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Fertility restoration in cytoplasmic genic male sterile line of pigeonpea [*Cajanus cajan* (L.) Millsp.] derived from *Cajanus scarabaeoides*

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Abstract

For commercial exploitation of heterosis, efficient and stable cytoplasmic genic male sterility system is developed using first CGMS line GT-288A/B alongwith fertility restoration mechanism from interspecific hybridization. To identify perfect pollen fertility restorers, 543 derivative lines of F_5 and F_6 populations of *Cajanus scarabaeoides* × *Cajanus cajan* and 1365 germplasm accessions were used as pollen parent on stable cytoplasmic genic male sterile line GT-288A during *kharif* 1997 to 2003. The F_1 progenies of all the crosses were evaluated during *kharif* 1998 to 2003 for their pollen fertility. The promising pollen fertility restoring parents were advanced and purified through selection and selfing. Finally eighteen fertility restorers were identified and characterized.

Key words: Pigeonpea, fertility restoration, cytoplasmic genic male sterility system, inbred lines

Introduction

The discovery of genetic male sterility system [1] and its utilization resulted in release of few hybrids such as ICPH-8 by International Crop Research Institute for Semi-Arid Tropics [2], PPH-4 by Punjab Agricultural University, Ludhiana [3], IPH-732 by Tamilnadu Agricultural University, Coimbatore [4], AKPH-4101 and AKPH-2022 by Punjabrao Krishi Vidhyapeeth, Akola [5]. However, these GMS based hybrids could not be commercialized because of labour intensive seed production and seed purity concerns. Reddy and Faris [6] reported maternal inheritance of male sterility in progenies derived from wild (*Atylosia scarabaeoides*) × cultivated pigeonpea (*Cajanus cajan*), which was associated with high degree of female sterility and other defects. Ariyanayagam *et al.*, [7] at ICRISAT, Hyderabad utilizing *Cajanus sericeus* showed the presence of cytoplasmic male sterility, however, stable CMS lines for commercial exploitation could not be developed until 1997 when, Tikka *et al.*, [8] reported first stable cytoplasmic male sterile line GT-288A with its maintainer line GT-288B utilizing *Cajanus scarabaeoides* as source of cytoplasm [9, 10]. This cytoplasmic genic male sterility system (CGMS) contains A line with S(rr), B line with genotype F(rr) and R lines with genotype S/F(RR) [11], which can be used to develop CGMS based hybrids. The utilization of this system for the development of commercial hybrids would be possible only if the perfect fertility restoration mechanism is available. The present investigation was therefore initiated to identify suitable fertility restorer lines.

Materials and methods

Some of the material (543 inbred lines in number) for the present study was derived from fertile plants of F_2 derivatives of a cross between *Cajanus scarabaeoides* × *Cajanus cajan* [10]. Secondly, germplasm accessions of cultivated pigeonpea (1365 in number) were crossed with CGMS line GT-288A during *kharif* 1997 to 2003. These F_1's were tested in subsequent *kharif* seasons. Crosses of each plant-to-plant mating were harvested and further studied separately. Parental plants were individually selfed for purification. Lines, which have shown lower than 80 per cent restorability, were rejected.

The pollen fertility test of all the F_1's was carried out assessing pollen stainability using 2 per cent acetocarmine solution [12]. The resultant hybrids having good dehiscence, abundant pollen dusting and higher pollen fertility in the stain were identified and pollen grains of these hybrids were dusted on stigma of male sterile plant to get pod setting on male sterile plants for confirmation of pollen fertility. These inbred lines and germplasm lines used to synthesize such hybrids were designated as restorers. After identification of inbred line or germplasm line as restorer, the fertility restorer were tested at multi-locations in replicated manner having two rows of four meter length for three years. The restorers, which were found stable for fertility restoration, were characterized for agro-morphological characters. The observations on agro-morphological characters were recorded on five randomly selected plants in each restorer at multi-locations for consecutive three years and average are presented.

Results and discussion

The utilization of diverse germplasm accessions may lead to identification of fertility restorer lines [13]. During 1997 to 2003, large number of hybrids were synthesized using 543 derivative inbred lines of F_5 and F_6 populations of *Cajanus scarabaeoides* × *Cajanus cajan* and 1365 germplasm lines to identify fertility restoring lines. The promising lines, which showed restoration, were repeatedly crossed for three years with GT-288A for confirmation (Table 1). Finally, 18 lines were identified which produced hybrids with high pollen fertility. The pollen fertility of F_1 hybrids was further tested for pod setting on the CGMS line GT-288A (Table 2).

Table 1. Number of crosses attempted to identify fertility restorer lines

Pollinators used			No. of	Restorer lines			Pollen
Year	Inbred lines	Germ-plasm lines	F_1 tested	Identi-fied	Rete-sted	Confir-med	fertility % of hybrids
1997	341	25	-	-	-	-	-
1998	202	85	366	59	-	-	23-98
1999	-	192	346	24	59	38	20-100
2000	-	161	254	48	62	42	56-100
2001	-	277	251	38	90	30	80-100
2002	-	625	345	17	68	28	80-100
2003	-	-	670	56	45	18	80-100

Table 2. Pod setting on CGMS line using F_1 (CGMS line × inbred line or germplasm line) as pollinator for confirmation of fertility restoration

Sr. No.	Year	No. of F_1 used as pollinator on GT-288A	No. of flowers attempted on GT-288A	No. of F_1 confirmed for pod setting
1.	1999	59	50	38
2.	2000	62	50	42
3.	2001	90	50	30
4.	2002	68	50	28
5.	2003	45	50	18

The identified 23 restorers were maintained through selfing. Their hybrids were repeatedly produced in subsequent years and were tested for pollen fertility. During selfing, some of the restorer lines could not maintain higher level of restoration as reflected in case of GTR-1, GTR-4, GTR-5, GTR-7 and GTR-17, while most of the lines maintained higher level of pollen fertility (Table 3).

Eighteen phenotypically diverse restorers were characterized for agromorphological characters (Table 4) and designated as Gujarat Tur Restorer-1 (GTR-1) to Gujarat Tur Restorer-23 (GTR-23). Among 18 restorers, 8 were of early maturing (135-150 days) and 10 belongs to medium (150-175 days) maturity group. Only one restorer (GTR-11) had determinate growth

Table 3. Pollen fertility status of F_1 hybrids using different inbred lines or germplasm lines in respective years

Sr. No.	Lines derived from	Name of restorer	Pollen fertility in F_1					
			1998	1999	2000	2001	2002	2003
1.	ICPL-8710	GTR-1	96.8	92.1	90.4	89.6	48.2	55.6
2.	(*Cajanus scarabaeoides* × *Cajanus cajan* F_2 × QMS-2 BC_2F_2---BC_9	GTR-2	85.6	88.2	84.8	80.6	92.3	93.2
3.	(*Cajanus scarabaeoides* × *Cajanus cajan*) F_2 - 17 G-II-F_{10}	GTR-3	87.7	94.8	91.2	74.0	96.4	97.1
4.	SKNP-9813	GTR-4	97.5	82.7	70.4	60.4	58.3	51.7
5.	(*Cajanus scarabaeoides* × *Cajanus cajan*) F_2 × ICPL-87119 BC_1 F_2 × ICPL-87119 BC_2F_2-- BC_7	GTR-5	97.3	94.2	87.6	90.0	42.8	48.3
6.	(*Cajanus scarabaeoides* × *Cajanus cajan*) F_2 × GT-100 BC_1F_2 × GAUT-88-9 BC_2F_2---BC_9	GTR-6	95.8	96.1	83.7	89.6	77.3	82.6
7.	ICP-8976	GTR-7	96.9	96.6	83.7	60.8	53.4	50.4
8.	AGS-70	GTR-8	93.7	94.2	96.5	62.1	91.7	92.4
9.	GAUT-92-17	GTR-9	93.7	95.8	92.2	80.9	89.4	90.1
10.	ICP-9260	GTR-10	89.8	91.7	96.5	90.8	87.4	89.8
11.	GT-100	GTR-11	96.3	95.1	92.8	90.7	89.3	90.7
12.	ICP-14722	GTR-12	-	-	93.4	96.5	94.6	95.1
13.	ICP-13	GTR-13	-	-	81.7	85.2	85.2	84.4
14.	ICP-10650	GTR-14	-	-	84.7	94.8	85.1	83.9
15.	ICP-10874	GTR-15	-	-	91.3	82.8	75.9	81.4
16.	ICP-11376	GTR-16	-	-	94.8	87.6	95.4	92.5
17.	ICP-11492	GTR-17	-	-	89.9	92.1	61.6	58.2
18.	AGS-10	GTR-18	-	-	89.2	96.5	78.9	83.0
19.	P 376-1	GTR-19	-	-	-	84.6	85.4	89.2
20.	ICP-50	GTR-20	-	-	-	90.2	88.3	90.1
21.	ICP-132	GTR-21	-	-	-	86.4	87.1	89.8
22.	ICP-204	GTR-22	-	-	-	88.3	90.2	91.3
23.	ICP-95	GTR-23	-	-	-	87.1	89.2	90.6

GTR = Gujarat Tur Restorer

Table 4. Characteristics of identified fertility restorer lines

Sr. No.	Restorer	Characters											
		DF	DM	PH	BP	PP	SP	PL	TW	PT	FC	PC	SC
1.	GTR-2	118	172	137	6.3	176	4.2	4.6	9.5	NDT	RS	GBS	Brown
2.	GTR-3	102	138	146	5.6	165	4.3	4.3	10.2	NDT	Y	Green	White
3.	GTR-6	111	155	172	9.1	188	4.3	4.2	8.5	NDT	Y	GDBS	White
4.	GTR-8	97	145	127	10.3	143	3.4	4.1	9.2	NDT	Y	BSM	White
5.	GTR-9	92	137	139	9.2	162	4.2	4.2	9.5	NDT	Y	GBS	White
6.	GTR-10	103	148	153	13.1	207	3.7	4.0	10.1	NDT	Y	Green	Orange
7.	GTR-11	95	145	90	4.2	130	3.9	4.1	10.1	DT	Y	GBS	White
8.	GTR-12	110	165	100	4.3	122	3.5	4.2	9.7	NDT	Y	GBS	Orange
9.	GTR-13	88	142	110	3.4	140	3.6	3.9	9.7	NDT	Y	GBS	Orange
10.	GTR-14	89	146	125	5.3	123	3.3	4.3	10.1	NDT	Y	GBS	Orange
11.	GTR-15	105	159	125	4.2	130	3.6	4.2	10.2	NDT	Y	GBS	Orange
12.	GTR-16	100	156	110	6.4	160	3.8	4.1	9.9	NDT	Y	GBS	Orange
13.	GTR-18	100	158	150	6.1	155	3.9	4.0	10.2	NDT	Y	GBS	White
14.	GTR-19	109	158	136	7.0	112	4.3	4.5	9.1	NDT	Y	GBS	Orange
15.	GTR-20	101	148	122	8.0	84	3.3	3.4	9.0	NDT	Y	GBS	Orange
16.	GTR-21	118	168	140	6.0	96	4.0	4.3	8.9	NDT	Y	GBS	Orange
17.	GTR-22	110	162	154	8.0	107	3.0	4.1	9.5	NDT	Y	GBS	White
18.	GTR-23	94	136	128	12.0	142	3.0	3.8	9.0	NDT	Y	GBS	Orange

DF = Days to flower, DM = Days to mature, PH = Plant height (cm), BP = Branches/plant, PP = Pods/plant, SP = Seeds/pod, PL= Pod length (cm), TW = Test weight (100-seed weight - g.), PT = Plant type, FC = Flower colour, PC = Pod colour, SC = Seed colour, RS = Red streaks, BSM = Black streaks on suture, GBS = Green with black streaks, GDBS = Green with dark black streaks, Y = Yellow, NDT = Non determinate, DT = Determinate

habit while remaining were non-determinate type (Table 4). These restorer lines, which have shown stable restorability and are agronomically desirable types can be utilized for development of cytoplasmic-genic male sterility system based pigeonpea hybrids. Moreover, these lines could also be used for intercrossing to develop base population for breeding better restorer lines.

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