

IDENTIFICATION OF NEW MALE STERILE LINES IN PEARL MILLET GERMPLASM

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To identify naturally occurring cytoplasmic male sterility (CMS) in pearl millet [*Pennisetum glaucum* (L.) R. Br.], 4950 germplasm accessions were screened and identified 67 male-sterile plants and sterility maintainers from 16 different germplasm accessions. Of these, eight CMS lines were found to be stable over locations, seasons and years. CMS lines DSA 105A and DSA 134 A from Ghana flowered 7-8 days earlier than 5141A and are less sensitive to extended daylength indicating their wider adaptability. To study the cytoplasmic relationships, the maintainers (B lines) of new CMS lines were crossed with the A lines of existing CMS sources and vice versa. Since DSA 105B and PMC 23B maintain sterility in A₁, A₂ and A₃ CMS systems, they were considered to have different cytoplasm. When the new CMS lines were crossed with 18 diverse pollinators, hybrids with Ghana CMS lines flowered early and produced more grain than the check hybrids. Downy mildew resistant CMS lines DSA 105A and DSA 134 are used to produce hybrids which are being evaluated in India and Niger.

Key words : Male sterile lines, pearl millet, germplasm.

Most commercial hybrids of pearl millet [*Pennisetum glaucum* (L.) R. Br.] are produced using the A₁ cytoplasmic-genetic male sterility system (CMS), originally found in Tift 23A (Burton, 1958). Two additional CMS systems designated as A₂ and A₃ were found from Ludhiana, India (Burton and Athwal, 1967). However, they have not been widely used (Rai and Singh, 1987). As the hybrids produced on Tift 23A became susceptible to downy mildew, and was replaced by several downy mildew resistant CMS lines produced by genomic substitution or induced mutations (Anand Kumar and Andrews, 1984; Pokhriyal et al., 1976; Appadurai and Sambathkumar, 1976; Aken'Ova and Chheda, 1981; Akenova, 1985). Chemical mutagens like ethidium bromide (Burton and Hanna, 1976) and mitomycin and streptomycin (Burton and Hanna, 1982) were used to produce CMS lines. CMS lines were also developed by genomic substitution of *Pennisetum glaucum* subspecies *monodii* with the cultivated line Thiotande (Marchais and Pernes, 1985). Mutations for fertility restoration and maintaining sterility were also reported (Burton, 1972; 1977).

Stable sterile cytoplasm identified from the wild relative *monodii* designated as A4 (Hanna, 1989) was also found to be highly susceptible to downy mildew when grown at ICRISAT Asia Center (IAC), Patancheru during 1990.

To overcome the problems of narrow cytoplasm base, it is desirable to identify new sources of stable downy mildew resistant CMS lines. Hence, we searched for spontaneously occurring CMS lines in exotic introductions. This paper describes the method of identifying spontaneously occurring CMS lines in pearl millet, their morphological characteristics, and stability over seasons, years, and locations, cytoplasmic relationships with the existing CMS lines and the performance of hybrids produced using these CMS lines with 18 diverse pollinators.

MATERIALS AND METHODS

ICRISAT had assembled 21,919 pearl millet germplasm accessions from 44 countries. As and when they were received, all the exotic pearl millet introductions to ICRISAT were space planted in Post-Entry Quarantine Isolation Area (PEQIA) for quarantine inspection and seed increase. To prevent outcrossing, emerging heads were bagged before stigma emergence (Mengesha and Appa Rao, 1986). When these growouts were carefully observed, we found naturally occurring male sterile plants. During the first growout in PEQIA at IAC, Patancheru, about 200 plants per accession were space planted. Before stigma emergence, heads were bagged and observed 10 days later for seed set. Those heads which did not produce seed were carefully examined for anther morphology and those plants with shrunken, shrivelled anthers and did not produce any pollen were isolated. In such plants, seed set under open-pollinated spikes was also observed. Such selected plants were given extra care by providing irrigation and fertilizers. Sister plants with desirable agronomic characters were also given extra care. Newly emerging spikes of the suspected male sterile plants were pollinated with the sister plants and their progenies were tested for fertility after bagging. Progenies in which there was no seed set were considered as male sterile (A line) and the male parent as the sterility maintainer (B line). These plants in which there was no seed set in selfed heads in A lines and which produced seed upon crossing with sister plant (new B lines) with desirable agronomic characters were advanced. To improve the level of downy mildew resistance, these A and B lines were also grown in downy mildew sick plot (Singh and Gopinath, 1985). Pollen from downy mildew free B plants only were used to pollinate downy mildew free A plants.

For morphological characterization, A and B lines were grown along with the popularly used CMS lines 5141A and ICMA 1 (81A) and the morphological characters were recorded according to the pearl millet descriptors (IBPGR/ICRISAT, 1993).

To study the cytoplasmic relationships with A₁, A₂ and A₃ sources, B lines of these three sources were crossed with the newly developed CMS lines. Similarly, the B lines of the new CMS lines were crossed to the A lines of A₁, A₂ and A₃ sources. Tift 23 d₂, Tift 239 and L67 were used for A₁, A₂ and A₃ sources, respectively. Some of the known restorers were also used as male parents on the new male sterile plants. Fifty F₁ plants from each cross were grown and seed set was observed in 5-10 bagged heads. Considering seed set in bagged heads they were classified as restorers, maintainers or partial steriles where seed set was about 20%. To ascertain whether the new CMS lines produce good hybrids, they were crossed with 18 diverse elite pollinators and the F₁ hybrids were grown along with the parents for comparison. These CMS lines along with hybrids produced on them were evaluated at four locations in India; Bhavanisagar (11°00'), Patancheru (17°22'), Gwalior (26°12'), and Hisar (29°10') during three years.

RESULTS AND DISCUSSION

Identification of male sterile plants

CMS plants were morphologically conspicuous as they were producing more than 10 tillers per plant compared to 3-5 in the fertile plants. These CMS plants with profuse tillers were sequential in flowering, and seed set was absent in the selfed heads. Open-pollinated heads of such plants or those heads pollinated with fresh pollen produced full seed set. The anthers of CMS plants were shrivelled, shrunken and did not emerge completely out of the florets compared to those fertile anthers which were large, plump and emerged completely. When the shrunken anthers were crushed and pollinated on the established male-sterile line 5141A, there was no seed set. These pollen grains did not stain with acetocaramine or iodine solution. Those plants which did not set seed after bagging but showed good seed set after open pollination or after hand pollination were considered as male sterile. The fertile plants which produced sterile plants in the next generation after crossing with the fertile plants were considered as a sterility maintainer or B line. Even though, we found male sterility in 16 different accessions during the first generation, the progenies of eight accessions produced 5-20 seeds inside the bagged heads. We advanced only 8 accessions which did not produce seeds in bagged heads. Partial sterility of some of these lines might be due to the fact that sterility was influenced by environment or there were several modifier genes. Stable male-sterile plants (A lines) and sterility maintainer plants (B lines) were derived in 5 accessions from Ghana, 2 accessions from Botswana and 1 accession from Togo.

All the accessions which had segregated for CMS were collected by ICRI SAT. The precise locations from where these CMS lines collected were

described elsewhere (Appa Rao *et al.*, 1989). All the CMS lines from Ghana which segregated for male sterility were collected around Bolgatanga in northern Ghana. While both the CMS lines from Botswana were collected in Kgatleng district which is a very dry area. The CMS line ARD 132 was collected from northern Togo which is relatively a high rainfall area (Appa Rao *et al.*, 1990).

To identify new sources of CMS, we screened an estimated one million plants from 4950 accessions from 18 countries. Of the 4950 accessions screened, 16 accessions from Botswana, Central African Republic, Ghana, Sudan, Tanzania and Togo had CMS plants (Table 1). The spontaneous mutation rate calculated per accession was 0.032. However, when mutation rate was calculated as percentage of mutant (CMS) plants to the total plants screened, the mutation rate was 0.0067. Spontaneous mutations for CMS appear to occur at a very low frequency. The percentage of mutant plants was 2.17 which was very low probably because we collected seeds from at least 30 plants. CMS plants must be in the original population because we found them during the first

Table 1. Frequency of spontaneous male sterile (CMS) plants identified in pearl millet germplasm from different countries.

Pedigree	Origin	Frequency of plants		
		Normal	CMS	% of CMS
PMC 23	Botswana	170	4	2.35
PMC 30	Botswana	180	3	1.67
AK 140	Central Africa	192	4	2.08
DSA 27	Ghana	175	1	0.57
DSA 59-1	Ghana	151	5	3.31
DSA 105	Ghana	172	3	1.74
DSA 118	Ghana	152	7	4.61
DSA 134	Ghana	168	5	2.98
DSA 144-1	Ghana	145	4	2.76
DSA 194-1	Ghana	176	3	1.70
PGI 2	Sudan	193	2	1.04
ACC 544	Sudan	158	3	1.90
M 70-1	Tanzania	161	2	1.24
ARD 51	Togo	174	6	3.45
ARD 56	Togo	182	2	1.10
ARD 132	Togo	538	13	2.42
	Total	3087	67	2.17

growout and they must have been maintained because of cross pollination. Such mutations appear to be occurring in a relatively high frequency in the material collected from Ghana and Togo.

Morphological characteristics

Morphological characteristics of the new CMS lines when evaluated at IAC, Patancheru in two contrasting seasons are given in Table 2. The new CMS lines flowered in 42-60 days in the rainy season and are less sensitive to day length compared to 5141A (Table 2). They grew to an height of 130-200 cm in the rainy and 120-140 cm in the postrainy season. Spike length varied from 14.5 to 25 cm in the rainy season with a slight reduction in the postrainy season. Grain number per spike varied from, 460 to 1936 and grain size from 5.8 to 14.3 mg. CMS lines from Ghana have certain desirable agronomic traits such as early flowering and large grain size. Estimated mean grain yield of CMS lines at Patancheru, Bhavanisagar and Hisar ranged from 465 kg ha⁻¹ in PMC 30A to 2352 kg ha⁻¹ in DSSA 134A.

The CMS lines DSA 105A and DSA 134A from Ghana, flowered 7-8 days earlier than 5141A and were resistant to downy mildew. Under extended daylength, a minimum delay of 2 days was found in DSA 105A compared to 15 days in 5141A and 9 days in MS 81A, revealing their wider adaptability. Early maturity, less sensitivity to daylength and large grain size of DSA 105 and DSA 134 are the agronomically desirable characters of these CMS lines. High tillering of PMC 23 and PMC 30 make them potential good forage types.

Cytoplasmic relationships

Seed set in F₁ plants of crosses involving new CMS lines with B lines of A₁, A₂, A₃ are given in Table 3. The sterile progeny was considered as maintainer and hence possessing the same cytoplasm, while fertile progeny was considered to have different cytoplasm. The cytoplasmic relationships of 5 CMS lines from Ghana and 2 from Botswana indicated that DSA 144 possess cytoplasm different from A₁ because the B line of this (maintainer) restored the fertility on A₁ cytoplasm. The B lines of DSA 105 and PMC 23 maintain sterility on A₁, A₂ and A₃ systems.

The sterile cytoplasm from *P. violaceum* in M₁ and M₂ (Marchais and Pernes, 1985) showed considerable differences with the new CMS lines. The B lines of both M₁ and M₂ restored fertility in all the new CMS lines except on PMC 30A. Reciprocal differences were observed in crosses involving DSA 59-1, DSA 105, DSA 144 and OMC 30. No reciprocal differences were observed in DSA 118.

Table 2. Characteristics of the newly identified male-sterile lines of pearl millet from Ghana and Botswana compared with 5141A

Character	DSA 59-1A		DSA 105A		DSA 118A		DSA 134A		DSA 144A		PNC 23A		PNS 30A		ARD 132A		5141A	
	X	SD	X	SD	X	SD	X	SD	X	SD	X	SD	X	SD	X	SD	X	SD
Days to 50% flowering-R	50	4.3	43	1.4	47	1.4	42	0.7	53	2.3	55	1.4	60	0.5	46	1.8	50	0.7
" "-PR	58	3.8	45	0.7	52	1.4	49	2.1	55	3.1	62	2.1	13	1.4	43	2.6	66	1.4
Plant height (cm)-R	140	2.0	140	2.2	140	7.7	130	11	140	1.8	145	7.1	120	1.5	200	1.7	131	2.8
" "-PR	120	1.6	120	1.6	125	11	130	21	100	2.4	135	3.5	120	1.7	140	2.6	70	0.7
Spike length (cm)-R	16.0	2.6	22.5	2.3	14.5	5.7	16.0	1.4	17.0	1.2	24.0	3.5	20.0	1.5	25.0	1.4	17.0	2.1
" "-PR	15.0	3.4	17.0	1.4	15.0	2.8	17.0	0.7	13.0	1.8	22.0	0.7	17.0	1.6	17.0	2.1	11.0	1.4
Spike thickness (mm)-R	25.0	1.5	24.5	3.5	24.0	0.7	19.0	2.1	22.0	3.2	23.0	1.4	18.5	2.0	34.0	2.6	14.0	2.5
" "-PR	26.0	2.7	28.0	4.2	23.0	0.7	23.0	4.2	17.0	2.6	22.0	3.5	20.0	1.7	28.0	2.1	12.0	0.7
No. of spikes/plant-R	4.5	3.4	2.5	1.8	3.5	2.4	4.2	1.3	4.3	1.8	3.4	2.4	6.8	2.1	2.8	1.2	5.3	2.6
Spike exertion (cm)-PR	3.0	1.4	8.0	1.6	9.0	2.3	9.0	1.5	2.0	4.5	3.0	1.4	2.0	1.8	10.0	1.6	5.0	0.6
No. of grains/spike-PR	1062	2.8	1485	3.4	1615	2.7	1450	2.5	460	2.8	1281	1.9	1485	2.8	1936	4.7	2056	1.8
1000 grain mass (g)-PR	12.8	1.8	14.1	2.0	12.5	1.9	14.3	0.7	9.0	0.8	12.6	1.5	5.8	2.5	14.1	1.2	6.2	0.5

R= rainy (kharif) season, PR = post-rainy (rabi) season.

Table 3. Cytoplasmic relationships of newly developed CMS lines with existing sources of cytoplasm

Pedigree	MS	Tift	L			DSA	DSA	DSA	DSA	DSA	PMC	PMC
	81B	239B	67B	M1B	M2B	59-1B	105B	105B	118B	134B	144B	23B
DSA 59-1A	M*	R*	M	R	R	M	M	M*	P	M*	M	M
DSA 105A	P	M	M*	R	R	M	M	M	M	M	M*	M*
DSA 118A	R	R*	P	R	R	M	M	M	M*	M*	M	M*
DSA 134A	P	P	M*	R	R	P	M	M*	M	P	M	V
DSA 144A	X	R	M*	R	X	M	M	M	R	M	M	V
PMC 23A	R*	V	M*	R	R	P	V	M*	M*	M	M	M*
PMC 30A	M*	R	R	M	M	M	M	P	M*	R	M*	M
M1A	M	R	R	M	M	M	M*	R	M*	M	V	M*
M2A	M*	R	R	C	M	M	M	R	V	X	X	R
MS 81A (A1)	M	R	M*	M*	M	M	M	V	M	R	M*	V
T 239A (A2)	R	M	P	R	R	R*	M*	R	V	M	M*	V
L67A (A3)	R	V	M	R	R	R	M*	P	M*	M*	M*	V

M = maintainer, M* = maintainer with 3-7 grains per spike, R = restorer, R* = restorer with incomplete seed set, P = partial sterile, V = variable, X = not available.

DSA 105B is also a maintainer on PC 23A and vice versa indicating that these two have the same cytoplasm. Reciprocal differences were observed in crosses involving Tift 239 (A₂) and DSA 144; crosses involving 239A with DSA 144B, maintains sterility in F₁ whereas the F₁ of DSA 144A with Tift 239B behaved as fertility restorer. Similarly, crosses involving L 67A with DSA 59-1B behaved as a restorer while the reciprocal cross behaved as a maintainer. Such reciprocal differences were also reported by (Rai and Hash, 1990). These reciprocal differences may be due to differences in cytoplasm but they may have the same genes for male sterility. The inbred J 104 which is a restorer on 5141A (A₁ system behaved as maintainer on DSA 105A confirming that the cytoplasm of DSA 105A is different from A₁ system.

Agronomic performance of hybrids

To assess the potential of these CMS lines, 18 diverse pollinators were crossed to the CMS lines and were evaluated for the agronomic traits including grain yield at five locations. The F₁ hybrids flowered 13 days earlier than the CMS lines, grew 35-100 cm taller, and produced longer spikes (Table 4). The increase in grain yield was from 300 to 1400 kgs thus indicating heterosis in the F₁ hybrids. The CMS lines from Ghana and Botswana were found to be resistant to downy mildew in India and Niger.

Seeds of these CMS lines were supplied to several plant breeders in private and public sectors in India and elsewhere. Hybrids produced on DSA

134A with indigenous inbreds from Niger appeared very promising at Sadore in Niger. Early maturity of DSA 105A is being exploited to produce early-maturing hybrids in India.

Table 4. Comparison of CMS lines with the hybrids produced on them with 18 diverse pollinators*

Character**	DSA	DSA	DSA	DSA	DSA	PMC	PMC	MS	
	59-19A	105A	118A	134A	144A	23A	30A	5141A	81A
Flowering - P	47.2	47.9	48.9	40.2	54.2	55.0	56.5	54.2	51.8
Flowering - H	46.8	45.7	47.5	45.7	X	X	X	X	50.2
Plant height (cm) - P	168	128	170	150	140	189	155	140	114
" " - H	230	231	223	233	X	X	X	X	228
Tiller number - P	4.2	3.0	2.6	3.8	3.4	3.2	7.0	2.8	2.7
Spike length (cm) - P	17.4	21.7	20.2	18.9	15.9	18.1	18.2	18.9	21.7
" " -H	22.5	22.7	24.1	21.5	X	X	X	X	24.3
1000 grain mass (g) - P	11.2	12.3	13.4	9.4	9.9	10.5	4.8	6.2	6.6
Grain yield (kg-1)-P	2012	1096	1472	2352	2043	1624	465	1389	2003
" " -H	2709	2475	2387	2664	X	X	X	X	2542

* = data based on mean for five locations in India.

**P = seed parent; H = mean of hybrids produced with 18 diverse pollinators, X = no data.

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