2000. Consequently, in 2006 the first CMS hybrid GTH 1 was developed by SDAU, Gujarat, and released by CVRC for cultivation in the central zone. Thirty-nine CGMS lines have been registered with ICAR-NBPGR and six CMS-based hybrids were released for cultivation as of 2019 (Table 3).

BRIEF ACCOUNT OF THE PIGEONPEA HYBRID BREEDING PROGRAMME AT ICAR-IIPR

CMS diversification and conversion of elite breeding lines into CMS lines at IIPR: Once a stable CMS system is established, the sterilizing cytoplasm may be combined with different nuclear backgrounds using standard backcross protocol. The cytoplasm donor is once used in the crossing scheme as a female parent and the preferred nuclear background is used as a recurrent parent. Several lines including UPAS 120A, PA 163A, PDA 89-2E A, Hy 4A, and H 28B A were converted at IIPR, Kanpur using A_2 cytoplasm (Singh *et al.* 2009). More recently, Pusa 992A, ICPL 88039A, and DPP 3-2A were converted at IIPR. The former sterile line contains A_4 cytoplasm derived from ICP 2089 A, whereas the latter two CMS lines carry A_2 cytoplasm from GT 288A. Additional ten lines AL 15, ICP 7148, AL 201, Pusa 2002-2, MN 5, ICP 88034, ICP 20338, ICP 11255, ICP 20340 and PAU 881 are being targeted for CMS diversification. As described by Chase (2007), a standard backcross protocol that enables cytoplasmic substitution is shown below (Table 4).

Evaluation of developed pigeonpea hybrids under AICRP scheme: During the last nine years, several hybrids were evaluated under AICRP on pigeonpea across different locations. The information on these entries is provided below (Table 5).

Development of hybrids: After evaluation in station trials and AICRP trials (IHT, AHT 1 and AHT 2) conducted at multi-locations, two CMS-based hybrids namely IPH 15-3 and IPH 09-5 developed by IIPR Kanpur were released and notified by CVRC in 2019 and 2021 respectively for cultivation in NWPZ (Western U P, Punjab, Haryana, Part of Rajasthan and Delhi) of country. Both hybrids are of early maturity duration (145-150 days), having indeterminate plant type, semi-spreading growth pattern, average yield of 15-16 q/ha, and possessing resistance to wilt and sterility mosaic disease.

Maintenance of CMS lines, their cognate maintainers and restorers: The CMS lines are maintained through the 'A × B' crossing scheme, while the B- and R- lines are maintained through self-pollination at ICAR-IIPR (Table 6 & 7). For large-

Table 4. Backcross method adopted for CMS line conversion program (modified from Chase 2007)

Generation	Organelle genomes of the original pollen-sterile seed parent (%)	Nuclear genome of the original pollen-sterile seed parent (%)	Nuclear genome of the recurrent pollen-fertile parent (%)	Pollen phenotype
F1	100 (GT288A/ICPA 2089)	50 (GT288A/ICPA 2089)	50(PA163/ ICPL 88039)	Sterile
BC2F1	100 (″)	12.5 (″)	87.5 (″)	Sterile
BC3F1	100 (″)	6.2 (″)	93.8 (″)	Sterile
BC4F1	100 (″)	3.1 (″)	96.9 (″)	Sterile
BC5F1	100 (″)	1.6 (″)	98.4 (″)	Sterile

Table 5. List of hybrids evaluated under AICRP pigeonpea from year 2010-11 to 2018-19

Trial	Tested zone	No of entries	Details of entries (Short duration)
IHT	North	24	IPH 10-01, IPH 10-02, IPH 10-03, IPH 10-04, IPH 10-05, IPH 11-01, IPH 11-02, IPH 11-03, IPH 12-01, IPH 12-02, IPH 12-03, IPH 12-04, IPH 13-01, IPH 13-02, IPH 13-03, IPH 13-04, IPH 14-01, IPH 15-01, IPH 15-02, IPH 15-06, IPH 16-02, IPH 17-02, IPH 18-01, IPH 18-02
	Central	8	IPH 10-06, IPH 10-07, IPH 10-08, IPH 10-09, IPH 10-10, IPH 11-04, IPH 13-05, IPH 13-06
	South	4	IPH 10-11, IPH 10-12, IPH 10-13, IPH 10-14
	Central & South	4	IPH 15-04, IPH 15-05, IPH 15-06, IPH 17-03
AHT 1	North	3	IPH 10-03, IPH 11-01, IPH 17-01
	Central	1	IPH 10-06
AHT 2	North	3	IPH 09-5, IPH 10-02, IPH 15-03

scale production, these lines are planted in isolation to prevent contamination from cross-pollination.

Table 6. CMS lines maintained at ICAR-IIPR, Kanpur

Sl. No.	Name	Type
1	ICP 84023 A	Early
2	ICP 67 A	Early
3	PA 163 A	Early
4	UPAS 120 A	Early
5	CORG 990047 A	Early
6	CORG 990050 A	Early
7	ICP 2039 A	Early
8	ICP 2089 A	Early
9	GT 33 A	Early
10	GT 288 A	Early
11	GT 290 A	Early
12	AL 101 A	Early
13	PDA 89-2E	Late
14	H-28 A	Late
15	Hy 4 A	Late
16	ICP 2043 A	Late
17	GT 301 A	Late
18	GT 307 A	Late
19	GT 308 A	Late

HYBRID PIGEONPEA SEED PRODUCTION METHODOLOGY

Large-scale and cost-effective hybrid seed production in pigeonpea: The often cross-pollinating nature of the pigeonpea crop demands strict maintenance of isolation distance to allow large-scale generation of pure seeds of hybrid and parental lines cost-effectively. The commercial seed production of pigeonpea involves large-scale seed production of the female line (A), maintainer (B), restorer (R), and hybrid (A × R). An isolation distance of at least 500 m is required to completely avoid the possibility of pollen contamination from neighboring pigeonpea crop. Relying solely on the insect (pollinator) activities, the female line (A) and maintainer line (B) are planted in a 4: 1 or 6:1 (female to male) ratio. A similar ratio is adopted in the case of hybrid seed production in which the female line (A) and restorer line (R) are planted in a 4:1 or 6:1 ratio. The extent of seed harvest from 6:1 planting is subjected to the higher activity of pollinators, while the 4:1 ratio is known to provide optimum harvest in general. To ensure genetic purity, the seeds are harvested first from fertile line(s) i.e. B and R lines. Subsequently, A-line is also harvested. Experiments were conducted at IIPR, Kanpur for two seasons to standardize the technique that enables cost-effective seed production of A-line. For example, the relative efficiencies of planting at different ratios were examined for UPAS 120 A and its cognate maintainer (UPAS 120B) using three ratios viz. 4A:1B, 6A:1B and 8A:1B. Notably, the 6:1 ratio witnessed the highest seed yield of A line. Similarly, at ICRISAT, a planting ratio of 4:1 gave good yield in both A × B and A × R seed production programs. Using this technology an average of 1135 kg ha-1 of A-line seed (A × B) was harvested. Similarly, an average of 975 kg ha^{-1} yield was recorded for hybrid seed (A × R). Regular monitoring of the seed production plot and removal of the off-types (roguing) is essentially needed to maintain genetic purity, and roguing is performed at the branching, flowering, and podding stages.

GENOMIC ADVANCES IN PIGEONPEA HYBRID BREEDING

Recent years have witnessed the generation of cutting-edge genomic tools to assist hybrid breeding in pigeonpea. The key molecular tools engaged in hybrid breeding are molecular markers or quantitative trait loci (QTL) facilitating accurate selection of fertility-restoration (*Rf*) traits, mitochondrial *orfs* leading to the development of non-functional pollen grains, and molecular markers for examining the genetic purity of the hybrid and the parents.

Table 7. Restorer lines maintained at ICAR-IIPR, Kanpur

S. No.	Name of the restorer	S. No.	Name of the restorer	S. No.	Name of the restorer	S. No.	Name of the restorer	S. No.	Name of the restorer
1	AK 250043R	21	AK 261429R	41	AKPR 273	61	GTR 5	81	GTR 25
2	AK 250083R	22	AK 261504R	42	AKPR 277	62	GTR 6	82	GTR 51
3	AK 250137R	23	AK 261505R	43	AKPR 292	63	GTR 7	83	GTR 52
4	AK 250157R	24	AK 261506R	44	AKPR 303	64	GTR 8	84	GTR 53
5	AK 250159R	25	AK 261514R	45	AKPR 312	65	GTR 9	85	GTR 54
6	AK 250165R	26	AK 261515R	46	AKPR 319	66	GTR 10	86	GTR 55
7	AK 250167R	27	AK 261526R	47	AKPR 324	67	GTR 11	87	GTR 524
8	AK 250173R	28	AK 261536R	48	AKPR 335	68	GTR 12	88	GTR 526
9	AK 250189R	29	AK 261561R	49	AKPR 344	69	GTR 13	89	GTR 529
10	AK 250204R	30	AK 261581R	50	AKPR 348	70	GTR 14		
11	AK 260479R	31	AK 261594R	51	AKPR 359	71	GTR 15		
12	AK 260748R	32	AK 261622R	52	AKPR 364	72	GTR 16		
13	AK 261233R	33	AK 261354R	53	AKPR 402	73	GTR 17		
14	AK 261243R	34	AKPR 02	54	AKPR 420	74	GTR 18		
15	AK 261264R	35	AKPR 12	55	CO 1R	75	GTR 19		
16	AK 261321R	36	AKPR 178	56	CO 2R	76	GTR 20		
17	AK 261322R	37	AKPR 210	57	GTR 1	77	GTR 21		
18	AK 261394R	38	AKPR 215	58	GTR 2	78	GTR 22		
19	AK 261409R	39	AKPR 224	59	GTR 3	79	GTR 23		
20	AK 261411R	40	AKPR 250	60	GTR 4	80	GTR 24		

***Molecular mapping of fertility-restoration loci*:** Genetic analysis of the mapping populations has enabled the identification of genomic regions controlling key traits in pigeonpea. The presence of such QTL/genes provides an exciting opportunity to facilitate indirect selection of the target traits. Research on various CMS systems has shown that the CMS phenotypes are rescued by the action of nuclear genes called restoration-of-fertility (*Rf*) genes. Pollen fertility is restored through the reconciliation of the "lost" harmony between the two genomes with different inheritance. Researchers suggest a metaphorical "molecular arms race" between the newly evolving mitochondrial *orfs* and the corresponding *Rf* genes (Touzet and Budar 2014). Molecular markers tightly associated with the fertility restoration (*Rf*) trait will allow accurate and rapid discrimination between the restore and maintainer lines. Rigorous field testing is required for the identification of a potential restorer line. Molecular markers thus circumvent the requirement of the labor-intensive and arduous field screening and equally importantly, the molecular assays can be conducted much earlier. Otherwise, the identification of the restorer cannot be done before the occurrence of flowering. Bohra et al. (2012) were the first to report the genetic linkage of the DNA markers with fertility-restoration traits. The authors developed three F2 populations (ICPA 2039 × ICPR 2447, ICPA 2043 × ICPR 2671, and ICPA 2043 × ICPR 3467) segregating for the fertility trait and genetic analysis of the led to the detection of four QTL that controlled pollen fertility restoration in the segregating populations. The four QTLs viz QTL-RF-1 (CcM1522–CcM1821), QTL-RF-2 (CcM0047–CcM2332), QTL-RF-3 (CcM2542–CcM1277) and QTL-RF-4 (CcM0374–CcM1506) located on LGs 06, 11, 06 and 06 explained 14.85%, 15.84%, 20.89%, and 24.17% of the phenotypic variation (PV), respectively for the fertility restoration trait. Later, Saxena *et al.* (2018) recorded pollen fertility data from an F_2 population (ICPA 2039 × ICPL 87119) assayed with GBS technology. QTL analysis identified one major QTL on CcLG08 that explained up to 28.5% phenotypic variance (PV) for A_4 fertility restoration (Saxena *et al.* 2018).

***Mitochondrial sequencing and identification of CMS-inducing elements*:** A compromised crosstalk between cytoplasm and nucleus is reported to cause CMS, and several mitochondrial CMS-associated genes (MCAGs) have been found in plants. Resulting from the extensive rearrangement events in plant mitochondrial genomes, these MCAGs show a chimeric structure comprising fragments of a mitochondrial gene and alien (unknown) sequences and "translate into peptides with membrane-spanning domains" (Bohra *et al.* 2016). The high-throughput sequencing methods have facilitated the generation of whole genome sequences of the mitochondria in different flowering

plants, especially those with CMS cytoplasm (Heng *et al.* 2014). A comparison of these mitochondrial sequences between the CMS line and cognate maintainer line establishes the role of these MCAGs in CMS induction. A high-quality mitochondrial (mt) genome (545.7-kb) was assembled for A_4-CMS line ICPA 2039 with Roche/ 454 FLX and Sanger platforms (Tuteja et al. 2012). Further comparison of this mt genome with cognate fertile line (ICPB 2039), hybrid (ICPH 2433), and *C. cajanifolius* (ICPW 29) identified a set of 13 chimeric MCAGs for CMS in pigeonpea. Further research on expression and structural variation in 34 mitochondrial genes between ICPA 2039 and ICPB 2039 led authors to associate a 10-bp deletion in the *nad7* gene with A_4-CMS. Subsequently, a gel-based marker was developed and successfully validated in a set of nine A_4 CMS lines and cognate fertile lines (Sinha *et al.* 2016). Analysis of the other CMS cytoplasms with the gene-based marker system alludes to the occurrence of diverse mechanisms underlying different CMS cytoplasms of pigeonpea (Sinha *et al.* 2016, Bohra *et al.* 2017). More recently, differential RNA editing patterns in mitochondrial transcripts of male fertile and CMS lines causing no major change in the protein structure were elucidated in pigeonpea (Kaila *et al.* 2019).

***Molecular markers for testing genetic purity of the hybrids and parental lines*:** To support grow-out test (GoT) in hybrid breeding, genetic purity testing kits based on DNA markers have also been developed in pigeonpea. Saxena *et al.* (2010) reported two diagnostic simple sequence repeat (SSR) markers (e.g., CCB4 and CCttc006) for purity testing of the pigeonpea hybrid ICPH 2438, with purity index of CCB4 being 94.2 and 95.6 while CCttc006 showing index of 98.7 and 97.8 for two different seed lots. Similarly, a set of 42 SSRs was identified each for two hybrids (ICPH 2671 and ICPH 2438) for their genetic purity assessment, and importantly, multiplexing of eight SSRs to a single PCR assay was shown as a cost-effective assay to detect genetic purity (Bohra *et al.* 2011). Bohra et al. (2017) demonstrated the utility of hypervariable SSRs for DNA profiling and seed purity testing of pigeonpea hybrids and their inbred lines. Deployment of these genomic tools and technology may help improve the efficiency of hybrid breeding in pigeonpea (Bohra *et al.* 2016, 2017).

***Genomic prediction for high heterosis in pigeonpea*:** Genomic selection (GS) was proposed by Meuwissen *et al.* (2011) to predict the genetic worth of breeding individuals by using the genome-wide genetic marker data. GS does not require the phenotypic data for performing selection in the breeding population. The selections are based on the genomic-estimated breeding values (GEBVs) which are calculated based on the marker effects summed up across the genome. The marker effects are calculated by using the phenotypic and genotypic data of the training population. Genomic prediction was used in pigeonpea to predict the hybrid performance and to identify the high heterotic patterns (Saxena *et al.* 2021). The authors used whole-genome resequencing (WGRS) data for genomic prediction in pigeonpeas. The prediction accuracy of 0.24 was obtained based on the application of ridge regression best linear unbiased prediction model on a training population comprised of 7 females, 35 males, 160 hybrids and 292 inbred lines. Further, two groups of 20 lines each were formed by applying a simulated annealing algorithm on the predicted performance of all more than 78,000 hybrids, and crossing between the lines between the two groups resulted in a 25% higher than average yield of all tested hybrids.

FUTURE STRATEGIES FOR HYBRID PIGEONPEA BREEDING

The following areas need to be emphasized for planning and implementing hybrid pigeonpea breeding in India.

- Develop high-yielding hybrids for specific agro-ecological regions
- Diversify hybrid parents to suit a variety of target environments
- Incorporate resistance to biotic stresses especially wilt, sterility mosaic, and phytophthora blight
- Develop cost-effective hybrid seed production technology with increased efficiency
- Develop seed quality parameters for hybrids and parents
- Conduct basic breeding and agronomy research for increased productivity
- Construction of high heterotic groups and identification of heterotic patterns
- Undertake molecular characterization of hybrids and their parents
- Employment of next-generation genomic tools to increase hybrid breeding efficiency

- Exploring the potential of modern breeding techniques like genomic selection in hybrid breeding

PROSPECTS OF HYBRID PIGEONPEA

Increase in area under pigeonpea: Development and release of pigeon pea hybrids in early and mid-early maturity groups will result in increased coverage of the area and enhanced production under pigeonpea. Since at present there are only a few varieties of pigeonpea under these maturity groups and farmers are demanding more varieties of these groups therefore, hybrids of the early and mid-early groups will be better choices for farmers as hybrids yield more than varieties in the same maturity duration because of early vigor (faster early vegetative growth) which results in completion of vegetative growth in lesser period and crop gets longer period for reproductive phase which leads to increase in grain yield as compared to varieties.

Minimum losses due to attack of insect pests and diseases: Results of limited experiments show that hybrids offer more resistance to disease attack than pure lines by virtue of their greater resilience. Also, the hybrids recover faster and assimilate greater biomass. Evaluation of a few wilt and sterility mosaic-resistant pigeonpea hybrids and pure line cultivars in disease-free and sick plot conditions indicated that under sick conditions in hybrids and pure lines, the level of disease resistance expressed was high with hybrids was enhanced to 60% under disease sick conditions. Hence, it is interpreted that in addition to the specific anti-fungal resistance mechanisms, the hybrids have an extra degree of genotypic plasticity, which helps them to tolerate and produce higher yields under stress conditions compared to the pure lines. In general, the hybrids in most crops express better environmental buffering capacity compared to pure line cultivars. The yield fluctuations brought about by various biotic and biotic stresses could be reduced by introducing hybrids.

Productivity and production of pigeonpea will be increased: Enhanced seedling vigor will result in the completion of vegetative growth at a faster rate in less time and the reproductive phase of the plant life cycle will get more time for flowering, grain development and pod-filling stages. The longer reproductive phase will result in increased grain yield as compared to varieties. Thus per unit area productivity of hybrids will be more as compared to the varieties resulting in higher grain production of pigeonpea.

Early hybrids maturing within 150 days will fit well in the pigeon pea-wheat cropping system in North India: In NWPZ of the country, pigeonpea-wheat crop rotation is in common practice. Some early varieties like UPAS 12 and Pusa 992 sometimes mature in more than 150 days which delays the sowing of wheat crops resulting in lesser production. In this condition, the early hybrids maturing within 150 days will be very much suitable for the timely sowing of wheat in pigeonpea-wheat cropping system.

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