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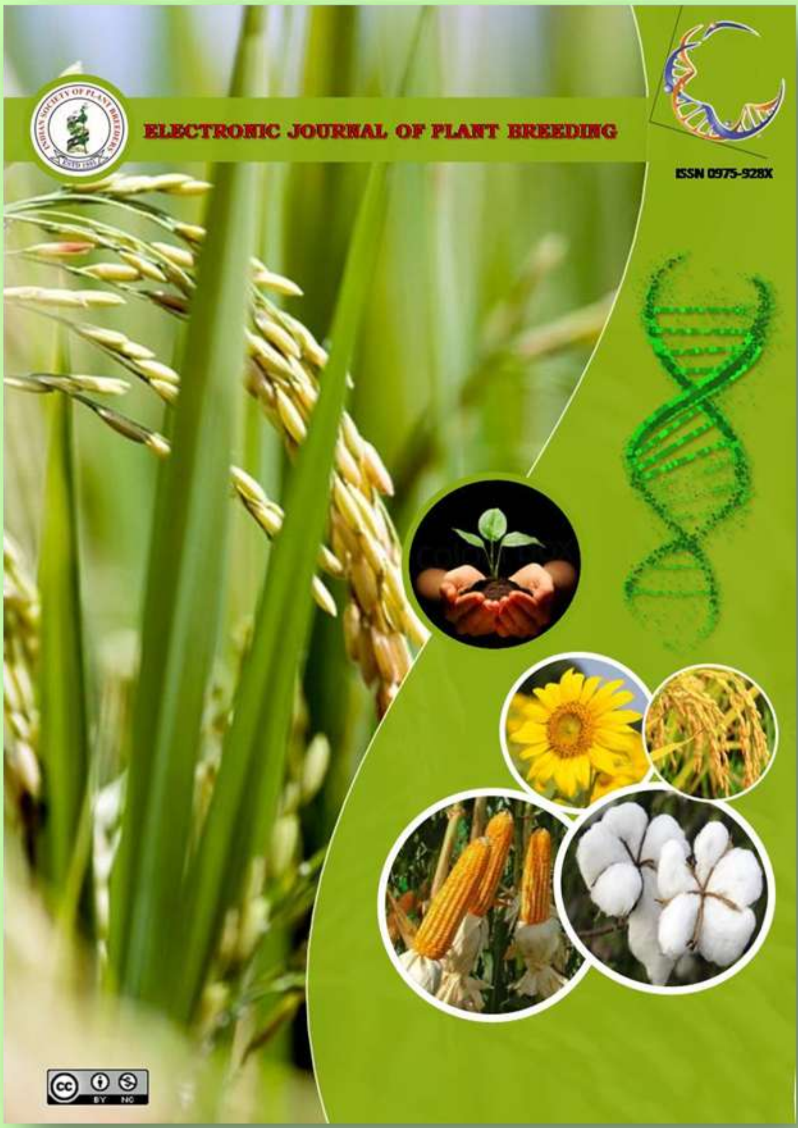

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Research Article

Identification of restorers and maintainers for A_2 and A_4 cytoplasm based CGMS lines in early maturing pigeonpea (*Cajanus cajan* (L.) Millsp.)

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Abstract
Fertility restoration is a crucial requirement for successful hybrid synthesis using CGMS system in pigeonpea. A total of 35 inbreds were crossed with three CGMS lines, where, two belong to A_2 cytoplasm(CORG 990047A and CORG 990052A), while other one belong to A_4 cytoplasm (CORG 7A)to identify maintainers and restorers. On the basis of pollen fertility analysis in 105 hybrids, seven inbreds partially restored the fertility of CORG 990047A. One and four inbreds partially restored the fertility of CORG 990052A and CORG 7A, respectively. A total of 28 inbreds maintained the sterility of CORG 990052 A followed by 16 inbreds for CORG 990047 A. Only seven inbreds maintained the sterility of A_4 CGMS line viz., CORG 7 A. In the present study, potential restorers was not identified for all the three CGMS lines. However, potential maintainers identified will be used in the backcross breeding to develop new CGMS lines with different genetic background.

Key words
Pigeonpea - CGMS lines - Early maturity - Fertility restoration.

Introduction

Pigeonpea (*Cajanus cajan* (L.) Millsp.) 2n = 2x = 22, is an important pulse crop of India. It is an often cross pollinated (20-70%) crop. It is cultivated in tropical and subtropical region of Africa, Asia and America. Among all the pulses, pigeonpea is the fourth important pulse crop in the world and second important pulse crop in India after chickpea. However, productivity wise it is low, that is 780 kg/ha (Saxena *et al*., 2014). The yield advantage of 40 per cent shown by hybrid technology has opened a new path to break the years old yield barrier issue in pigeonpea (Saxena *et al*., 2014).

The development of genetic male-sterility system (Reddy *et al*., 1978), resulted in release of ICPH 8, the first Genetic Male Sterility (GMS) based hybrid in pigeonpea by International Crop Research Institute for Semi-Arid Tropics (Saxena *et al*., 1992). The disadvantages of Genetic male sterility (GMS) based hybrids are labour-intensive seed production and seed purity. As rouging of 50 per cent fertile plants from the female rows resulted in loss of population and low hybrid seed yield, hence commercially not successful. Tikka *et al*. (1997) developed, the first cytoplasmic genetic male sterile (CGMS) line, GT-288A using *Cajanus scarabaeoides* (A_2) cytoplasm. CGMS-based hybrids SKNPH-10 (GTH-1) and ICPH-2671 (Pushkal) were released for cultivation in Gujarat and Madhya Pradesh, respectively (Saxena *et al*., 2013).

Past years CGMS lines were developed using eight different cytoplasmic sources (A_1 to A_8) from wild species of pigeonpea (Saxena *et al.*, 2013). Out of these, A_2 (*Cajanus scarabaeoides*) and A_4*(C. cajanifolius)* cytoplasm based CGMS system was found to be more stable under various environmental conditions (Saxena *et al*., 2005). Kalaimagal *et al.* (2008) developed two stable CGMS lines viz., CORG 990047 A and CORG 990052 A from the wild species of *Cajanus scarabaeoides* (A_2 cytoplasm). In heterosis breeding programme using CGMS system, availability of best maintainers and restorers is the basic requirement. Hence, the investigation was undertaken to identify restorers and maintainers for $A_2$and A_4CGMS sources with different male parents such as inbreds.

Materials and Methods

During *kharif* 2018, CGMS lines CORG 990047 A and CORG 990052 A (A_2 cytoplasm)and CORG 7 A (A_4 cytoplasm) were crossed with 35 inbreds as male parents, at the experimental plots of Department of Pulses, TNAU, Coimbatore. The resultant 105 hybrids were raised in field during *rabi*2018-19 along with parents and standard check variety CO (Rg) 7 in Randomized Block Design (RBD) with two replications. Each hybrid was raised in one row of 4 m length with spacing of 60 × 25 cm. All the recommended package of practices were followed to raise healthy crop.

The pollen fertility was examined for each hybrid and parents using unopened matured flower buds selected randomly from five plants and their anthers were crushed on glass slide with a drop of 1 per cent of iodine potassium iodide (IKI) and viewed under a light microscope. The counts of fertile (round and well stained) and sterile (shrivelled, hyaline and unstained) pollen grains in three microscopic fields under 10X magnification was observed. The mean was calculated for all the microscopic fields and pollen fertility percentage was calculated as per the formula given below.

$$\text{Pollen fertility (\%)} = \frac{\text{Number of fertile pollen grains}}{\text{Total number of pollen grains examined}} \times 100$$

Based on pollen fertility status, the hybrids were categorized as restorers (>80% pollen fertility), partial restorers (40 - 79% pollen fertility), partial maintainers (10 - 39% pollen fertility) and maintainers (<10% pollen fertility) (Vanishree *et al*., 2018). The pod set per cent was observed under selfing in each hybrid to confirm the results.

Results and Discussion

In this study, all the parents, hybrids and check variety were falling under early duration group with the duration of 120-130 days. The pollen fertility of parents recorded more than 94 per cent(Table 1). The hybrids derived from CORG 990047A ranged from 0 to 77.19 per cent. Of the 35 inbreds tested, 16 inbreds were completely maintained the sterility, 12 inbreds partially maintained the sterility while, the other seven inbreds partially restored the fertility (Table 2).

The mean pollen fertility of hybrids between CGMS line, CORG 990052 A (A_2 cytoplasm)and 35 inbreds is presented (Table 2). It ranged from 0 to 77.54 per cent. Out of 35 inbreds, 28 inbreds were completely maintained the sterility, six inbreds partially maintained the sterilityand one inbred partilly restored the fertility.The pollen fertility status of hybrids derived from A_4 cytoplasm based CGMS line viz., CORG 7 A is given in (Table 2). It ranged from 0 to 71.26 per cent. Among the 35 inbreds, seven inbreds were completely maintained the sterility, 24 inbreds partially maintained the sterility and four inbreds partially restored the fertility.

The frequency of maintainers was high for CORG 990052 A (80 per cent) followed by CORG 990047 A (46 per cent). Among the inbreds only 20 per cent were maintained the sterility of CGMS line viz., CORG 7 A (A_4 cytoplasm). Partial restorers obtained for different CGMS lines was also found to below. A total of seven and one inbreds partially restored the fertility of CGMS lines viz., CORG 990047 A, CORG 990052 A, respectively. Only four inbreds partially restored the fertility of CORG 7 A.

The results revealed that no inbreds restored the fertility of all the three CGMS lines. The frequency of partial restorers for CGMS lines viz., CORG 990047 A (7), CORG 990052 A (1) and CORG 7 A (4) was low. The low frequency of restorers for CGMS lines were reported by Saroj *et al.* (2015), Sultana *et al.* (2017), Sharma *et al.* (2018), Milind *et al*. (2018). The inbreds with partial restoration ability could be used as a source to develop potential fertility restorers which can be further used in developing good heterotic CGMS based hybrids in pigeonpea.

The wide range of variability was observed for frequency of maintainers. Low frequency of maintainers was observed for CORG 7 A (A_4 cytoplasm) when compared to CGMS lines with A_2 cytoplasm (CORG 990047 A and CORG 990052 A). However, the inbreds *viz*., IVT (E)-312, CRG 16-07, CRG 18-07 and IVT (E)-306 were maintained the sterility in all the three CGMS lines. The potential maintainers identified could be used in the development of new male sterile lines through backcross breeding. It is concluded that separate breeding program is highly essential to develop restorers and to sustain the CGMS based hybrid technology in pigeonpea.

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Table 1. Pollen fertility status of parents

Sl. No	Parents	Pollen fertility (%)
	I. Female parents	
1	CORG 990047 B	96.32
2	CORG 990052 B	97.44
3	CORG 7 B	98.00
	II. Male parents	
1	IVT (E)-312	99.10
2	ICPL 12337	100.00
3	IVT (E)-319	95.07
4	IVT (E)-322	100.00
5	CRG 2013-12/1	100.00
6	IVT (E)-315	97.62
7	IVT (E)-311	98.20
8	IVT (E)-313	99.33
9	CO 5	100.00
10	CRG 14-07/1	97.67
11	CRG 2013-12/2	98.23
12	CRG 14-07/2	100.00
13	CRG 16-07	100.00
14	CRG 16-03	98.65
15	CRG 18-07/1	99.63
16	CRG 18-02/1	95.72
17	CRG 18-08	97.66
18	CRG 18-02/2	98.17
19	CRG 18-05	100.00
20	CRG 18-09	95.34
21	CRG 18-06	97.83
22	CRG 18-03/1	99.51
23	CRG 18-07/2	98.18
24	CRG 18-04/1	100.00
25	CRG 18-01	99.00
26	CRG 18-03/2	94.39
27	CRG 18-04/2	98.69
28	CRG 2013-02/1	100.00
29	IVT (E)-308	99.11
30	IVT (E)-307	96.72
31	IVT (E)-306	100.00
32	IVT (E)-305	96.52
33	IVT (E)-318	97.64
34	IVT (E)-321	99.72
35	CRG 2013-02/2	98.79
	III. Check variety	
1	CO(Rg) 7	95.04

Table 2.Pollen fertility and restoration status of hybrids.

Sl. No	Hybrids	Pollen fertility (%)	Restoration reaction
1	CORG 9900 47A × IVT (E)-312	0.00	M
2	CORG 9900 47A × ICPL 12337	12.37	PM
3	CORG 9900 47A × IVT (E)-319	3.36	M
4	CORG 9900 47A × IVT (E)-322	12.35	PM
5	CORG 9900 47A × CRG 2013-12/1	6.66	M
6	CORG 9900 47A × IVT (E)-315	74.44	PR
7	CORG 9900 47A × IVT (E)-311	24.52	PM
8	CORG 9900 47A × IVT (E)-313	15.70	PM
9	CORG 9900 47A × CO 5	0.00	M
10	CORG 9900 47A × CRG 14-07/1	73.92	PR
11	CORG 9900 47A × CRG 2013-12/2	40.57	PR
12	CORG 9900 47A × CRG 14-07/2	70.34	PR
13	CORG 9900 47A × CRG 16-07	3.61	M
14	CORG 9900 47A × CRG 16-03	0.00	M
15	CORG 9900 47A × CRG 18-07/1	0.00	M
16	CORG 9900 47A × CRG 18-02/1	77.19	PR
17	CORG 9900 47A × CRG 18-08	23.59	PM
18	CORG 9900 47A × CRG 18-02/2	21.70	PM
19	CORG 9900 47A × CRG 18-05	14.84	PM
20	CORG 9900 47A × CRG 18-09	9.38	M
21	CORG 9900 47A × CRG 18-06	19.34	PM
22	CORG 9900 47A × CRG 18 -03/1	0.00	M
23	CORG 9900 47A × CRG 18-07/2	71.44	PR
24	CORG 9900 47A × CRG 18-04/1	75.19	PR
25	CORG 9900 47A × CRG 18-01	18.93	PM
26	CORG 9900 47A × CRG 18-03/2	26.55	PM
27	CORG 9900 47A × CRG 18-04/2	7.59	M
28	CORG 9900 47A × CRG 2013-02/1	9.78	M
29	CORG 9900 47A × IVT (E)-308	5.11	M
30	CORG 9900 47A × IVT (E)-307	12.02	PM
31	CORG 9900 47A × IVT (E)-306	5.30	M
32	CORG 9900 47A × IVT (E)-305	0.00	M
33	CORG 9900 47A × IVT (E)-318	13.45	PM
34	CORG 9900 47A × IVT (E)-321	4.00	M
35	CORG 9900 47A × CRG 2013-02/2	0.00	M
36	CORG 9900 52A × IVT (E)-312	0.00	M
37	CORG 9900 52A × ICPL 12337	0.00	M
38	CORG 9900 52A × IVT (E)-319	0.00	M
39	CORG 9900 52A × IVT (E)-322	14.48	PM
40	CORG 9900 52A × CRG 2013-12/1	8.06	M
41	CORG 9900 52A × IVT (E)-315	0.00	M
42	CORG 9900 52A × IVT (E)-311	7.47	M
43	CORG 9900 52A × IVT (E)-313	0.00	M
44	CORG 9900 52A × CO 5	0.00	M
45	CORG 9900 52A × CRG 14-07/1	0.00	M
46	CORG 9900 52A × CRG 2013-12/2	0.00	M
47	CORG 9900 52A × CRG 14-07/2	7.53	M
48	CORG 9900 52A × CRG 16-07	0.00	M
49	CORG 9900 52A × CRG 16-03	8.20	M
50	CORG 9900 52A × CRG 18-07/1	15.77	PM
51	CORG 9900 52A × CRG 18-02/1	77.54	PR
52	CORG 9900 52A × CRG 18-08	0.00	M
53	CORG 9900 52A × CRG 18-02/2	0.00	M
54	CORG 9900 52A × CRG 18-05	0.00	M
55	CORG 9900 52A × CRG 18-09	4.55	M
56	CORG 9900 52A × CRG 18-06	13.06	PM
57	CORG 9900 52A × CRG 18-03/1	16.67	PM

58	CORG 9900 52A × CRG 18-07/2	9.99	M
59	CORG 9900 52A × CRG 18-04/1	9.65	M
60	CORG 9900 52A × CRG 18-01	12.07	PM
61	CORG 9900 52A × CRG 18-03/2	8.94	M
62	CORG 9900 52A × CRG 18-04/2	0.00	M
63	CORG 9900 52A × CRG 2013-02/1	4.61	M
64	CORG 9900 52A × IVT (E)-308	11.67	PM
65	CORG 9900 52A × IVT (E)-307	9.80	M
66	CORG 9900 52A × IVT (E)-306	0.00	M
67	CORG 9900 52A × IVT (E)-305	0.00	M
68	CORG 9900 52A × IVT (E)-318	3.86	M
69	CORG 9900 52A × IVT (E)-321	8.56	M
70	CORG 9900 52A × CRG 2013-02/2	9.60	M
71	CORG 7A × IVT (E)-312	18.46	PM
72	CORG 7A × ICPL 12337	22.29	PM
73	CORG 7A × IVT (E)-319	9.34	M
74	CORG 7A × IVT (E)-322	6.87	M
75	CORG 7A × CRG 2013-12/1	17.34	PM
76	CORG 7A × IVT (E)-315	20.92	PM
77	CORG 7A × IVT (E)-311	24.74	PM
78	CORG 7A × IVT (E)-313	21.60	PM
79	CORG 7A × CO 5	9.73	M
80	CORG 7A × CRG 14-07/1	16.32	PM
81	CORG 7A × CRG2013-12/2	12.97	PM
82	CORG 7A × CRG 14-07/2	13.02	PM
83	CORG 7A × CRG 16-07	8.03	M
84	CORG 7A × CRG 16-03	11.92	PM
85	CORG 7A × CRG 18-07/1	16.79	PM
86	CORG 7A × CRG 18-02/1	14.05	PM
87	CORG 7A × CRG 18-08	21.70	PM
88	CORG 7A × CRG 18-02/2	12.84	PM
89	CORG 7A × CRG 18-05	14.74	PM
90	CORG 7A × CRG 18-09	45.71	PR
91	CORG 7A × CRG 18-06	74.56	PR
92	CORG 7A × CRG 18 -03/1	8.12	M
93	CORG 7A × CRG 18-07/2	70.16	PR
94	CORG 7A × CRG 18-04/1	71.26	PR
95	CORG 7A × CRG 18-01	17.73	PM
96	CORG 7A × CRG 18-03/2	9.21	M
97	CORG 7A × CRG 18-04/2	18.71	PM
98	CORG 7A × CRG 2013-02/1	16.54	PM
99	CORG 7A × IVT (E)-308	17.21	PM
100	CORG 7A × IVT (E)-307	15.38	PM
101	CORG 7A × IVT (E)-306	4.39	M
102	CORG 7A × IVT (E)-305	16.01	PM
103	CORG 7A × IVT (E)-318	8.58	M
104	CORG 7A × IVT (E)-321	11.12	PM
105	CORG 7A × CRG 2013-02/2	14.63	PM

M - Maintainer PM- Partial maintainer PR- Partial restorer

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