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Fertility Restoration in Cytoplasmic-Nuclear Male-Sterile Lines Derived from 3 Wild Relatives of Pigeonpea

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Three cytoplasmic-nuclear male-sterile (CMS) lines, one each derived from *Cajanus sericeus* (A_1 cytoplasm), *Cajanus scarabaeoides* (A_2 cytoplasm), and *Cajanus cajanifolius* (A_4 cytoplasm), were crossed to 7 pigeonpea (*Cajanus cajan* (L.) Millsp.) cultivars in a line × tester mating scheme to study the fertility restoration of the CMS lines. Twenty-one F_1 hybrid combinations were planted in unreplicated 3-row plots in 3 environments. There was no effect of environments on the expression of fertility restoration. Pigeonpea cultivar ICPL 129-3 restored fertility in A_1 cytoplasm and maintained male sterility in the other 2 (A_2 and A_4) cytoplasms. Among crosses involving CMS line (of A_4 cytoplasm) ICPA 2039 one hybrid combination was male-sterile and another male fertile. The remaining 5 combinations segregated for male-fertility (66–84% fertility restoration). Such testers can easily be purified for use in hybrid breeding programs by selfing and single-plant selection for 2–3 generations.

Introduction

Pigeonpea (*Cajanus cajan* (L.) Millsp.) is an important high-protein food legume of rainfed agriculture in Asia, Africa, and the Caribbeans. Predominantly, the crop is cultivated with low inputs that, on average, produce about 700 kg/ha. In spite of releasing dozens of pigeonpea varieties over the past few decades, no significant improvement could be realized in its productivity. Exploitation of hybrid vigor has been suggested to overcome this constraint (Saxena *et al.* 1996; Stakstad 2007). Besides hybrid vigor, there are 2 prerequisites to breed commercial hybrids; an economic means of mass pollen transfer and availability of a stable male-sterility system. Pigeonpea is known to have a considerable extent of natural outcrossing (Saxena *et al.* 1990). The search for male-sterility system started during early 1980s. The recent achievements in breeding cytoplasmic-nuclear male-sterile (CMS) systems have paved the way to develop commercial hybrids in this crop. The success in development of nuclear or genetic male-sterile (GMS) lines showed a ray of hope for development of CMS lines. At the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) at Patancheru, India, CMS lines are available with different maturity groups. The experimental hybrids developed at ICRISAT have shown the possibility of exploiting the hybrid vigor in pigeonpea (Saxena *et al.* 2006). The present study deals with the fertility restoration in F_1 hybrid combinations, developed by crossing the CMS lines derived from 3 wild relatives of pigeonpea and 7 pigeonpea cultivars as testers.

Materials and Methods

The experimental materials comprised 3 diverse early maturing CMS lines. These were ICPA 2067 with A_1 cytoplasm derived from *Cajanus sericeus* (Saxena *et al.* 1997), ICPA 2052 with A_2 cytoplasm of *C. scarabaeoides* (Saxena and Kumar 2003), and ICPA 2039 with A_4 cytoplasm of *C. cajanifolius* (Saxena *et al.* 2005). The male-sterile line (A-line) seeds were obtained by manual hand pollination under cages.

Among the 3 wild species, *C. sericeus* is a small erect shrub, more or less densely branched. This wild species, collected from Satpura mountains and western hills of India, is less preferred by pod borer (*Helicoverpa armigera* Hub) pest. The other wild species *C. scarabaeoides* is a creeper climber. It exhibits antibiosis as well as mechanical resistance to pod borers (van der Maesen 1986). This species was collected from Australia. The third wild species *C. cajanifolius* (Haines) van der Maesen *comb. nov* was collected from central India that differs from the cultivated type (De 1974). The somatic chromosome number of all these wild relatives of pigeonpea resembles that of the cultivated types ($2n = 2x = 22$).

Seven popular pigeonpea varieties were selected as testers on the basis of their combing ability in a previous

study (Phad 2003). Among these, BSMR 175, BDN 2, BWR 23, BSMR 736, and BSMR 853 originated from Marathwada Agricultural University, Parbhani (Maharashtra, India); ICPL 129-3 was bred at ICRISAT, Patancheru (Andhra Pradesh, India); and Nirmal 2 was developed by Nirmal Seeds Pvt. Ltd, Jalgaon (Maharashtra, India). To protect the experimental materials from pollinating insects, all the CMS lines and testers were planted inside a nylon net (0.5 mm size) at Patancheru in June 2004. Individual plants of the CMS lines were examined for male sterility to avoid any pollen shedder in hybridization. At flowering, 4200 hand pollinations were made on the male-sterile lines using fresh pollen from the 7 cultivars in a line × tester mating scheme. In each cross, 80–120 pods were obtained with a mean crossing success of 55%. To study stability of fertility restoration, all the hybrid combinations were planted along with a control cultivar ICPL 87119 during 2005 rainy season in unreplicated 3-row plots, at Patancheru (17°N) on 28 June, Parbhani (19°N) on 12 July, and Latur (18°N) on 14 July. Two seeds were planted per hill that resulted in 90% plant stand at each location. At each location, the experimental materials were planted in black cotton soils with recommended inter- (75 cm) and intra- (25 cm) row spacings (Saxena 2006). Standard cultural practices were adopted to grow a healthy crop. To study pollen fertility at each location, 5 fully grown but unopened flower buds were randomly collected from 30 plants in each cross combination and their anthers were squashed in 2% acetocarmine solution. As the locations were far apart, the buds could not be collected on the same day. The observations were completed in 1-week intervals at the 3 locations. For each slide, 3 microscopic fields were examined and counts were made for male-fertile (round and red color stained) and male-sterile (shriveled and unstained) pollen grains. Plants with >10% stained pollen grains were classified as male-fertiles. To further confirm the fertility restoration, each plant with >10% pollen fertility was selfed with a musclin cloth bag (5 μm) to observe pod setting. The percentage of male-fertile plants in each F_1 population was considered as an indicator of fertility restoration.

Results and Discussion

The pod setting on the male-sterile plants after hand pollinations revealed that the crossing success was high (55%), which is in accordance with previous studies (Rao *et al.* 1996) conducted at Patancheru. The selfed individual hybrid plants, on average, produced 33 ± 0.35 pods per plant as compared to control cultivar ICPL 87119 (42 ± 0.47 pods per plant). This shows that even small proportion (10% or more) of fertile pollen grains in a plant was capable of setting a high number of pods. Data from 3 locations revealed that there was no influence of environments on the expression of fertility restoration and each cross combination exhibited more or less the similar fertility restoration (Table 1).

Among the testers, ICPL 129-3 was unique and exhibited perfect fertility restoration of A_1 cytoplasm and perfect male-sterility maintenance in A_2 and A_4 cytoplasms. Cultivar Nirmal 2 also expressed perfect fertility with A_1 cytoplasm and male sterility with A_2 cytoplasm as was observed in ICPL 129-3. But in contrast, it also exhibited moderate level (66%) of fertility restoration in A_4 cytoplasm. BSMR 175 maintained perfect male sterility in A_2 cytoplasm, whereas cultivars BWR 23 and BSMR 736 restored moderate levels of fertility in all 3 cytoplasms. Considering the performance of CMS lines in this experiment it was found that ICPA 2067 (A_1 cytoplasm) could not maintain male sterility with any of the testers and only in BSMR 175 cross 65% plants exhibited male sterility. Three crosses with ICPA 2052 (A_2 cytoplasm) maintained perfect male sterility, whereas the remaining 4 crosses had 61–78% male-fertile plants. Out of 7 crosses involving ICPA 2039 (A_4 cytoplasm), one maintained perfect male sterility and one restored perfect fertility. In the remaining crosses with ICPA 2039, the proportion of fertile plants ranged from 66 to 84%. In maize (*Zea mays*) 30 male-sterile lines were classified into various groups on the basis of fertility restoration (Beckett 1971). Similar classification in the present study was not possible as there were only 3 CMS lines. Worstell *et al.* (1984) observed variation in sorghum (*Sorghum vulagre*) for fertility among hybrids of the same female with specific males. Such differences in fertility restoration could be attributed to the presence/absence of one or more fertility restoring genes. Beckett (1971) also observed similar interactions in maize CMS lines. Jan *et al.* (2002) observed the differences for fertility restoration of same cytoplasm with different testers in sunflower (*Helianthus annuus* L.). Studies at ICRISAT showed the presence of 1–3 dominant genes for restoring male-fertility in all the CMS sources of pigeonpea (Dalvi VA, unpublished data).

Conclusions

The information generated from this experiment showed that ICPL 129-3 can be used to develop hybrids on CMS lines derived from A_1 cytoplasm, whereas cultivars BSMR 175, ICPL 129-3, and Nirmal 2 can be used for the development of new diverse CMS lines with A_2 cytoplasm. Because ICPL 129-3 maintained male sterility in A_4 cytoplasm, it could be used for development of a new A-line. Cultivars BDN 2, BWR 23, BSMR 736, and BSMR 853 showed more or less similar reaction for fertility restoration across the 3 CMS lines. There were differences among testers for fertility restoration of different cytoplasms and the same cytoplasm showed different fertility restoration behavior with different testers. The partial fertility restoration observed in some hybrid combinations could be attributed to genetic impurities in the male parents, which could be due to natural outcrossing and difficulties in the maintenance of genetic stocks under natural pollination. Such lines, however, can easily be purified by selfing and single-plant selection for 2–3 generations as has been demonstrated in the hybrid breeding program at ICRISAT.

Table 1. Fertility restoration in F_1 hybrids at 3 locations during 2005 rainy season (June/July)

		ICPA 2067		ICPA 2052		ICPA 2039	
Tester	Location	Total plants	Fertility restoration (%)	Total plants	Fertility restoration (%)	Total plants	Fertility restoration (%)
ICPL 129-3	Latur	41	100	35	0	29	0
	Parbhani	29	100	28	0	35	0
	Patancheru	35	100	39	0	36	0
	Total/Mean	105	100	102	0	100	0
Nirmal 2	Latur	42	100	48	0	29	66
	Parbhani	5	100	51	0	52	67
	Patancheru	58	100	52	0	37	65
	Total/Mean	105	100	151	0	118	66
BWR 23	Latur	49	100	53	80	46	70
	Parbhani	12	100	37	81	48	75
	Patancheru	32	100	42	71	47	70
	Total/Mean	93	100	132	78	141	72
BSMR 736	Latur	36	69	49	65	42	100
	Parbhani	13	69	49	63	52	100
	Patancheru	27	70	46	59	49	100
	Total/Mean	76	71	144	63	143	100
BSMR 175	Latur	51	35	52	0	36	80
	Parbhani	9	33	52	0	51	80
	Patancheru	45	36	28	0	38	76
	Total/Mean	105	35	132	0	125	79
BDN 2	Latur	54	70	52	75	37	85
	Parbhani	12	67	48	75	54	83
	Patancheru	49	69	38	76	42	81
	Total/Mean	115	70	138	75	133	84
BSMR 853	Latur	39	82	33	60	47	80
	Parbhani	10	80	54	61	49	76
	Patancheru	50	80	29	62	50	74
	Total/Mean	96	83	116	61	146	77
Mean (across testers)			80 ± 0.50		40 ± 0.80		68 ± 1.00

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