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Original article

Development of new cytoplasmic-genetic male-sterile lines in pigeonpea from crosses between *Cajanus cajan* (L.) Millsp. and *C. scarabaeoide*s (L.) Thouars

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Abstract. Exploitation of hybrid vigour is quite possible in cross-pollinated crops. However, pigeonpea is a grain legume crop with a moderate level of cross-pollination (20–70%), which is mainly aided by insect pollinators. As a first step, hybrids based on genetic male sterility (GMS) were developed in pigeonpea, but the hybrid seed production technique is not farmer-friendly, because in the hybrid seed production plot 50% of the population, which are male-fertile in the female rows, have to be eliminated in time before contamination. This requires skilled labour and is a time-consuming process, which increases the cost of hybrid seed production. Therefore, the objective of this study was to develop cytoplasmic-genetic male-sterile (CGMS) lines in pigeonpea through wide hybridization, which would be very suitable for hybrid seed production. Two CGMS lines, viz. CORG 990052 A and CORG 990047, were developed by interspecific hybridization of *Cajanus cajan* and *C. scarabaeoides.* Restorers were identified and three CGMS-based pigeonpea hybrids were developed. The hybrid COPH 3 is found to be promising in Tamil Nadu State, India.

Keywords: *Cajanus cajan*, *Cajanus scarabaeoides*, fertility restoration, hybrid, male sterility, pigeonpea.

Introduction

Pigeonpea or redgram is an important crop in India, where it is the second most important pulse crop after chickpea. Besides India, also Uganda, Kenya, the West Indies (Puerto Rico and the Dominican Republic in the Caribbean region), and Burma are the major pigeonpea-producing countries. It is grown in a wide range of soils (from sandy to heavy soils, pH of 5.0 to 8.0). It is a tropical or sub-tropical plant, extending between 30ºN and 30ºS latitude, and cannot tolerate even light frost during any stage of its growth. It appears to be better adapted to marginal climatic conditions than many other pulse crops.

Of all the pulse crops, pigeonpea is the only crop showing more than 70% outcrossing (Saxena 2001). Khan (1973) reported 3–4% outcrossing. A very high degree of outcrossing, up to 94.5%, has been reported from Kenya (Onim 1981). A large number of factors – such as the abundance of insect pollinators in relation to the number of flowers, flowering habit of varieties grown, location of fields in relation to insect habits, distance between the unlike varieties, barrier crops, and other environmental factors, like wind velocity – determine the amount of outcrossing in pigeonpea (Bhatia et al. 1981).

Pigeonpea research was started at the Tamil Nadu Agricultural University in the 1950s. Several varieties, viz. Co 1, Co 2, Co 4 and SA 1, were developed on the basis of pure line selection, although the crop is often cross-pollinated. Later on, in the 1970s, mutation breeding was exploited, and varieties Co 3, Co 5 and Co 6 were released. After mutation, recombination breeding was attempted and new varieties, viz. Vamban 1 [a double cross derivative of (Prabath HY 3A) (T 21

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102)] and Vamban 3 (Vamban 1 Gulbarga), were developed. Though several improved varieties were developed, a quantum jump in grain yield could not be realized in pigeonpea production for a long time.

Pigeonpea has a substantial amount of non-additive genetic variance (Sharma et al. 1973; Saxena et al. 1981) and hybrid vigour for yield (Solomon et al. 1957). The discovery of stable genetic male sterility (Reddy et al. 1978), coupled with its outcrossing nature (Saxena et al. 1983), has opened the possibility of commercial utilization of heterosis in pigeonpea. Hybrids COPH 1 and COPH 2 were developed by using genetic male sterility (GMS). However, the hybrid seed production with a genetically determined male-sterile parent in pigeonpea poses some problems with prompt identification and removal of male-fertile sibs, which account for 50% of the population within female rows of a the hybrid seed production field. It is time- and labour-intensive, involving 40–50% of the seed production cost (Muthiah et al. 1998). Inefficiency in eliminating the fertile sibs reduces the quality of the hybrid seed. Further, the removal of 50% of the population (fertile sibs) results in reduced yields. The first attempt to develop cytoplasmic-genetic male-sterile (CGMS) lines in pigeonpea by using the crossable wild relatives of pigeonpea was made by Reddy and Faris (1981). Ariyanayagam et al. (1995) reported *Cajanus sericeus* as the cytoplasmic male sterility (CMS) source. The first CGMS line of GT 288A was developed by using *C. scarabaeoides* at Gujarat Agricultural University, S. K. Nagar, India (Tikka et al. 1997). Consequently, several scientists have succeeded in identifying male-sterile segregants from the interspecific crosses involving *C. volubilis* (Wanjari et al. 2001), *C. acutifolius* (Rathnaswamy et al. 1998a; Mallikarjuna and Saxena 2002), and *C. cajanifolius* (Saxena et al. 2005b), while Mallikarjuna and Saxena (2005) reported a CMS source from the cultivar itself (*C. cajan*). The experience with GMS hybrid technology has conclusively demonstrated that in pigeonpea the exploitation of hybrid vigour is possible if the seed production techniques are optimized (Saxena et al. 1998; Rathnaswamy et al. 1998b). Hence, it was felt that hybrid breeding could be revolutionized if the CGMS system is used (Saxena et al. 1998; Sharma and Saxena 2003; Saxena et al. 2005a; Pandey et al. 2005; Saxena et al. 2006). Therefore, development of CGMS lines in pigeonpea was attempted in this study, conducted at the Department of Pulses, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore, India.

Material and methods

Five cultivars, viz. Co 5, ICPL 83027, ICPL 87, CORG 9060 and CORG 9061, were selfed for 2 seasons to ensure genetic purity. Three wild species were obtained from the International Crop Research Institute for Semi-Arid Tropics (ICRISAT), Patancheru, India. The cultivars were then crossed with the wild species *C. acutifolius* (Accession No. ICPW 2), *C. scarabaeoides* (Accession No. ICPW 280), and *C. cajanifolius* (Accession No. ICPW 28) by artificial emasculation followed by hand crossing in 1997. They were crossed in a paired cross fashion in the crossing block at the Department of Pulses, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore, India.

The interspecific F_1 hybrids were sown in 1998. The mature anthers of the F_1 plants were observed under a microscope by using 2% acetocarmine. One F_1 plant from the combination *C. scarabaeoides* (Acc. No. ICPW 280) CORG 9060, which showed 96.% male sterility under a microscope, was backcrossed with CORG 9060 for 6 generations for genome substitution. One plant from the combination *C. scarabaeoides* (Acc. No. ICPW 280) CORG 9061, which showed 96% male sterility, was also back-crossed with its recurrent parent CORG 9061 for 6 generations in order to get the whole genome of CORG 9061 in the background cytoplasm of *C. scarabaeoides.* The male-sterile BC_6F_1 population of the combination *C. scarabaeoides* CORG 9060 was designated as CORG 990052 A and the maintainer as CORG 990052 B, while the male-sterile BC_6F_1 population of the combination *C. scarabaeoides* CORG 9061 was designated as CORG 990047 A and the maintainer as CORG 990047 B. For large-scale seed multiplication of these 2 male-sterile lines, they were grown in isolation in 2003. Lines A and B were sown in 4:1 ratio. These plants were sown in 60 cm × 20 cm spacing in order to record the morphological characters. Five plants were randomly selected from each line A and B. The data on days to first flower, plant height, number of primary branches per plant, pods per plant, pod length (cm), seeds per pod, and 100-seed weight (g), were recorded and analysed statistically. During flowering, the an-

thers of all the plants of both lines A were observed for the presence of stained (fertile) and unstained (sterile) pollen grains. Simultaneously, both lines A were studied for their stability in various locations of Tamil Nadu.

In order to identify restorers for both lines A, the male-sterile BC_6 F_1 plants were crossed with 80 early-maturing lines in 2003. The F_1 plants were grown during the summer of 2004 and studied for fertility restoration. The F_1 plants of combinations CORG 990052 A × CORG 9904 and CORG 990047 A × ICPL 84031 and ICPL 90028, recorded 96–98% male fertility. In 2004 the same hybrid combinations were made, and more hybrid seeds were produced by hand crossing for confirmation of restorers. The restorer for CORG 990052 A was designated as CO 1 R, and the restorers ICPL 84031 and ICPL 90028 were designated as CO 2 R and CO 3 R, respectively.

During the summer of 2005, a hybrid trial was performed, consisting of 3 CGMS hybrids, viz. COPH 3 (CORG 990052 A × CO 1 R), COPH 4 (CORG 990047 A × CO 2 R), and COPH 5 (CORG 990047 A × CO 3 R), and 2 controls, namely COPH 2 (ms Co 5 × ICPL 83027, a GMS-based hybrid), and Co(Rg) 7 (a high-yielding local variety). They were evaluated in a randomized block design with 5 replications. In 2005, the test hybrids COPH 3 and COPH 4 and controls COPH 2 and Co(Rg) 7 were evaluated in multilocation trials in Tamil Nadu.

Results and discussion

Development of CGMS lines CORG 990052 A and CORG 990047 A

A total of 200 crosses were made in the 15 cross combinations with the 3 wild species and 5 cultivars. The pod set percentage was very low (2–4%). Since they were interspecific hybrids, the pods were shrivelled and mostly single-seeded (90–100%). The number of male-fertile/sterile plants in various F_1 combinations is presented in Table 1.

Out of 23 plants in the F_1 combination of *C. scarabaeoides* × CORG 9060, 22 plants showed yellow anthers and were male-fertile when stained and observed under a microscope. Only one plant of the same combination had pale yellow, small and sticky anthers and recorded 100% pollen sterility. Therefore, this plant was back-crossed with the recurrent parent (CORG 9060) up to BC_6 F_1. The male-sterile BC_6 F_1 population was designated as CORG 990052 A and the maintainer as CORG 990052 B.

Among all the 22 F_1 plants of the combination *C. scarabaeoides* × CORG 9061, 20 plants exhibited 90–96% pollen fertility, while 2 plants exhibited 94–96% pollen sterility. The plants that exhibited pollen sterility were backcrossed with CORG 9061 up to 6 generations. The male-sterile BC_6F_1 population was designated as CORG 990047 A and the maintainer as CORG 990047 B.

The male-sterile lines of BC_1 F_1 to BC_6F_1 had sterile pollen grains, while lines B exhibited 100% pollen fertility.

Stability of CORG 990052 A and CORG 990047 A

A total of 100 seeds of CORG 990052 A and CORG 990047 A each were supplied to various research stations of the Tamil Nadu Agricultural University (viz. Regional Research Station, Paiyur; Agricultural Research Station, Pattukkottai; Agricultural Research Station, Bhavanisagar; Agricultural Research Station, Virinjupuram; and National Pulses Research Centre, Vamban) for multilocation trials and evaluated in 2003. In all the locations, plants of both lines were 100% male-sterile without pollen production, inferring that they were stable over locations. At Coimbatore, lines A were sown on 15th July, 31st January and 4th April, and the anthers were observed during flowering. All pollen grains were sterile in the male-sterile plants, and this enabled concluding that lines A were stable over locations and seasons (Table 2).

Morphological description

The lines CORG 990052 A and B were isogenic except for anther colour, reflecting male sterility/fertility. The plants were erect, compact and indeterminate in growth habit. The stems were green and flowers had faint red veins on the dorsal side of the standard petal. The anthers of line A were small, pale yellow and sticky, with sterile pollen grains. The plants of CORG 990052 A grew up to a height of 110.5 ± 1.1 cm and started to flower 68.1 ± 0.3 days after sowing. The pods were green with purple streaks, 5.80 ± 0.05 cm long, and produced 3.45 ± 0.058 seeds/pod. The seeds were oval and orange in colour, with 100-seed weight of 8.1 ± 0.1 g (Table 3).

First flowering was, on average, 3 days earlier in CORG 990052 B than in CORG 990052 A, which was similar to the case of male-sterile and male-fertile segregants of the GMS system, viz.

Table 1. Pigeonpea pollen fertility/sterility in interspecific F1 hybrid combinations.

Cajanus hybrid combinations	No. of plants		
	total	male-sterile	male-fertile
C. acutifolius (Acc. No. ICPW 2) × Co 5	26	0	26
C. scarabaeoides (Acc. No. ICPW 280) × Co 5	30	0	30
C. cajanifolius (Acc. No. ICPW 28) × Co 5	25	0	25
C. acutifolius (Acc. No. ICPW 2) × ICPL 83027	20	0	20
C. scarabaeoides (Acc. No. ICPW 280) × ICPL 83027	20	0	20
C. cajanifolius (Acc. No. ICPW 28) × ICPL 83027	20	0	20
C. acutifolius (Acc. No. ICPW 2)× ICPL 87	23	0	23
C. scarabaeoides (Acc. No. ICPW 280) × ICPL 87	22	0	22
C. cajanifolius (Acc. No. ICPW 28) ×ICPL 87	20	0	20
C. acutifolius (Acc. No. ICPW 2) × CORG 9060	25	0	25
C. scarabaeoides (Acc. No. ICPW 280) × CORG 9060	23	1	22
C. cajanifolius (Acc. No. ICPW 28) × CORG 9060	24	0	24
C. acutifolius (Acc. No. ICPW 2) ×CORG 9061	20	0	20
C. scarabaeoides (Acc. No. ICPW 280) × CORG 9061	22	2	20
C. cajanifolius (Acc. No. ICPW 28) × CORG 9061	22	0	22

Table 2. Male sterility of pigeonpea CGMS lines CORG 990052 A and CORG 990047 A over locations and seasons

No.	Location and season	CORG 990052 A			CORG 990047 A		
		total no. of plants	male-sterile plants	male sterility (%)	total no. of plants	male-sterile plants	male sterility (%)
1	Regional Research Station, Paiyur: June–July	98	98	100	96	96	100
2	Agricultural Research Station, Pattukkottai: June–July	99	99	100	94	94	100
3	Agricultural Research Station, Bhavanisagar: June–July	98	98	100	96	96	100
4	Agricultural Research Station, Virinjipuram: June–July	96	96	100	98	98	100
5	National Pulses Research Centre, Vamban: June–July	94	94	100	94	94	100
6	Tamil Nadu Agricultural University, Coimbatore						
	15th June sowing	100	100	100	98	98	100
	31st January sowing	98	98	100	96	96	100
	1st April sowing	100	100	100	96	96	100

ms Co 5, ms Prabhat DT, ms Prabhat NDT, and ms T 21 (Rathnaswamy et al. 1997, 1998b; Kalaimagal and Muthiah 2004). Flowering duration was also longer in line A than in line B, as lines A reached maturity after 121 days, while line B after 110 days. Such differences were also observed in the case of male-sterile and male-fertile segregants of the GMS system, as reported by Rathnaswamy et al. (1996, 1997) and Kalaimagal and Muthiah (2004). The actual causes for these differences need to be studied.

The lines CORG 990047 A and B were isogenic except for male sterility/fertility, too. The plants were semi-spreading, with branches 12.9 ± 0.4 cm long, indeterminate, green stem, and yellow flowers with faint red veins on the dorsal side of standard petals. The plants grew up to 137.0 ± 2.0 cm and started to flower 74.2 ± 0.4 days after sowing. The pods were green with purple streaks, 5.81 ± 0.2 cm long, and produced 3.9 ± 0.68 seeds/pod. The seeds were oval and orange in colour, with 100-seed weight of 9.4 ± 0.1 g (Table 3).

A difference of 3–4 days between lines A and B for first flowering (line B flowered first) and a difference of 12 days for days to maturity was observed. The reason was the extension of the flowering period in line A. The overall pod set of a line B plant is 5.9–9.4% (which was observed by marking the flowers formed every day), while that

Table 3. Morphological description of pigeonpea CMS lines CORG 990052 A and B, as well as CORG 990047 A and B

Character	CORG 990052 A	CORG 990052 B	CORG 990047 A	CORG 990047 B
Days to first flowering	68.1 ± 0.3	65.2 ± 0.2	74.2 ± 0.4	71.0 ± 0.3
Plant height (cm)	110.5 ± 1.1	112.0 ± 2.0	137.0 ± 2.0	134.1 ± 0.18
Primary branches per plant	8.9 ± 0.20	9.0 ± 0.18	12.9 ± 0.4	13.2 ± 0.38
Pods per plant	138.1 ± 3.86	135.2 ± 4.0	188.2 ± 4.0	185.1 ± 5.0
Pod length (cm)	5.80 ± 0.05	5.85 ± 0.045	5.78 ± 0.06	5.86 ± 0.04
Seeds per pod	3.45 ± 0.058	3.50 ± 0.10	3.81 ± 0.68	3.76 ± 0.56
100-seed weight (g)	8.1 ± 0.10	8.2 ± 0.15	9.4 ± 0.10	9.8 ± 0.20
Plant type	erect, compact	erect, compact	semi-spreading	semi-spreading
Growth habit	indeterminate	indeterminate	indeterminate	indeterminate
Stem colour	green	green	green	green
Flower colour Standard petal	yellow faint red veins on dorsal side	yellow faint red veins on dorsal side	yellow faint red veins on dorsal side	yellow faint red veins on dorsal side
Anther	small, pale yellow, sticky	yellow, normal size with fertile pollen grains	small, pale yellow, sticky	yellow, normal size with fertile pollen grains
Seed colour	orange	orange	orange	orange
Days to maturity	120–130 days	120–130 days	120–130 days	120–130 days

of line A plants is 6.1–9.4%. There was more flower drop in line A during the initial flowering phase, while in line B during the late flowering phase. A detailed physiological study needs to be undertaken to throw more light on this aspect.

Identification of restorers

In any hybrid breeding program, identification of potential restorers forms an important part. Therefore, 80 cross combinations with both lines A were developed and tested for their restorability. One restorer CORG 9904 was identified for CORG 990052 A, with 96.2–98.6% fertility restoration, and 2 restorers, viz. ICPL 84031 and ICPL 90028, were identified for CORG 990047 A, with 90.2–98.2% and 91.0–96.0% fertility restoration, respectively. Although the CMS source was the same, these lines A did not share a common restorer, but this may be due to differential intergenomic or cytoplasmic–genomic interactions. Also within a hybrid combination the plants showed differential fertility restoration, and this may be due to the heterogeneity within the restorer. To override this problem, the plants that show a higher fertility restoration alone have to be selected to form the seed source for the restorer. The restorer CORG 9904 is designated here as CO 1 R, while ICPL 84031 as CO 2 R and ICPL 90028 as CO 3 R. The restorers ICPL 84031 R and ICPL 90028 R were determinate in growth habit, while CORG 9904 R was indeterminate in growth habit.

Out of the 160 F_1 combinations of both lines A, 6 combinations showed 100% male sterility, inferring that the corresponding pollen parents can be developed into new male-sterile lines.

Performance of the hybrids

The hybrid trial with COPH 3 (CORG 990052 A × CO 1 R), COPH 4 (CORG 990047 A × CO 2 R), COPH 5 (CORG 990047 A × CO 3 R), and the controls COPH 2 and Co(Rg) 7 showed that the yield of COPH 3 was 16.2% higher than in COPH 2 and 29.4% higher than in Co(Rg) 7, while that of COPH 4 was on a par with COPH 2 and 11.3% higher than in Co(Rg) 7. The hybrid COPH 5 was on a par with the control variety Co(Rg) 7 and did not show any hybrid vigour (Table 4). Similar trials were conducted at various locations (viz. Bhavanisagar, Melalathur, Paiyur, Pattukkottai, Vamban and

Table 4. Grain yield and fertility status of pigeonpea hybrids at Coimbatore – summer 2005

Yield and fertility status	COPH 3	COPH 4	COPH 5	COPH 2	Co(Rg) 7
Grain yield (kg/ha)	1255	1080	970	1080	970
Yield increase over COPH 2 (%)	16.2	0	–10.2	0	–10.2
Yield increase over Co(Rg) 7 (%)	29.4	11.3	0	11.3	0
Fertility status	96.0	93.0	96.2	100.0	100.0

Table 5. Grain yield and fertility status of pigeonpea hybrids at various locations of Tamil Nadu in June–July 2005

No.	Location	COPH 3		COPH 4		COPH 5		Co(Rg) 7	
		yield (kg/ha)	fertility (%)	yield (kg/ha)	fertility (%)	yield (kg/ha)	fertility (%)	yield (kg/ha)	fertility (%)
1	Tamil Nadu Agricultural University, Coimbatore	1300	96.2	1250	94.7	1020	100	950	100
2	National Pulses Research Centre, Vamban	1078	92.1	887	94.5	980	100	1008	100
3	Agricultural Research Station, Bhavanisagar	1031	95.1	963	93.1	788	100	985	100
4	Regional Research Station, Paiyur	1294	95.6	1283	94.0	898	100	964	100
5	Agricultural Research Station, Pattukkottai	792	92.5	416	92.7	475	100	696	100
6	Sugarcane Research Station, Melalathur	1100	92.3	1000	93.1	980	100	875	100

Coimbatore) in June–July 2005. The results showed that the grain yield of COPH 3 was 20.4% higher than in the highest control Co(Rg) 7, while that of COPH 4 was 5.9% higher than in that control (Table 5). Pollen fertility was also studied in all the locations. The results revealed that the pollen fertility of COPH 3 and COPH 4 ranged from 92.30 to 96.20 and from 92.70 to 94.70, respectively. There was normal pod set in all the hybrid plants, and seed filling was also good.

Conclusions

Improved pigeonpea varieties were gradually developed by pure line selection, mutation breeding, and recombination breeding, but afterwards no quantum jump in grain yield could be realized in pigeonpea production. Therefore, exploitation of heterosis was considered useful, and hybrids COPH 1 and COPH 2 were developed by using GMS. Though the hybrids recorded good yield and were stable, they could not be popularized to the expected level because of the constraints of hybrid seed production and higher hybrid seed cost, which was primarily due to GMS. A new promising CGMS pigeonpea hybrid COPH 3 was developed in this study by using *C. scarabaeoides* as a CMS source. Therefore, the development of CGMS-based pigeonpea hybrids should open new vistas in pigeonpea production of Tamil Nadu and India.

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