Sustainable Agriculture for Food, Bio-energy and Livelihood Security

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Breeding Hybrids for Enhancing Productivity in Pigeonpea

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INTRODUCTION

Pigeonpea [*Cajanus cajan* (L.) Millsp.] is a versatile plant species, which can grow successfully in a range of soil types, temperatures, and photoperiods. Its deep and strong root system enables it to overcome intermittent droughts and other stresses. Pigeonpea is globally cultivated on 5.25 million hectares with an average production of 3.2 million tonnes. It is grown mainly in semi-arid regions of Asia, Africa, Latin America, and Caribbeans. In India it is predominantly grown in the states of Maharashtra, Uttar Pradesh, Karnataka, Andhra Pradesh, Madhya Pradesh, Bihar, and Gujarat. The adoption of new varieties in India has helped in increasing pigeonpea area from 2.3 million hectares in 1950 to 3.58 million hectares in 2005. However, no increase has been witnessed in the productivity of the crop that has remained unchanged at about 700 kg ha^{-1} and the country is still short in supply of grains by a margin of over 500,000 tonnes annually.

The quantum jump in yield potential observed in some cereals and vegetable crops in the past was primarily due to exploitation of hybrid vigour. In pulses, however, this breeding approach could not be used due to their highly self-pollinating nature and inability to produce large quantities of hybrid seed economically. Pigeonpea, however, is a unique crop where a reasonable extent of natural out-crossing occurs and ICRISAT scientists believe that this trait can be used in breeding commercial hybrids. For a successful commercial hybrid breeding, it is imperative that the hybrids have significant yield advantages over the existing varieties, and their seed production is cost-effective. To achieve this, the most crucial components are (i) a stable CMS system, (ii) a set of diverse fertility restorers, and (iii) a mass pollen transfer mechanism. In pigeonpea, all these components are now available and this has made the commercial hybrids a reality.

NATURAL OUT-CROSSING

The efficiency of any hybrid seed production programme depends on the effectiveness of controlled natural mass pollen transfer mechanism from male to female parent. This ensures economical production of quality hybrid seed. Williams (1977) reported that *Apis mellifera* and *Megachile* species of insects play an important role in natural out–crossing in pigeonpea. In the process of collecting nector from flowers large quantities of pollen get attached to their bodies, which affect cross-pollination. On an average, the extent of natural out-crossing is about 25% but it may vary in either direction depending upon the intensity of insect population. In a review conducted by Saxena *et al.* (1990) it was revealed that in most places where pigeonpea is grown the natural out-crossing occurs and the level of out-crossing is sufficient to undertake an effective hybrid-breeding programme.

CYTOPLASMIC-NUCLEAR MALE-STERILITY (CMS) SYSTEMS

The cytoplasmic - nuclear male-sterility (CMS) system is the most widely accepted means of producing commercial hybrids in field crops. The expression of CMS, in part, is controlled by the factors carried only through the female parent, which is never lost or diluted in the succeeding generations. Nuclear genes generally influence the expression of this trait. The male-sterility effects are conditioned by interactions among various nuclear and cytoplasmic factors. The cytoplasmic factor is referred to as 'N' for normal fertile cytoplasm and 'S' for the sterile cytoplasm. The CMS lines are maintained by these two cytoplasmic factors. The CMS (A-line) must have 'S' cytoplasm and nuclear genes '*msms*', and the maintainer B-line must have a normal cytoplasm with '*msms*' nuclear gene. The F1 between A- and B-line, therefore, is always male-sterile since the 'N' cytoplasm responsible for fertility in the B-line is not transferred to the F1. For producing fertile hybrid seed, the A-line in 'S' cytoplasm is crossed with a fertile line with fertility restorer nuclear genes, commonly known as '*Fr*' genes. To sum up, the three-line system is geared for multiplying A-line with the help of B- line and for producing the hybrid seed, the A-line is crossed with R-line.

The development of pure breeding CMS system in pigeonpea would facilitate the commercial seed production. Since, the search for a functional male-sterility in the germplasm succeeded in identifying only genetic male-steriles, the efforts to develop CMS were made through planned plant breeding. The first such unsuccessful attempt was made by crossing *C. scarabaeoides,* a wild relative of pigeonpea, with a cultivated type (Reddy and Faris, 1981). Ariyanayagam *et al.* (1995) crossed *C. sericeus* with a cultivated type and in the backcross populations male-sterility was observed. The reversion of some male-sterile plants to fertility always complicated the selection and stabilization of this trait. Intensive selection in additional backcross generations, however, resulted in the identification of a promising CMS line (Saxena *et al.,* 1996). This was designated as A_1 cytoplasm. The scientists at Gujarat Agricultural University and ICRISAT simultaneously developed a CMS system with *C. scarabaeoides* as cytoplasm donor (Tikka *et al.,* 1997; Saxena and Kumar, 2003) and it was designated as A_2 cytoplasm. At Akola, another CMS system was developed (Wanjari *et al.,* 2001) from an inter-specific crosses involving *C. volublis* (A_3 cytoplasm). The recently developed CMS system, designated as A_4 cytoplasm (Saxena *et al.,* 2005), has the cytoplasm of *C. cajanifolius,* another wild relative of pigeonpea (Table 1). The male-sterile plants in this material show no morphological deformity and produce plenty of pollen grains in hybrid combinations with fertility restorers. This male-sterile source is stable and capable of producing high-yielding hybrids (Saxena *et al.,* 2006). Therefore, it has a good potential in commercial hybrid breeding.

TABLE 1: Segregation of male-sterility over generations in A4 CMS system

Generation	Total plants	Fertile
BC_7F_1	17186	0
BC_6F_1	1133	0
BC_5F_1	67	0
BC_4F_1	7	0
BC_3F_1	165	0
BC_2F_1	5	1

HIGH-YIELDING HYBRIDS

In 2005 season, a number of short-duration hybrids involving *A4* cytoplasm were evaluated at five locations (Table 2). Of these, ICPH 2438 ranked first with mean yield of 2893 kg ha^{-1} and mean superiority of 106% over the control ICPL 88039 (1403 kg ha^{-1}). The other promising hybrids were ICPH 2363 (2447 kg ha^{-1}, 74% superiority), ICPH 2429 (2032 kg ha^{-1}, 45% superiority), and ICPH 2364 (1940 kg ha^{-1}, 38% superiority). In the medium-duration group also several hybrids were evaluated at four locations, during 2005 (Table 3). Among these, hybrid ICPH 2744 (2790 kg ha^{-1}) at Patancheru, ICPH 2788 (4166 kg ha^{-1}) at Jalna, ICPH 3331 (3476 kg ha^{-1}) at Manoharabad, and ICPH 3331 (3185 kg ha^{-1}) at Medchal were found promising. Overall, the promising hybrid combinations were ICPH 2788 (3263 kg ha^{-1}), ICPH 2740 (3066 kg ha^{-1}), and ICPH 3331 (2917 kg ha^{-1}). These hybrids exhibited 47 - 64% superiority over the control Asha (1989 kg ha^{-1}).

TABLE 2: Yield (kg ha^{-1}) of top five short-duration pigeonpea experimental hybrids in multi-location trials, 2005

Hybrid	Patancheru (ICRISAT)	Jalna (MAHYCO)	Manoharabad (Zuari Seeds)	Medchal (JK Seeds)	Nagpur (Ankur Seeds)	Mean	Superiority over control (%)
ICPH 2438	3414	3792	1518	2236	3504	2893	106
ICPH 2363	2736	3958	1012	2060	2471	2447	74
ICPH 2429	2177	2625	1577	2024	1758	2032	45
ICPH 2364	2375	2300	1101	1696	2229	1940	38
ICPH 2460	2454	2708	1429	1238	1479	1862	33
ICPL 88039 (c)	1306	1750	1042	736	2179	1403	-
SEm (±)	472.2	NR	149.3	288.5	197.6		
CV (%)	28.2	9.5	16.0	24.4	12.1		

NR- not reported

TABLE 3: Yield (kg ha^{-1}) of top five medium-duration pigeonpea experimental hybrids in multi-location trials, 2005

Hybrid	Patancheru (ICRISAT)	Jalna (MAHYCO)	Manoharabad (Zuari Seeds)	Medchal (JK Seeds)	Mean	Superiority over control (%)
ICPH 2728	2525	4166	3238	3124	3263	64
ICPH 2740	2761	4041	2524	2936	3066	54
ICPH 3331	2216	3791	3476	3185	2917	47
ICPH 2671	2671	3416	2571	2996	2913	46
ICPH 2751	2171	3125	2381	3493	2793	40
Asha (c)	1927	3124	1333	1570	1989	-
SEm (±)	207.7	NR	540.7	331.0		
CV (%)	13.7	7.3	32.2	21.1		

NR- not reported

SEED PRODUCTION TECHNOLOGY OF HYBRIDS AND THEIR PARENTS

Isolation specifications: Since the extent of natural out-crossing in pigeonpea is determined by the population of pollinating insects, its extent may vary from one place to another. Therefore, it is difficult to specify isolation distances for different seed classes that would be effective at every location. We, however, need to develop some safe and standard guidelines to maintain the purity standards of the hybrids. Overall we feel that an isolation distance of 500 m will be sufficient for quality hybrid seed production in pigeonpea.

Seed production of A-lines: The nucleus seed of parental lines of hybrids should be produced with highest standards of genetic purity. For multiplying nucleus seed both A- and B- lines should be grown, preferably inside an insect proof cage. Each and every plant of A- and B- lines should be examined for various parameters and off-types be rogued and crossing should be done to produce the desired number of seeds. For the production of breeder's seed of A-line, a field with appropriate isolation distance is selected and A- and B- lines are grown with recommended agronomic package. At ICRISAT, a ratio of 4 rows of A-line: 1 row of B-line has been found effective in producing seed of a given A-line. In the seed multiplication of ICPA 2039 at ICRISAT 27 kg of crossed seed was harvested from 225 m^2 block with an estimated yield of 1111 kg ha^{-1}. In another isolation of the same male-sterile line, 200 kg seed @ 877 kg ha^{-1} was harvested. The seed thus harvested be dried and packed after treating with Malathion powder @ 1 g kg^{-1} seed. In the short-duration materials, the mature pods on male-sterile and fertile plants be harvested by pod picking or by cutting the top pod bearing portion of the plants. The perennial nature of species will force the plants to re-generate and produce a second flush of flowers and pods.

Seed production of B- and R- lines: The maintainer (B) and restorer (R) lines are male-fertile in nature and their genetically pure seed can be produced without much difficulty. For nucleus seed production of B- and R- lines, about 100 plants are harvested from the central portion of the breeder's seed production plot and their progenies be grown in the subsequent generation. After assessing their purity aspects, the selected progenies are bulked to serve as nucleus seed. For breeder seed production the nucleus seed should be multiplied in separate isolation. It is also important to multiply seed in large quantities so that foundation seed production is feasible. Always sufficient care should be taken to rogue the off-types as and when such plants are identified in the seed production plot.

Seed production of hybrids: In a three-line hybrid seed production system, the hybrid seed produced by crossing A- line with R- line, is commonly called certified seed and it is grown on a larger scale. For the production of certified seed of hybrids the A-line and its pollen parent (R- line) are grown in 4:1 ratio in an isolated block. Some additional rows of pollen parent can also be sown on each side of the plot. This will enhance pollen availability for cross-pollination. At flowering the pollinating insects will visit the male and female flowers in a random fashion and in the process collect pollen from fertile plants and carry out hybridization on the male-sterile plants. Using this technology in 2005 rainy season, from a small (120 m^2) isolation a total of 15 kg seed @ 1250 kg ha^{-1} of hybrid ICPH 2671 was produced. For optimum seed yields the male : female row ratio (1:4) should not be recommended for all the environments and depending on the insect activity and time of sowing, it should be modified.

Seed production cost of hybrids: Seed cost plays an important role in adoption of hybrids. Both, the technology itself and crop management practices are critical in determining the production costs. So far no study has been conducted to determine the production cost of CMS-based hybrids and their parents. A detailed cost of seed production study undertaken by Tamil Nadu Agricultural University, Coimbatore using the GMS-based hybrid system it was found that 813 kg ha^{-1} of hybrid seed was produced at the cost of Rs 6.25 kg^{-1}. (Murugrajendran *et al.,* 1990). Since in the CMS-based hybrid technology the rouging operation is eliminated and hence the production cost of both female parent and hybrid will certainly reduce by a significant margin. A detailed study on this aspect is needed.

DIVERSIFICATION OF HYBRID PARENTS

ICRISAT has a strong hybrid pigeonpea breeding programme with increased emphasis on disease and insect resistance, stability and yield. At present we have 31 CMS lines with a large variation for important agronomic traits (Table 4). Seven of these A-lines also have high level of resistance to wilt and sterility mosaic diseases. We have also developed 91 fertility restorers in different maturity groups (Table 5). Of these, 37 restorers are resistant to wilt and sterility mosaic diseases. This genetic materials can be used to develop hybrids suitable for diverse environments.

TABLE 4: Variation for important agronomic traits among A-lines

Characters	Range
Days to flower	55 – 135
Days to maturity	100 – 198
Plant height (cm)	74 – 245
100-seed mass (g)	8.6 – 13.8
Seeds pod-1	3.2 – 5.6

TABLE 5: Fertility restorers of A4 cytoplasm available in different maturity groups at ICRISAT

Maturity group	Lines	Days to flower	Seed pod^{-1}	100-seed mass (g)
Extra-short	15	<70	3.0 – 4.1	6.2 – 12.7
Short	17	71 – 80	3.0 – 4.1	6.7 – 12.1
Medium	45	81 – 130	3.4 - 4.5	7.5 – 14.7
Long	14	>130	3.3 – 4.9	7.7 – 14.0

NEW FRONTIERS AND STRATEGIES

The development and promotion of pure line varieties has helped in stabilizing the productivity and in increasing the production at the national level. But unfortunately the productivity has shown no sign of improvement. The advent of hybrid technology has given us hope that the stagnated yield could be increased. To achieve a quantum jump in the productivity level of pigeonpea a good beginning has been made by ushering in the hybrid breeding. The development of stable CMS system in pigeonpea is a real boon to the breeders. The present achievements have established the hybrid technology but a lot needs to be done in the next decade to show the impact of this technology in farmers' field and for this more resources will be required to develop the parental materials suitable for diverse environments. The next most important target will be to develop hybrids with insecticidal genes with high quality.

To enhance the pace of research and development of hybrid pigeonpea ICRISAT is actively involved in technology transfer to national research system, including both public and private sector seed companies. Now, our major responsibility is to take this product to our clients - the farmers. We expect that both small- and large-holder farmers will show interest in the hybrids. However, since pigeonpea is predominantly cultivated by small, resource-poor farmers, we need to keep the seed costs within the reach of our clients. In this partnership the sharing of knowledge, breeding materials, and experience are our major strength.

The partnership between ICRISAT and private sector seed companies is strong and it has evolved over time. In the early years, ICRISAT played a nurturing role to the fledgling industry through informal networks. As private seed industry grew, it started to develop a significant research capability of its own and now they are our research partners – both as a source of funds and complementary expertise, especially in the area of development (Gowda *et al.*, 2004).

Considering that each partner will contribute small amount of funds, ICRISAT initiated the concept of "consortia", under which a consortium member provides a small grant to augment research efforts. So far 12 private and one public sector seed companies (Table 6) have joined the Pigeonpea Hybrid Parents Research Consortium. Since last three years this consortium model is operating smoothly, and over 3000 seed samples of breeding materials and hybrid parents have been made available (Table 7) to the partners.

TABLE 6: List of pigeonpea hybrid parents research consortium members

Sr. No	Name of seed company
1.	Ankur Seeds Pvt. Ltd., Nagpur, Maharashtra
2.	Biogene Agri-tech., Ahmedabad, Gujarat
3.	Bioseed Research India Pvt. Ltd., Hyderabad, Andhra Pradesh
4.	JK Agri Genetics Ltd., Hyderabad, Andhra Pradesh
5.	Krishidhan Seeds Ltd., Jalna, Maharashtra
6.	Maharashtra Hybrid Seeds Co. Ltd., Jalna, Maharashtra
7.	Maharashtra State Seed Corporation Limited, Akola
8.	Nath Seeds Pvt. Ltd., Aurangabad, Maharashtra
9.	Nimbkar Seeds Pvt. Ltd., Phaltan, Maharashtra
10.	Nuziveedu Seeds Ltd., Secunderabad, Andhra Pradesh
11.	Pradham-Biotech Pvt Ltd., Hyderabad, Andhra Pradesh
12.	SM Sehgal Foundation, ICRISAT, Hyderabad, Andhra Pradesh
13.	Zuari Seeds Limited, Bangalore, Karnatka

TABLE 7: Summary of pigeonpea hybrid breeding materials supplied by ICRISAT

	2006	2005	2004	Total
Private seed companies	1881	788	60	2729
Public seed companies	32	-	-	32
ICAR institutes	47	40	3	90
Agricultural Universities	154	66	45	265

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