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Short Communication

A new cytoplasmic-nuclear male sterility system in pearl millet

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With 2 tables

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Abstract

Among the cytoplasmic-nuclear male sterility (CMS) systems reported in pearl millet, *Pennisetum glaucum* (L.) R. Br., the $A_m = A_4$ system produces the highest frequency of male-sterile hybrids. A CMS source identified in a large-seeded gene pool (LSGP) was compared with the A_4 system. Seven diverse restorer lines of the A_4 system produced hybrids with $81A_4$ that were all fertile (pollen-shedding score 4 and 68–89% selfed seedset). In contrast, all the hybrids of these inbreds made with the isonuclear line with the LSGP cytoplasm were sterile (pollen-shedding score 1 and 0–3% selfed seedset). Topcross hybrids of four diverse composites made with $81A_4$ had 10–35% plants that had good fertility (> 50% selfed seedset). In comparison, no plant of any topcross hybrid with the isonuclear line having LSGP cytoplasm exceeded 20% selfed seedset, and it was rare for a plant to exceed even 10% selfed seedset. These differential fertility restoration patterns of hybrids indicate that the LSGP cytoplasm represents a CMS system that is different from the A_4 and, by implication, from all those reported to date. This new CMS system is designated A_5.

Key words: *Pennisetum glaucum* — cytoplasm — fertility restoration — hybrids — male sterility

The discovery of the Tift $23A_1$ cytoplasmic-nuclear male sterility (CMS) and its successful utilization in breeding male-sterile lines of commercial hybrids is a landmark in pearl millet (*Pennisetum glaucum* (L.) R.Br.) improvement (Athwal 1965, Burton 1965, Burton and Powell 1968). Among the several additional CMS sources that have been identified, the classified systems include A_2 and A_3 (Burton and Athwal 1967) and $A_m = A_4$ (Hanna 1989). Of all these four CMS systems, the A_4 system, developed from a Senegalese accession of *P. glaucum* subsp. *monodii* (= *violaceum*), a wild relative of the cultivated pearl millet, produces the highest frequency of male-sterile hybrids (Hanna 1989, Cereals Program, ICRISAT 1992). A CMS source has recently been identified from a male-sterile plant of the large-seeded gene pool (LSGP) jointly constituted by Genetic Enhancement and Genetic Resources Divisions of ICRISAT Asia Center (IAC). Almost all the plants grown from the open-pollinated seeds of male-sterile plants of this CMS source were observed to be male-sterile in bulk plantings. The objective of this research was to determine if this CMS source from LSGP represents a new CMS system.

Male-sterile lines: In a bulk planting of about 10,000 plants of LSGP at IAC during the 1989 cool-dry season, 67 were observed not to shed pollen but had 70–100% seedset in the open-pollinated panicles. These plants were, therefore, male sterile and female fertile. During the 1990 rainy and cool-dry seasons, almost all the plants grown from the open-pollinated seeds of a male-sterile plant, designated as LSGP-66, were male sterile.

A dwarf male-sterile line $81A_4$ that possesses the nuclear genome of the inbred line 81B and the A_4 cytoplasm was developed at IAC in 1990. As genetic background has been shown to have a significant effect on the expression of male sterility in pearl millet (Rai and Hash 1990), a near-isonuclear line (hereafter referred to as isonuclear line) was developed by three generations of backcrossing of 81B into the LSGP cytoplasm. This isonuclear line is designated $81A_L$.

Single-cross hybrids: Seven diverse IPC (ICRISAT Pollinator Collection) lines developed from a composite and six different crosses (Table 1) that had been identified as restorers of the A_4 CMS system, were crossed with $81A_4$ and $81A_L$ during the 1994 cool-dry season. Bulk pollen collected from a pollen parent was divided into two lots and used to cross with 2–3 plants of each A-line. During the 1994 rainy season, 14 single-cross hybrids were planted in single-row plots of 4 m (about 30 plants) at IAC. At 75% anthesis, each plot was scored for pollen shedding, following the procedure of Rai and Hash (1990). A plot was rated as 1 (all plants at anthesis had shrunken anthers and did not shed pollen), 2 (> 90% plants at anthesis had shrunken anthers and did not shed pollen), 3 (> 90% plants at anthesis had plump anthers and shed pollen), and 4 (all plants at anthesis had plump anthers and shed pollen). No consideration was given to the quantitative variation in pollen production. Five to 10 panicles were also selfed in each plot to determine selfed seedset, following the ergot rating scale of Thakur and Williams (1980).

Topcross hybrids: Four composites (IVC-C_5, ExBD_2, BSEC, and MC-C_{10}) of diverse origin were crossed with $81A_4$ and $81A_L$ to produce eight topcross hybrids. IVC-C_5 is a cycle 5 bulk of an intervarietal composite that was developed by intermating 73 superior intervarietal crosses of Indian × African origin. ExBD_2 is a dwarf version of a landrace variety from the Gashva region of Bornu province in northern Nigeria. BSEC is a bold-seeded early composite, based mostly on the early-maturing landraces from Togo and Ghana. MC-C_{10} is a cycle 10 bulk of medium composite, developed by random mating 197 geographically diverse and medium-maturing lines.

The bulk pollen collected from a composite was divided into two lots and used to cross with each A-line. Over 1 week, about 70–80 plants of each composite were used as pollen sources to cross 10 panicles of each A-line. The eight topcross hybrids were planted during the 1994 rainy season in 12-row plots of 4 m (about 200 plants) and over 150 plants of each hybrid were selfed to determine seedset, following the same procedure as for single-cross hybrids.

Crop and weather: Both single-cross and topcross hybrid nurseries were grown at IAC in a field that had been fertilized with 60 kg N/ha and

Table 1: Pedigree of pollen parents, pollen-shedding scores, and mean selfed seedset (%) of single-cross hybrids made with two isonuclear lines of pearl millet, ICRISAT Asia Center, rainy season 1994

Pollen parent		Pollen-shedding score		Mean selfed seedset (%)	
Identity	Parentage	$81A_4$	$81A_L$	$81A_4$	$81A_L$
IPC 319-4	SC_1-FS-135-4	4	1	89	1
IPC 827-1	(5054B × F_4FC 1498-1-1-1)-3-1-1-1	4	1	88	3
IPC 804-2	(S10LB-30 × LCSN-1225-6-3-1)-1-2-1-1-2	4	1	85	0
IPC 931-1	(F_4FC 1498-1-1-1 × J 104)-6-1-2-1-1-1	4	1	85	2
IPC 458	(J 260-1 × 700557)-1-4-10-5-1)-1-2-2-1	4	1	76	1
IPC 492	(B 282 × J 804-1-3-9)-7-2-2	4	1	68	3
IPC 501	(NEP 7-5603 × SS 48)-47-7-1	4	1	75	0

Score: 1 = All plants at anthesis had shrunken anthers and shed no pollen; 2 = > 90% plants at anthesis had shrunken anthers and shed no pollen; 3 = > 90% plants at anthesis had plump anthers and shed pollen; 4 = All plants at anthesis had plump anthers and shed pollen

40 kg P/ha. The anthesis occurred between 10 and 24 August. During the period from 1 week before to 1 week after anthesis, there was 19.5 cm of rain with a mean minimum temperature of 22°C, mean maximum temperatures of 29°C, and mean relative humidities of 90% in the mornings and 69% in the afternoons.

All seven inbred pollen parents produced fertile hybrids with $81A_4$ with a pollen-shedding score of 4 (i.e., all plants had plump anthers and shed pollen) and 68–89% selfed seedset (Table 1). In contrast, all hybrids with $81A_L$ were sterile, with no plant shedding pollen (score 1) and 0–3% mean seedset. A low seedset of this order in some of the otherwise male-sterile single-cross hybrids is more likely to be caused by contamination with the alien pollen. The possibility of scant pollen, leading to some self-pollination and low degree of selfed seedset, however, cannot be ruled out. Male-sterile × maintainer hybrids, expected to be sterile, have been found in some cases to have about a 1% seedset in pearl millet (Rai and Hash 1990). Thus, the differential fertility restoration patterns of hybrids of these two A-lines indicate that the LSGP cytoplasm represents a CMS system that could be different from that of A_4. Of the other three CMS systems (A_1, A_2, and A_3), none is known to produce only sterile hybrids in crosses with diverse inbred lines (Hanna 1989, Cereals Program, ICRISAT 1992). Thus, the LSGP source, by implication, is also different from these three CMS systems.

Although the pollen parents used in single-cross hybrids of this study are from diverse sources, they represent a small sample of genetic diversity. The haploid pollen from composites (used for producing topcross hybrids) represent a much larger genetic diversity of potential inbred lines. In topcross hybrids of four diverse composites with $81A_4$, 54–87% plants were in the sterile class (8–62% plants with no seedset and 25–46% plants with 1–5% seedset) (Table 2). In topcross hybrids with $81A_L$, 97–99% plants were in the sterile class (45–82% plants with no seedset and 17–52% plants with 1–5% seedset). While 10–35% plants of hybrids based on $81A_4$ were highly fertile (> 50% selfed seedset), only 1–3% plants of topcross hybrids made with $81A_L$ had as much as 10% selfed seedset, and no plant had more than 20% selfed seedset. This differential fertility restoration pattern of hybrids of $81A_4$ and $81A_L$ supports the conclusion, based on single-cross hybrids, that the LSGP source represents a CMS system different from that of A_4. Three composites (IVC, MC and $ExBD_2$) used in this study have been found to produce higher frequencies of fertile hybrids with $81A_1$ than $81A_4$, and the A_2 and A_3 systems produce far higher frequencies of fertile hybrids with a diverse range of inbred pollen parents than even the A_1 system (Cereals Program, ICRISAT 1992), implying that the LSGP system is different from A_1, A_2 and A_3. This new CMS system from LSGP is designated A_5.

Results of this study show that it will be difficult to find restorers of the A_5 CMS system. The rare occurrence of fertile plants in bulk plantings of open-pollinated seeds harvested from male-sterile plants of this system indicates that it may be possible to find restorers in an extensive survey of the germplasm. The most immediate source could be the LSGP itself in which this CMS source was identified. Other possible sources could be broad-based gene pools developed at IAC, and accessions of *P. glaucum* subsp. *monodii*, a wild relative of cultivated pearl millet. Use of the A_5 CMS system in breeding male-sterile lines of grain hybrids will depend on finding its restorers. However,

Table 2: Selfed seedset (%) in topcross hybrids of isonuclear lines of pearl millet, ICRISAT Asia Center, rainy season 1994

Topcross hybrid		Number of hybrid plants	Per cent hybrid plants in seedset class					
Composite[1]	A-line		0	1–5	6–10	11–20	21–50	> 50
IVC-C5	$81A_4$	178	8	46	6	2	3	35
	$81A_L$	168	45	52	3	0	0	0
$ExBD_2$	$81A_4$	190	40	25	2	0	1	32
	$81A_L$	152	64	34	2	0	0	0
BSEC	$81A_4$	126	62	25	2	< 1	< 1	10
	$81A_L$	176	82	17	1	0	0	0
MC-C10	$81A_4$	187	28	35	6	1	3	27
	$81A_L$	156	57	40	1	2	0	0

[1]IVC = Inter-Varietal Composite; $ExBD_2$ = Ex-Bornu (dwarf sidecar version); BSEC = Bold-Seeded Early Composite; MC = Medium Composite

this A_5 cytoplasm, in terms of identifying its maintainers, provides an excellent opportunity for both genetic and cytoplasmic diversification of male-sterile lines of forage hybrids.

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