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Restorers and Maintainers of A₂ and A₄ Cytoplasm-Based CMS Lines in Pigeonpea

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ABSTRACT

Keywords

Fertility restoration, Sterility maintenance, A₂ and A₄ Cytoplasmbased CMS Lines

Article Info

Accepted: 07 November 2018 Available Online: 10 December 2018 In the present investigation, 78 genotypes were evaluated for their fertility restoration or sterility maintenance behaviour with two A₂ cytoplasm-based CMS lines (BRG 1A and Hy 3CA) and 36 genotypes with three A₄ cytoplasm-based CMS lines (BRG 1A, Hy 3CA and BRG 3A) in pigeonpea during 2012 and 2013. It was observed that 16 genotypes *viz.*, GCB 4, GCB 15, GCB 18, GCB 45, GCB 50, GCB 55, GCB 61, GCB 68, ICPL 87119, GRG 206, GRG 215, GRG 818, JKM 207, WRP 1, BDN 2 and BDN 2010 restored fertility of both A₂ cytoplasm-based CMS lines *viz.*, BRG 1A and Hy 3CA while three genotypes *viz.*, ICPL 20116, ICPL 20093 and BRGL 13-171 restored fertility of all the three A₄ cytoplasm- based CMS lines *viz.*, BRG 1A, Hy 3CA and BRG 3A. The identified restorers and maintainers could be further utilized in developing new restorer lines/hybrids and CMS lines, respectively.

Introduction

Pigeonpea (*Cajanus cajan* (L.) Mill spp.) is an important pulse crop of India. In spite of releasing many varieties in pigeonpea over the past few decades, no significant improvement has been realized in its productivity which has been hovering around 600-700 kg/ha (Nadarajan *et al.*, 2008). Exploitation of hybrid vigour is one of the ways to overcome this constraint. The recent achievements in breeding CMS systems have paved the way to develop commercial hybrids in pigeonpea. In

heterosis breeding programme using CMS system, availability of best maintainers and restorers is the prior requirement. Restorers for different CMS sources will increase the cytoplasmic diversification, which in turn can prevent genetic vulnerability due to the use of single CMS source. Keeping these points in view, the investigation was undertaken to explore the extent of fertility restoration and sterility maintenance for A₂ and A₄ CMS sources across different male parent sources such as germplasm, advance breeding lines and varieties.

Materials and Methods

The male parental material used in the study is detailed in Tables 1a and 1b. The first set of experiment comprised of two A2 cytoplasmbased CMS lines viz., BRG 1A and Hy 3CA, and 78 male parental lines (16 germplasm lines and 62 breeding lines/varieties, Table 1a). The second set of experiment consisted of three A₄ cytoplasm-based CMS lines viz., BRG 1A, Hy 3CA and BRG 3A, and 36 male parental lines (six germplasm lines and 30 breeding lines/varieties, Table 1b). All these experimental material were procured from Pigeonpea, AICRP on UAS, GKVK. Bangalore.

During kharif 2012, two A₂ cytoplasm-based CMS lines viz., BRG 1A and Hy 3CA were crossed with 78 genotypes as male parents. Similarly, three A₄ cytoplasm-based CMS lines viz., BRG 1A, Hy 3CA and BRG 3A were crossed with 36 genotypes as male parents at the experimental plots of AICRP on Pigeonpea, UAS Bangalore. The resultant 264 hybrids (156 hybrids from A₂ and 108 hybrids from A₄ CMS lines) were raised in field during kharif 2013 and evaluated for pollen fertility status. Each hybrid was planted in two rows of 4 m length with spacing of 90 × 30 cm. All the recommended agronomic practices were followed to raise healthy crop. Protective irrigations were provided at the time of sowing, flowering and pod formation stage

For assessing the pollen fertility or sterility status of each hybrid, five well developed unopened flower buds were collected randomly from five plants from each of the hybrids and their anthers were squashed on glass slide with a drop of 2% aceto-carmine stain and examined under a light microscope. The counts of fertile (round and well stained) and sterile (shrivelled, hyaline and unstained) pollen grains in five microscopic fields under 10X magnification was noted. The means for

all the microscopic fields were estimated and the proportion of fertile and sterile pollens was expressed in percentage as mentioned below.

Pollen fertility (%) =

Number of fertile pollen grains × 100 Total number of pollen grains examined

Based on pollen fertility status of hybrids, the respective pollen parents/genotypes were categorised as restorers (>80% pollen fertility), partial restorers (40 - 79% pollen fertility), partial maintainers (10 - 39% pollen fertility) and maintainers (<10% pollen fertility) (Saxena, 2013).

Results and Discussion

Restoration of fertility in the male sterility based hybrid is crucial for exploiting hybrid vigour in sexually reproducing crop species.

Restorers and maintainers for A_2 cytoplasm-based CMS lines

The per cent pollen fertility in hybrid combinations with CMS line BRG 1A ranged from 0.00 to 99.00 per cent, while that in hybrid combinations with CMS line Hy 3CA ranged from 0.00 to 98.00 per cent (Table 2). Of the 78 genotypes tested, 24 genotypes viz., GCB 4, GCB 11, GCB 15, GCB 18, GCB 45, GCB 50, GCB 55, GCB 61, GCB 68, ICPL 161, ICPL 11376, ICPL 20108, ICPL 20116, ICPL 87119, GRG 206, GRG 215, GRG 818, BDN 2, BDN 2009, BDN 2010, BRG 2-5, JKM 207, WRP 1 and BRGL 13-68 restored complete fertility in the hybrid combinations with BRG 1A, while the genotype GRG 2009 partially restored the fertility and the remaining 53 genotypes maintained sterility.

In the hybrid combinations with CMS line Hy 3C A, 17 genotypes *viz.*, GCB 4, GCB 15, GCB 18, GCB 45, GCB 50, GCB 55, GCB 61, GCB 68, ICPL 87119, GRG 206, GRG 215,

GRG 818, BDN 2, BDN 2001-9, BDN 2010, WRP 1 and JKM 207 restored complete fertility, while the genotype GCB 11 was the partial restorer and the remaining 60 genotypes maintained sterility in the hybrids. Among the two A₂ cytoplasm-based CMS lines, high frequency of restoration was observed for BRG 1A (32.05%) compared to Hy 3CA (23.07%). However, the frequency of fertility restorers observed in the present investigation was low which is in parallel with the study of Saxena (2002).

The genotypes GCB 4, GCB 15, GCB 18, GCB 45, GCB 50, GCB 55, GCB 61, GCB 68, ICPL 87119, GRG 206, GRG 215, GRG 818, BDN 2, BDN 2010, JKM 207 and WRP 1 restored the fertility in hybrids derived from both the CMS lines BRG1 A and Hy 3CA. These genotypes could be utilized in the heterosis breeding after testing their ability and heterosis. combining These observations made with 156 hybrids involving germplasm, advanced breeding lines and varieties indicated that the restorers were available in germplasm lines as well as advanced breeding lines and varieties, however their frequency was low. The similar report was made by Saxena, (2004). This suggests that intensive exploration germplasm, breeding lines and varieties could be fruitful in identifying new restoration sources.

Restorers and maintainers for A₄ cytoplasm-based CMS lines

The mean pollen fertility of hybrids between three A₄ cytoplasm-based CMS lines (BRG 1A, BRG 3A and Hy 3CA) and 36 genotypes is presented in Table 3. The mean fertility ranged from 0.00 to 96.00 per cent in hybrid combinations with CMS line BRG 1A; 0.00 to 94.00 per cent in hybrid combinations with BRG 1A and 0.00 to 92.00 per cent in hybrid combinations with Hy 3C A.

Out of 36 genotypes, eight genotypes viz., GCB 126, TS-3R, ICPL 81-3, ICPL 11376, ICPL 20093, ICPL 20096, ICPL 20177 and BRGL 13-171 restored complete fertility in the hybrid combinations with CMS line, BRG 1A while four genotypes viz., GCB 58, ICPL 20116, ICPL 20128 and BRGL 13-170 partially restored the fertility and remaining 24 genotypes maintained sterility in the hybrids. In the hybrid combinations with CMS line, BRG 3A, six genotypes viz., TS-3R, ICPL 20093, ICPL 20116, BRGL 13-167, BRGL 13-170 and BRGL 13-171 behaved as restorers. Three genotypes viz., ICPL 20098, ICPL 20096, BRGL 12-246 behaved as partial restorers, while remaining 27 genotypes maintained sterility of CMS line, BRG 3A. Eight genotypes viz., GCB 73, GCB 126, ICPL 81-3, ICPL 20096, ICPL 20098, ICPL 20116, BRGL 13-167 and BRGL 13-171 restored the fertility completely in hybrid combinations with CMS line Hy 3CA. Only one genotype viz., GCB 58 partially restored fertility, while remaining 27 genotypes maintained the sterility of CMS line, Hy 3CA. Among the three CMS lines, the line BRG 1A (33.33%) registered high frequency of fertility restoration compared to BRG 3A (25.55%) and Hy 3CA (25.55%).

Three genotypes *viz.*, ICPL 20093, ICPL 20116 and BRGL 13-171 restored the fertility of hybrids from all the three CMS lines. Six genotypes *viz.*, TS 3R, ICPL 20093, ICPL 20096, ICPL 20116, BGRL 13-170 and BRGL 13-171 behaved as restorers for two CMS lines, BRG 1A and BRG 3A. For combination of BRG 1A and Hy 3CA, six genotypes *viz.*, GCB 58, GCB 126, ICPL 20093, ICPL 20116, ICPL 81-3 and BRGL 13-171 acted as restorers. Similarly, five genotypes *viz.*, ICPL 20093, ICPL 20098, ICPL 20116, BRGL 13-167 and BRGL 13-171 behaved as restorers for another combination of two CMS lines, BRG 3A and Hy 3CA.

Table.1a Germplasm and breeding lines/varieties used to study their maintainer and restorer reaction with A₂ cytoplasm-based CMS lines in pigeonpea

Sl. No.	Genotypes	Source	Sl. No.	Genotypes	Source
	mplasm lines	Source	39	BRGL 12-3	Bangalore
1	GCB 4	Bangalore	40	BRGL 12-4	Bangalore
2	GCB 6	Bangalore	41	BRGL 12-6	Bangalore
3	GCB 11	Bangalore	42	BRGL 12-7	Bangalore
4	GCB 15	Bangalore	43	BRGL 12-12	Bangalore
5	GCB 16	Bangalore	44	BRGL 12-14	Bangalore
6	GCB 18	Bangalore	45	BRGL 12-16	Bangalore
7	GCB 27	Bangalore	46	BRGL 12-17	Bangalore
8	GCB 36	Bangalore	47	BRGL 12-18	Bangalore
9	GCB 41	Bangalore	48	BRGL 12-20	Bangalore
10	GCB 45	Bangalore	49	BRGL 12-21	Bangalore
11	GCB 48	Bangalore	50	BRGL 12-30	Bangalore
12	GCB 50	Bangalore	51	BRGL 12-45	Bangalore
13	GCB 55	Bangalore	52	BRGL 12-174	Bangalore
14	GCB 61	Bangalore	53	BRGL 12-175	Bangalore
15	GCB 68	Bangalore	54	BRGL 12-176	Bangalore
16	GCB 75	Bangalore	55	BRGL 12-177	Bangalore
Breeding lines/varieties		8	56	BRGL 12-192	Bangalore
17	BRG 2-5	Bangalore	57	BRGL 12-204	Bangalore
18	GRG 206	Gulbarga	58	BRGL 12-205	Bangalore
19	GRG 215	Gulbarga	59	BRGL 12-229	Bangalore
20	GRG 818	Gulbarga	60	BRGL 12-230	Bangalore
21	GRG 2009	Gulbarga	61	BRGL 12-242	Bangalore
22	WRP 1	Gulbarga	62	BRGL 12-246	Bangalore
23	PT-221	Gulbarga	63	BRGL 12-248	Bangalore
24	KPL 43	Kanpur	64	BRGL 12-251	Bangalore
25	JKM 207	Khargoan	65	BRGL 12-257	Bangalore
26	BSMR 736	Badnapur	66	BRGL 12-261	Bangalore
27	BSMR 853	Badnapur	67	BRGL 12-282	Bangalore
28	BDN 2	Badnapur	68	BRGL 13-68	Bangalore
29	BDN 2001-9	Badnapur	69	BRGL 13-174	Bangalore
30	BDN 2010	Badnapur	70	BRGL 13-184	Bangalore
31	BDN 2029	Badnapur	71	BRGL 13-186	Bangalore
32	ICPL 161	ICRISAT	72	BRGL 13-191	Bangalore
33	ICPL 11376	ICRISAT	73	BRGL 13-198	Bangalore
34	ICPL 20108	ICRISAT	74	BRGL 13-200	Bangalore
35	ICPL 20116	ICRISAT	75	BRGL 13-201	Bangalore
36	ICPL 87119	ICRISAT	76	BRGL 13-203	Bangalore
37	BRGL 12-1	Bangalore	77	BRGL 13-263	Bangalore
38	BRGL 12-2	Bangalore	78	BRGL 13-256	Bangalore

Table.1b Germplasm and breeding lines/varieties used to study their maintainer and restorer reaction with A₄ cytoplasm-based CMS lines in pigeonpea

Sl. No.	Genotypes	Source	Sl. No.	Genotypes	Source
Local germplasm lines			18	ICPL 81-3	ICRISAT
1	GCB 2	Bangalore	19	ICPL 20128	ICRISAT
2	GCB 47	Bangalore	20	ICPL 88039	ICRISAT
3	GCB 58	Bangalore	21	ICPL 20107	ICRISAT
4	GCB 73	Bangalore	22	ICPL 20177	ICRISAT
5	GCB 88	Bangalore	23	BRGL 13-167	Bangalore
6	GCB 126	Bangalore	24	BRGL 12-68	Bangalore
Breed	ling lines/varieties		25	BRGL 12-174	Bangalore
7	TS-3R	Gulbarga	26	BRGL 13-171	Bangalore
8	ICP 7035	ICRISAT	27	BRGL 13-170	Bangalore
9	ICPL 20108	ICRISAT	28	BRGL 12-208	Bangalore
10	ICPL 20116	ICRISAT	29	BRGL 12-211	Bangalore
11	ICPL 11376	ICRISAT	30	BRGL 13-176	Bangalore
12	ICPL 161	ICRISAT	31	BRGL 12-246	Bangalore
13	ICPL 20205	ICRISAT	32	BRGL 13-186	Bangalore
14	ICPL 20098	ICRISAT	33	BRGL 13-187	Bangalore
15	ICPL 11811	ICRISAT	34	BRGL 13-190	Bangalore
16	ICPL 20096	ICRISAT	35	BRGL 12-268	Bangalore
17	ICPL 20093	ICRISAT	36	BRGL 12-290	Bangalore

Table.2 Pollen fertility (%) of hybrids and restoration status of genotypes in combination with A_2 cytoplasm-based CMS lines in pigeonpea

		BRG 1A		Hy 3C A		
Sl.	Genotypes	Pollen Fertility	Reaction	Pollen Fertility	Reaction	
No.		(%)		(%)		
1	GCB 4	91.00	R	90.00	R	
2	GCB 6	5.00	M	0.00	M	
3	GCB 11	84.00	R	43.00	PR	
4	GCB 15	89.00	R	93.00	R	
5	GCB 16	0.00	M	0.00	M	
6	GCB 18	92.00	R	96.00	R	
7	GCB 27	0.00	M	0.00	M	
8	GCB 36	4.00	M	6.00	M	
9	GCB 41	0.00	M	0.00	M	
10	GCB 45	97.00	R	86.00	R	
11	GCB 48	0.00	M	0.00	M	
12	GCB 50	80.00	R	91.00	R	
13	GCB 55	97.00	R	88.30	R	
14	GCB 61	83.00	R	84.00	R	
15	GCB 68	87.00	R	89.00	R	
16	GCB 75	0.00	M	0.00	M	
17	BRG 2-5	83.00	R	8.00	M	
18	GRG 206	99.00	R	98.00	R	
19	GRG 215	80.00	R	83.00	R	
20	GRG 818	90.00	R	88.00	R	
21	GRG 2009	58.75	PR	23.00	PM	
22	WRP 1	97.00	R	92.00	R	
23	PT-221	0.00	M	0.00	M	
24	KPL 43	9.00	M	0.00	M	
25	JKM 207	93.00	R	94.00	R	
26	BSMR 736	0.00	M	0.00	M	
27	BSMR 853	8.00	M	6.00	M	
28	BDN 2	92.00	R	95.00	R	
29	BDN 2001-9	16.00	PM	86.00	R	
30	BDN 2010	98.00	R	94.00	R	
31	BDN 2029	93.00	R	21.00	PM	
32	ICPL 161	93.00	R	29.00	PM	
33	ICPL 11376	84.00	R	5.00	M	
34	ICPL 20108	86.00	R	38.00	PM	
35	ICPL 20116	93.00	R	32.00	PM	
36	ICPL 87119	89.00	R	92.00	R	

Table.2, Contd...

		BRG 1A Hy 3C				
Sl. No.	Genotypes	Pollen Fertility (%)	Reaction	Pollen Fertility (%)	Reaction	
37	BRGL 12-1	0.00	M	0.00	M	
38	BRGL 12-2	5.00	M	7.00	M	
39	BRGL 12-3	0.00	M	7.00	M	
40	BRGL 12-4	19.00	PM	9.00	M	
41	BRGL 12-6	8.00	M	0.00	M	
42	BRGL 12-7	0.00	M	0.00	M	
43	BRGL 12-12	7.00	M	0.00	M	
44	BRGL 12-14	27.00	PM	0.00	M	
45	BRGL 12-16	4.00	M	23.00	PM	
46	BRGL 12-17	8.00	M	0.00	M	
47	BRGL 12-18	0.00	M	0.00	M	
48	BRGL 12-20	0.00	M	0.00	M	
49	BRGL 12-21	6.00	M	33.00	PM	
50	BRGL 12-30	0.00	M	9.00	M	
51	BRGL 12-45	8.00	M	6.00	M	
52	BRGL 12-174	8.00	M	0.00	M	
53	BRGL 12-175	7.00	M	0.00	M	
54	BRGL 12-176	0.00	M	21.00	PM	
55	BRGL 12-177	13.00	PM	0.00	M	
56	BRGL 12-192	0.00	M	0.00	M	
57	BRGL 12-204	0.00	M	0.00	M	
58	BRGL 12-205	0.00	M	0.00	M	
59	BRGL 12-229	9.00	M	0.00	M	
60	BRGL 12-230	0.00	M	27.00	PM	
61	BRGL 12-242	0.00	M	8.00	M	
62	BRGL 12-246	0.00	M	6.00	M	
63	BRGL 12-248	26.00	PM	0.00	M	
64	BRGL 12-251	0.00	M	0.00	M	
65	BRGL 12-257	0.00	M	0.00	M	
66	BRGL 12-261	0.00	M	0.00	M	
67	BRGL 12-282	0.00	M	23.00	PM	
68	BRGL 13-68	99.00	R	31.00	PM	
69	BRGL 13-174	0.00	M	0.00	M	
70	BRGL 13-184 BRGL 13-186	31.00	PM M	0.00	M	
71		0.00	M	0.00	M	
72 73	BRGL 13-191 BRGL 13-198	0.00 8.00	M M	0.00	M M	
74	BRGL 13-198	8.00	M	6.00	M	
74 75	BRGL 13-200	4.00	M	9.00	M	
76	BRGL 13-201	36.00	PM	0.00	M	
77	BRGL 13-263	0.00	M	0.00	M	
78	BRGL 13-256	0.00	M	31.00	PM	
70	DKUL 13-230	0.00	IVI	31.00	PIVI	

M – Maintainer R- Restorer PM- Partial maintainer PR- Partial restorer

Table.3 Pollen fertility (%) of hybrids and restoration status of genotypes in combination with A_4 cytoplasm-based CMS lines in pigeonpea

Sl.		BRG 1A		BRG 3 A		Hy 3C A	
No	Genotypes	Pollen Fertility (%)	Reaction	Pollen Fertility (%)	Reaction	Pollen Fertility (%)	Reaction
1	GCB 2	5.00	M	9.00	M	0.00	M
2	GCB 47	0.00	M	6.00	M	7.00	M
3	GCB 58	73.00	PR	19.00	PM	72.00	PR
4	GCB 73	0.00	M	0.00	M	89.00	R
5	GCB 88	0.00	M	8.00	M	6.00	M
6	GCB 126	88.00	R	15.00	PM	86.00	R
7	TS-3R	81.00	R	83.00	R	19.00	PM
8	ICP 7035	12.00	PM	5.00	M	33.00	PM
9	ICPL 20108	5.00	M	0.00	M	0.00	M
10	ICPL 20116	68.00	PR	86.00	R	83.00	R
11	ICPL 11376	85.00	R	6.00	M	5.00	M
12	ICPL 161	14.00	PM	22.00	PM	16.00	PM
13	ICPL 20205	9.00	M	0.00	M	0.00	M
14	ICPL 20098	5.00	M	73.00	PR	96.00	R
15	ICPL 11811	5.00	M	6.00	M	9.00	M
16	ICPL 20096	96.00	R	66.00	PR	9.00	M
17	ICPL 20093	92.00	R	90.00	R	92.00	R
18	ICPL 81-3	88.00	R	9.00	M	90.00	R
19	ICPL 20128	71.00	PR	8.00	M	8.00	M
20	ICPL 88039	16.00	PM	4.00	M	0.00	M
21	ICPL 20107	0.00	M	31.00	PM	6.00	M
22	ICPL 20177	83.00	R	3.00	M	8.00	M
23	BRGL 13-167	8.00	M	81.00	R	87.00	R
24	BRGL 12-68	6.00	M	0.00	M	0.00	M
25	BRGL 12-174	6.00	M	9.00	M	9.00	M
26	BRGL 13-171	85.00	R	83.00	R	91.00	R
27	BRGL 13-170	71.00	PR	94.00	R	7.00	M
28	BRGL 12-208	5.00	M	0.00	M	8.00	M
29	BRGL 12-211	24.00	PM	6.00	M	9.00	M
30	BRGL 13-176	5.00	M	0.00	M	7.00	M
31	BRGL 12-246	8.00	M	72.00	PR	6.00	M
32	BRGL 13-186	7.00	M	23.00	PM	5.00	M
33	BRGL 13-187	5.00	M	9.00	M	9.00	M
34	BRGL 13-190	6.00	M	6.00	M	8.00	M
35	BRGL 12-268	33.00	PM	26.00	PM	9.00	M
36	BRGL 12-290	0.00	M	0.00	M	0.00	M

M - Maintainer

R- Restorer

PM- Partial maintainer

PR- Partial restorer

The results also revealed that the restorer for one CMS line behaved as maintainer of another CMS line and vice versa. For example, genotypes ICPL 11376 and ICPL 20177 behaved as restorers for BRG 1A while they maintained sterility of BRG 3A and Hy 3CA. Similar variable fertility restoration behaviour among a common set of male parents with different cytoplasmic sources has also been reported by Dalvi et al., (2008) and Such results Nithva (2008).unexpected as the very expression of CMS and its restoration is primarily based on the interaction of genes present in mitochondrial DNA and the corresponding nuclear restorer genes (Frei et al., 2004).

From the present study it can be concluded that the germplasm, advanced breeding lines and varieties can be used as a source to find out the potential fertility restorers which can be further used in developing good heterotic CGMS based hybrids in pigeonpea. However, availability of restorers in low frequency from these sources suggests the alternative way of developing new restorers by conventional breeding methods such repeated as backcrossing of the restorer lines with the recurrent parent, followed by the selection of the fertile plants. If molecular markers could be employed to tag the restorer genes, it would reduce the time required to develop new restorer lines.

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