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Seed parent breeding efficiency of three diverse cytoplasmic-nuclear male-sterility systems in pearl millet

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Abstract Pearl millet (*Pennisetum glaucum* (L.) R. Br.) hybrids, grown widely in India and to some extent in the US, are all based on an A_1 CMS source, leaving the pearl millet hybrids vulnerable to potential disease or insect pest epidemics. A comparison of this CMS system with two additional CMS systems (A_4 and A_5) in the present study based on isonuclear A-lines (seed parents) and their isonuclear hybrids showed that A-lines with the A_4 cytoplasm had much fewer pollen shedders and much reduced selfed seed set in visually assessed non-shedding plants as compared to those with the A_1 cytoplasm. A-lines with the A_5 cytoplasm had neither any pollen shedders nor did they set any seed when selfed. This showed that the A_5 CMS system imparts complete and most stable male sterility, followed by the A_4 and A_1 CMS systems. The frequency of maintainers, averaged across a diverse range of 26 populations, was highest for the A_5 CMS system (98%), followed by the A_4 (59%) and the A_1 (34%) system indicating the greatest prospects for genetic diversification of A-lines lies with the A_5 cytoplasm, and the least with the A_1 cytoplasm. Mean grain yield of hybrids with the A_1 cytoplasm was 5% more than the A_4-system hybrids, while there was no difference between the mean grain yield of hybrids based on A_1 and A_5 CMS systems. Based on these results, it is suggested that seed parents breeding efficiency will be the greatest with the A_5 CMS system, followed by the A_4 CMS system, and least with the currently commercial A_1 CMS system.

Keywords Breeding efficiency · Cytoplasm · Isonuclear A-lines · Male sterility · Pearl millet · *Pennisetum glaucum*

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Introduction

Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is a highly cross-pollinated crop grown on 26 million ha in Africa (15 million ha) and Asia (11 million ha) with India having the largest area (about 10 million ha) in the world. It is primarily grown for grain production, but is also valued for its fodder (both stover and green forage). Single-cross hybrids of pearl millet have 25–30% grain yield advantage over open-pollinated varieties (Rai et al. 2006). The availability of a commercially viable A_1 cytoplasmic-nuclear male-sterility (CMS) system, discovered in 1958 (Burton

1965), provided stimulus for hybrid breeding programs in India and also, to some extent, in Africa and the USA. The A_1 CMS system continues to be the only cytoplasm involved in all the commercial hybrids in India where more than 80 hybrids are cultivated on 50% of the total pearl millet area. It is also the only cytoplasmic source involved in commercial hybrids in the USA and in experimental hybrids being tested in Africa. Considering the risk of cytoplasmic uniformity associated with potential vulnerability to disease and insect pest epidemics, as witnessed in case of southern corn leaf blight epidemic on Texas CMS-based hybrids of corn [*Zea mays* (L.)] in the USA (Scheifele et al. 1970), concerted efforts were made to search for alternative CMS systems in pearl millet. This led to the identification of several CMS sources, of which two alternative sources, one (A_4) identified in a wild species (Hanna 1989) and the other one (A_5) identified in a large-seeded gene pool (Rai 1995) were found particularly interesting. Initial results based on pollen shedding and selfed seed set in visually-assessed non-shedding plants in isonuclear A-lines in the genetic background of 81B had shown male-sterility of these two alternative CMS systems to be greater and more stable than that of the A_1-system A-lines (Rai et al. 2001).

The commercial potential of a CMS system depends on several factors. The foremost among these is the stability of male-sterility of A-lines across various genetic backgrounds and environments. This attribute reduces the probability of discarding A/B pairs whose early generation backcross progenies may be sterile, but turn fertile in advance backcross stages. It also reduces the seed production cost of A-lines and hybrids, and contributes to the maintenance of genetic purity of A-lines and hybrids. High frequency of maintainers in the germplasm and breeding materials enhances the prospects of genetic diversification of A-lines. This, of course, would require greater efforts in breeding restorers of grain hybrids. The relative usefulness of alternative CMS sources also depends on their effect on grain yield and traits of agronomic and adaptation significance. Other factors that determine breeding efficiency of a CMS system include the nature of genetic inheritance and its impact on restorer development (in case of grain hybrids). This paper reports on the comparison of three diverse CMS systems (A_1, A_4 and A_5) for their breeding efficiency in terms of stability of male sterility, frequency of maintainers in a wide range of breeding populations and any effects on grain yield and agronomic traits.

Materials and methods

Experimental material

Isonuclear A-lines

Three isonuclear A-lines with the A_1, A_4 and A_5 cytoplasm in each of the three and diverse genetic backgrounds of the B-lines (81B, 5054B and ICMB 88004) (Table 1) used in this study were developed by more than seven generations of backcrossing of the B-lines into the respective cytoplasmic sources. These nine A-lines were used to study the stability of their male-sterility with respect to the frequency of pollen shedders and degree of selfed seed set in visually assessed non-shedding plants classified as male-sterile.

Isonuclear single-cross hybrids

Two sets of hybrids were made for two experiments. For experiment 1, the three B-lines (81B, 5054B and ICMB 88004) and their counterpart six A-lines with the A_1 and A_4 cytoplasms were crossed with each of the five diverse dual-restorer lines (those lines that restore male fertility of hybrids made on the A_1 and A_4-system male-sterile lines) (Table 1), leading to 45 hybrids. Dual-restorers identified in testcross nurseries prior to summer 1997 were subjected to three generations of single-plant selection for high levels of male-fertility restoration of both the $81A_1$ and $81A_4$. Bulk pollen from each restorer line was collected from panicles selfed with parchment paper bags when one-third of the panicles were out of the boot to pollinate the selfed panicles of A-lines and B-lines at full stigma emergence. For experiment 2, the same three B-lines and their counterpart six A-lines with the A_1 and A_5 cytoplasm were crossed with five diverse dual-restorer lines (Table 1), leading to 45 hybrids. These dual-restorers (restorers of A-lines with the A_1 and A_5 cytoplasm) were developed by 8–9 generations of backcrossing of an A_5 restorer gene from a genetic stock into five diverse A_1-system restorers, followed by three generations of selfing and single plant selection for high levels of male fertility restoration in the A_5 cytoplasmic background. The experi-

Table 1 Parentage of B-lines involved in isonuclear A-lines, and dual-restorers involved in hybrids of pearl millet

Parental line	Parentage/origin
B-line	
81B (ICMB 1)	Gamma radiation-induced downy mildew resistant selection from Tift $23D_2B$
5054B	Developed by Indian Agricultural Research Institute (IARI), New Delhi, India
ICMB 88004	Togo-11-5-2 + …
Dual-restorer (A_1 and A_4)	
IPC 577	[IP 2788 × (J 9347 × 700544-7-2-1)]-1-4-1 + …
IPC 804-2	(S 10LB-30 × LCSN 1225-6-3-1)-1-2-1-1 + …
IPC 827-1	(5054B × F_4FC 1498-1-1-1)-3-1-1 + …
IPC 873	(B Senegal-2-5 × EC 298-2)-2-3-1-1-3 + …
IPC 1518	ICRC-F_4-146-3 + …
Dual-restorer (A_1 and A_5)[a]	
ICMR 03111	IPC 655: (B Senegal-2-5 × 700651)-2-1-4-B + …
ICMR 03222	IPC 873: (B Senegal-2-5 × EC 298-2)-2-3-1-1-3 + …
ICMR 03333	IPC 882: (J 25-1 × J 1798-1-1)-16-4-2-2-2 + …
ICMR 03444	IPC 1518: ICRC-F_4-146-3 + …
ICMR 03555	IPC 1466: H 77/833-2-B + …

[a] Dual-restorers developed by backcrossing A_5 restorer source into IPC lines (A_1 restorers)

mental hybrid seed was produced following the same procedure as for experiment 1.

Isonuclear topcross hybrids

Three isonuclear A-lines with the A_1, A_4 and A_5 cytoplasm in the genetic background of the most widely used commercial seed parent 81B (i.e., $81A_1$, $81A_4$ and $81A_5$) were crossed with 26 diverse open-pollinated varieties (OPVs) and composites (Table 2) developed in Asia region (15) and African regions (11) to assess the frequency of potential maintainers that could be derived from inbreeding in these populations. These 78 topcross hybrids were developed by pollinating 20–25 panicles of each of the three A-lines with bulk pollen collected from 100 to 150 plants of each population. The crosses were made on the tiller panicles of those plants in A-lines that had been examined and found sterile on the basis of pollen shedding in the main panicles.

Field trials

Isonuclear A-lines

The nine isonuclear A-lines with three CMS sources were evaluated in isolated fields at least 1,000 m away from any other pearl millet field in seven year × season environments at Patancheru during 1999–2001 (Table 3). The three seasons (rainy season, early-dry summer season and late-dry summer season) exposed the plants to a wide range of temperature and humidity during the flowering time. The mean maximum temperature encountered during the period spanning from the initiation of flowering till end of the flowering varied from 28.9 to 29.8°C across the years during the rainy season, from 35.8 to 37.2°C during the early-dry summer, and from 37.8 to 39.9°C during the late-dry summer. The highest maximum temperatures encountered during the crop period were 32.8°C during the rainy season, 40.4°C during the early-dry summer, and 42.3°C during the late-dry summer season. The highest relative humidity at 800 h across the years was 89–91% during the rainy season, 63–73% during the early-dry summer season and it was 57% during the late-dry summer season.

More than 700 plants were evaluated for male-sterility at the flowering time by visually checking each A-line in the forenoon for plants shedding pollen. Those plants shedding pollen were classified as male-fertile. Of those visually found non-shedding in the main panicles, tiller panicles of generally more than 300 plants were selfed with the parchment paper bags at the heading time to evaluate for seed set under sel-

Table 2 Target region/parentage/origin of pearl millet population involved in topcross hybrids

Population	Target region/parentage/origin
Asia region	
MC CO	A medium composite developed at ICRISAT by random mating 197 geographically diverse lines from India and Africa
EDC	Early dwarf composite developed by crossing an early composite and a dwarf composite constituted at ICRISAT
HHVBC (tall)	High head volume B-composite (tall) developed by random mating 17 HHVBC S_5 progenies.
ICMR 312	A topcross pollinator developed by random mating 200 S_1 progenies selected from C_2 cycle bulk of Bold-Seeded Early Composite (BSEC)
ICMV 92901	An open-pollinated variety developed by random mating 272 S_1 progenies from BSEC selected at Aurangabad
ICMV 98109	An open-pollinated variety bred by random mating 14 S_1 progenies of EC II C_6 selected for grain yield and agronomic score at Patancheru
MC 94 C_2	Medium Composite second cycle random mated bulk of MC 94 that was developed by random mating 172 progenies selected from India and Western Africa
ICRC NI	Initial cycle random mated bulk of ICRISAT Restorer Composite II for Northern India
ICMP 97774	An open-pollinated variety developed from fourth cycle random mated full-sib bulk of Early Smut Resistant Composite II. Developed by random mating early flowering selections at Hisar from SRC II
ICMS 7704	An open-pollinated variety developed from six inbred lines derived from Indian × African crosses selected at Tandojam in Pakistan
Raj 171	An open-pollinated variety developed by random mating eight S_1 progenies from Inter-varietal composite selected at Patancheru
RCB-2	Rajasthan Composite Bajra developed from 20 inbreds of diverse genetic origin, developed by Rajasthan Agricultural University, India
ICTP 8203	An open-pollinated variety developed by random mating five S_2 progenies selected from an *Iniadi* landrace, originating from northern Togo
WC-C 75	An open-pollinated variety developed from seven full-sib progenies of World Composite (developed in Nigeria) selected at Coimbatore in 1975
RLDTCP	Restorer Lines Dwarf Topcross Pollinator
Africa region	
NC C_1 (tall)	C1 bulk of Nigerian composite developed by random mating 200 S_1 progenies derived from Nigerian and other West-African germplasm
SOSAT-C88	An open-pollinated variety developed by random mating 248 S_1 progenies from the composite *Souna* × *Sanio* developed in Western Africa
GB 8735	Open-pollinated variety developed by random mating four F_3 progenies derived from crosses involving *Iniadi* and *Souna* landraces in Westrern Africa
ICMV IS 89305	An open-pollinated variety developed by recombining nine S_1 progenies from ICRISAT-INRAN genepool (INMG-1) in Western Africa
Ankoutess	An open-pollinated variety developed by random mating 16 S_1 progenies derived from Ankoutess in Western Africa
SDMV 89004	An open-pollinated variety developed by mass selection in SDMV 87014 in Southern Africa
SDMV 90031	An open-pollinated variety developed by random mating five elite SADC varieties in Southern Africa
SDMV 92037	An open-pollinated variety developed by random mating eight S_1 progenies from SADAC genepool 2812 in Southern Africa
SDMV 93032	An open-pollinated variety developed by random mating backcross derivatives of a Zimbabwe landrace and two Togo-based genotypes in Southern Africa
SDMV 95022	An open-pollinated variety developed by random mating backcross derivatives of IBMV 8502 and ICMV 87901 in Southern Africa
SDMV 95045	An open-pollinated variety developed by random mating six late-maturing S_1 progenies from Okashana 1 in Southern Africa

Table 3 Maximum temperature and relative humidity (RH%) during flowering period of pearl millet isonuclear A-lines across seven years × season combinations at Patancheru

Environment		Date planted	Flowering period	Max. temperature (°C)		RH% (0800 h)	
Season	Year			Mean	Range	Mean	Range
Rainy	1999	19 Jul 1999	31 Aug–14 Sep	28.9	24.7–31.3	90	80–98
	2000	14 Jul 2000	26 Aug–24 Sep	29.8	27.2–32.8	91	85–98
	2001	10 Jul 2001	21 Aug–30 Aug	29.1	24.7–32.5	89	80–97
Dry summer (early)	2000	2 Feb 2000	18 Mar–11 Apr	37.2	34.2–40.4	63	41–85
	2001	7 Feb 2001	20 Mar–12 Apr	35.8	30.9–39.0	73	47–87
Dry summer (late)	2000	15 Mar 2000	28 Apr–19 May	37.8	29.8–42.1	57	31–100
	2001	15 Mar 2001	25 Apr–23 May	39.9	36.2–42.3	57	33–82

fing. The selfed panicles were harvested at the physiological maturity and scored for selfed seed set following the ergot rating scale (Thakur and Williams 1980), where seeds were considered equivalent to ergot-infected seeds.

Isonuclear single-cross hybrids

The 45 hybrids in experiment 1 were planted in 2-row plots of 4 m length during the 2002 and 2003 rainy season at Patancheru (18°N) in the southern India, and at Hisar (29°N) and Jamnagar (22°N) in the northern India. Similarly, the 45 hybrids in experiment 2 were planted in 2-row plots of 4 m length at Patancheru during the 2004 rainy season and 2005 summer season, and during the 2004 and 2005 rainy season at Jamnagar. Both experiments were planted in split plot design in four replications, except three replications in experiment 1 in 2002 at Hisar. The nine hybrids made with a pollinator on nine A-lines (3 cytoplasm × 3 nuclear genetic backgrounds of the female parents) were randomized as sub-plots and the pollinator base was assigned to the main plot. The trials received 80 kg N and 40 kg P ha^{-1}. The trials were over-planted at 75 cm row-to-row spacing during the rainy season at Patancheru, and at 60 cm spacing at other locations and at Patancheru during the 2005 summer season. About 15 days after planting, seedlings were thinned to 10 cm spacing. Time to 50% flower was recorded on the plot basis when main panicles of 50% of the plants in a plot had fully emerged stigmas. Plant height and panicle length was recorded on five random plants (three plants in Jamnagar for experiment 2). Plant and panicle counts were used to determine the number of panicles $plant^{-1}$.

Isonuclear topcross hybrids

The 78 topcross hybrids were evaluated for the frequency of male-fertile and male-sterile plants based on the visually assessed pollen shedding. Each hybrid was planted in four rows of 4 m with 10 cm spacing between the plants within the rows, giving approximately 150 plants of each hybrid in each of the two replications. The trial was conducted during the 2000 rainy season and 2001 dry-summer season at Patancheru. Each plant in the trial was rated either as fertile (shedding pollen) or sterile (shedding no pollen).

Statistical analyses

The frequency of male-sterile plants in topcross hybrids of the 26 populations had a very wide range both on $81A_1$ and $81A_4$ with a great deal of overlap. Thus, the difference between these two systems for the frequency of male-sterile plants in hybrids (and hence by implication the frequency of maintainers in each of the population) was tested for statistical significance using the χ^2-test for independence (Gomez and Gomez 1984). Grain yield and other agronomic traits in the hybrid trials were analyzed following a fixed model analysis of variance in a split-plot design, using the statistical package GenStat Release 8.1 (Payne 2002).

Results

Stability of male-sterility

Isonuclear A-lines with the A_1 cytoplasm had pollen shedders in all the three genetic backgrounds and in

Table 4 Frequency of pollen shedders (%)[a] in isonulcear A-lines across seven environments, Patancheru

Cytoplasm	Genetic background	Pollen shedder frequency in environment[b]						
		E1	E2	E3	E4	E5	E6	E7
A_1	81B	0.6	0.4	0.6	0.5	0.4	0.4	0.3
	5054B	1.2	2.1	2.5	0.1	0.4	0.1	0.3
	ICMB 88004	0.0	0.6	0.3	0.2	0.3	0.4	0.1
A_4	81B	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	5054B	0.0	0.0	0.3	0.0	0.1	0.0	0.0
	ICMB 88004	0.0	0.0	0.0	0.0	0.0	0.1	0.0
A_5	81B	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	5054B	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	ICMB 88004	0.0	0.0	0.0	0.0	0.0	0.0	0.0

[a] Based on more than 700 plants of each line in each environment

[b] Rainy season (E1 = 1999, E2 = 2000, E3 = 2001); early dry summer (E4 = 2000, E5 = 2001); late dry summer (E6 = 2000, E7 = 2001)

all the environments except in ICMA 88004 during the 1999 rainy season (Table 4). There were no more than 0.6% pollen shedders in $81A_1$ and $ICMA_1$ 88004 in any of the environments, and there were no clear patterns of pollen shedding across the three seasons in which these lines were tested. In $5054A_1$, however, there were relatively higher frequencies of pollen shedders during the rainy season (1.2–2.5%) compared to the early- and late-dry seasons (0.1–0.4% shedders). A-line with the A_4 cytoplasm had no pollen shedders in the genetic background of 81B in all the environments. Lines $5054A_4$ and $ICMA_4$ 88004 had a maximum of 0.1–0.3% pollen shedders in one or two environments. A-lines with the A_5 cytoplasm had no pollen shedders in any of the three genetic backgrounds and in any of the seven environments (Table 4).

A very high percentage of plants (95–100%) of A-lines with the A_1 cytoplasm visually assessed as no pollen-shedder did not set any seed when selfed (Table 5). However, a low frequency of plants with 1–5% selfed seed set was found in all the A-lines with this cytoplasm, and in all the environments except the late-dry summer of 2001. A few plants of all the three A-lines had even >10% selfed seed set in at least one of the environments. There was no clear pattern related to the effect of environment on selfed seed set. The seed set in $ICMA_1$ 88004 was lesser than in the other two A-lines with genetic backgrounds of 81B and 5054 B. A-lines with the A_4 cytoplasm had fewer plants than those with the A_1 cytoplasm in 1–5% selfed seed class, especially in the genetic backgrounds of 81B and ICMB 88004. Except for one plant in 6–10% seed set class in $5054A_4$ in 2001 early-dry summer, no plant of all the three A-lines had more than 5% selfed set. A-lines with the A_5 cytoplasm rarely set any seed in any of the three genetic backgrounds and in any of the seven environments.

Maintainer frequency

Since the pollen grains are haploid, frequency of male-fertile and male-sterile plants in topcross hybrids made on A-lines are indicators, respectively, of the frequency of restorers and maintainers that can be expected from inbreeding during line development from the pollinator populations. Averaged across all the 26 populations, there were 33% male-sterile plants (i.e., potential maintainers) in hybrids based on $81A_1$, 59% in hybrids based on $81A_4$ and 98% in hybrids based on $81A_5$. The frequency of maintainers of the A_1 and A_4 CMS systems, however, varied a great deal among the populations both from Asia and African regions. In the populations from Asia, the frequency of potential maintainers varied from 6 to 74% for the A_1 CMS system, from 28 to 96% for the A_4 and from 87 to 100% for the A_5 system (Fig. 1). In 12 of the 15 populations, the frequency of maintainers of the A_4 CMS system was significantly higher than those for the A_1 CMS system (χ^2 ranging from 30 to 815; $P < 0.01$). In the populations from Africa, the frequency of maintainers varied from 16 to 74% for

Table 5 Number of plants in selfed seed set class (percent seed set) in three isonuclear A-lines in three CMS systems of pearl millet across seven environments, Patancheru

Environment		Number of plants in A-lines with the cytoplasm														
		A_1					A_4					A_5				
		Total plants	Plants in seed set class				Total plants	Plants in seed set class				Total plants	Plants in seed set class			
Season	Year		0	1–5	6–10	>10		0	1–5	6–10	>10		0	1–5	6–10	>10
81B genetic background																
Rainy	1999	353	348	4	1	0	364	364	0	0	0	353	353	0	0	0
	2000	356	352	3	1	0	346	346	0	0	0	320	320	0	0	0
	2001	329	323	4	1	1	311	311	0	0	0	362	362	0	0	0
Dry summer (early)	2000	304	292	10	1	1	365	365	0	0	0	298	298	0	0	0
	2001	353	334	11	7	1	313	310	3	0	0	354	354	0	0	0
Dry summer (late)	2000	356	340	14	1	1	329	326	3	0	0	379	378	1	0	0
	2001	245	245	0	0	0	225	225	0	0	0	110	110	0	0	0
5054B genetic background																
Rainy	1999	371	357	12	2	0	373	373	0	0	0	365	365	0	0	0
	2000	335	325	9	1	0	317	308	9	0	0	330	330	0	0	0
	2001	346	335	4	4	3	300	285	15	0	0	331	331	0	0	0
Dry summer (early)	2000	304	301	3	0	0	304	304	0	0	0	302	302	0	0	0
	2001	424	418	4	1	1	372	370	1	1	0	356	356	0	0	0
Dry summer (late)	2000	303	294	6	3	0	301	298	3	0	0	312	312	0	0	0
	2001	269	269	0	0	0	216	214	2	0	0	147	147	0	0	0
ICMB 88004 genetic background																
Rainy	1999	320	315	5	0	0	355	355	0	0	0	358	358	0	0	0
	2000	354	350	3	1	0	283	283	0	0	0	348	348	0	0	0
	2001	302	300	2	0	0	307	307	0	0	0	284	284	0	0	0
Dry summer (early)	2000	354	353	1	0	0	314	314	0	0	0	343	343	0	0	0
	2001	500	497	1	0	2	350	350	0	0	0	286	286	0	0	0
Dry summer (late)	2000	345	341	4	0	0	328	328	0	0	0	313	310	3	0	0
	2001	272	272	0	0	0	157	155	2	0	0	167	167	0	0	0

the A_1 CMS system and from 27 to 88% for the A_4 CMS system (Fig. 2). The frequencies of maintainers of the A_4 CMS system were significantly higher than those for the A_1CMS system in eight of the 11 populations (χ^2 ranging from 29 to 368; $P < 0.01$). Again, the highest frequency of maintainers (88–99%, except one population having 55%) was observed for the A_5 CMS system.

Grain yield and agronomic traits

All the hybrids in both experiments had profuse pollen shedding and hence were highly male-fertile. Experiment 1 was conducted at varying productivity levels with the mean grain yield of hybrids in the trial ranging from 1.64 t ha^{-1} at Jamnagar in 2002 (E5) to 3.83 t ha^{-1} at Patancheru in 2002 (E1) (Table 6). The CMS effect on grain yield was highly significant ($P < 0.01$) (Table 7). Averaged across all the six environments and the genetic backgrounds of the A-lines and R-lines, the mean grain yield of the A_1-system hybrids was 2.57 t ha^{-1}, which was 5% more than the mean grain yield of the A_4-system hybrids, and 6% more than the mean grain yield of hybrids made on the B-lines. There was, however, highly significant ($P < 0.1$) CMS × genetic background interaction and significant ($P < 0.5$) CMS × environment interaction (Table 7). Averaged over environments and R-lines, the mean grain yield of the A_1-system hybrids in the genetic background of 81B was 2.60 t ha^{-1}, similar to

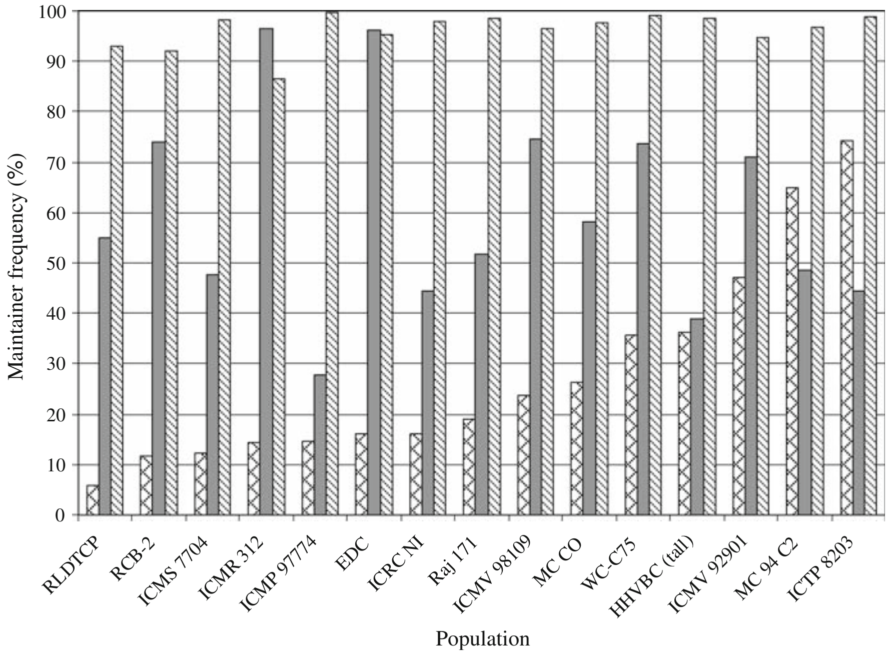

Fig. 1 Frequency of maintainer of $81A_1$, $81A_4$ and $81A_5$ in 15 diverse populations of pearl millet from Asia region. Mean of 2000 rainy season and 2001 summer season, Patancheru. ⊠: $81A_1$ CMS; ■: $81A_4$ CMS; ▧: $81A_5$ CMS

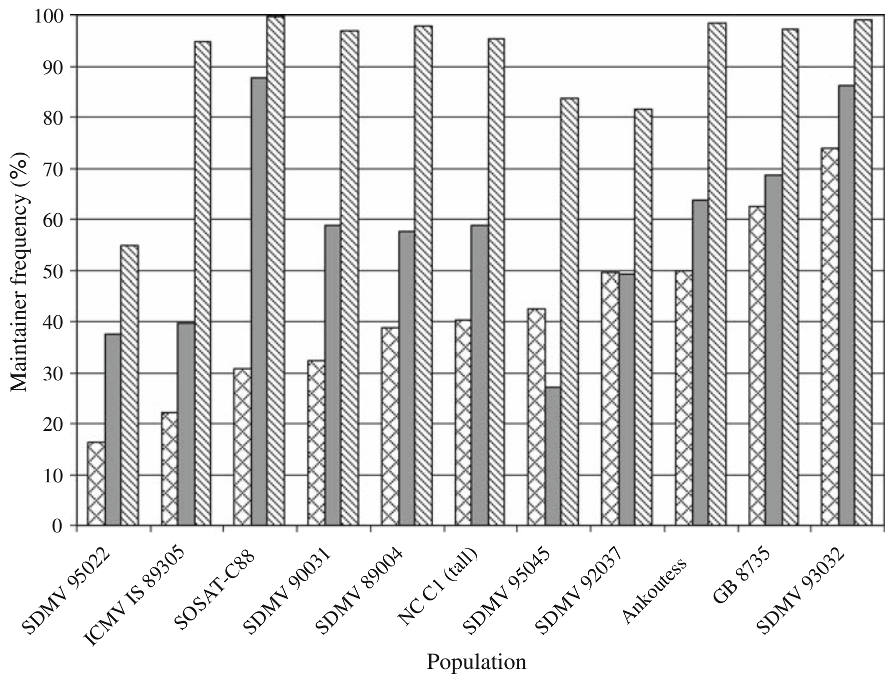

Fig. 2 Frequency of maintainer of $81A_1$, $81A_4$ and $81A_5$ in 11 diverse populations of pearl millet from Africa region. Mean of 2000 rainy season and 2001 summer season, Patancheru. ⊠: $81A_1$ CMS; ■: $81A_4$ CMS; ▧: $81A_5$ CMS

the mean grain yield of $81A_4$ hybrids. In the genetic background of 5054B and ICMB 88004, however, the A_1-system hybrids had 8–9% more grain yield than the A_4-system hybrids. In two environments (E3 and E5), CMS differences were non-significant, while in the remaining four environments, the mean grain yield of the A_1-system hybrids varied from being similar to the A_4-system hybrids to more than 9% yield advantage in one environment (E2). Averaged over the restorers, the mean gain yield advantage of the A_1-system hybrids over the A_4-system hybrids exceeded 10% in the genetic backgrounds of 5054B

Table 6 Grain yield of isonuclear hybrids of pearl millet in three diverse cytoplasmic and genetic backgrounds (Experiment 1)

Cytoplasm (CMS)	Genetic background	Mean grain yield (t ha^{-1})[a] in environment[b]						
		E1	E2	E3	E4	E5	E6	Mean
A_1	81B	3.67	2.27	3.77	2.02	1.74	2.09	2.60
	5054B	3.95	2.04	3.64	2.02	1.37	1.95	2.50
	ICMB 88004	4.15	2.17	3.67	1.92	1.87	1.83	2.60
A_4	81B	3.70	2.14	3.64	2.06	1.80	2.19	2.59
	5054B	3.73	1.82	3.34	1.72	1.34	1.90	2.31
	ICMB 88004	3.80	1.96	3.36	1.88	1.84	1.71	2.42
Fertile	81B	3.76	2.17	3.89	1.94	1.81	2.08	2.61
	5054B	3.71	1.86	3.32	1.67	1.25	1.92	2.29
	ICMB 88004	4.01	2.09	2.88	1.58	1.78	1.52	2.31
CMS background	A_1	3.92	2.16	3.70	1.99	1.66	1.95	2.57
	A_4	3.74	1.97	3.45	1.89	1.66	1.93	2.44
	Fertile	3.82	2.04	3.36	1.73	1.61	1.84	2.40
	Trial mean	3.83	2.09	3.50	1.87	1.64	1.91	2.47
	SE±[c]	0.04	0.03	0.11	0.05	0.04	0.04	–

[a] Mean of five hybrids

[b] E1 = Patancheru 2002; E2 = Patancheru 2003; E3 = Hisar 2002; E4 = Hisar 2003; E5 = Jamnagar 2002; E6 = Jamnagar 2003

[c] SE for comparing the effect of three CMS backgrounds averaged over nuclear backgrounds of the male and female parents

Table 7 Analysis of variance for grain yield (t ha^{-1}) of isonuclear single-cross hybrids in pearl millet

Source of variation	Experiment 1		Experiment 2	
	df	Mean square	df	Mean square
Environment (E)	5	149.89**	3	96.16**
Residual (a)	17	1.78	12	1.57
Restorer (R)	4	1.67*	4	2.63**
E × Restorer	20	1.66**	12	0.84**
Residual (b)	68	0.60	48	0.29
A-lines	8	2.05**	8	2.09
Genetic background (GB)	2	4.60**	2	7.29**
Cytoplasm (C)	2	2.27**	2	0.44*
GB × C	4	0.66**	4	0.32*
E × A-lines	40	0.61**	24	0.59
E × GB	10	1.77**	6	1.72**
E × C	10	0.28*	6	0.28*
E × GB × C	20	0.19	12	0.17
R × A-lines	32	0.51	32	0.41
R × GB	8	1.30**	8	1.3**
R × C	8	0.47**	8	0.16
R × GB × C	16	0.13	16	0.09
E × R × A-lines	160	0.19*	96	0.16
E × R × GB	40	0.23*	24	0.27**
E × R × C	40	0.22*	24	0.15
E × R × GB × C	80	0.15	48	0.10
Residual (c)	680	0.15	480	0.12

*, ** Significant at 0.05 and 0.01 probability levels, respectively

Table 8 Grain yield of iso-nuclear hybrids of pearl millet in three diverse cytoplasmic and genetic backgrounds (Experiment 2)

Cytoplasm	Genetic background	Mean grain yield (t ha^{-1})[a] in environment[b]				
		E1	E2	E3	E4	Mean
A_1	81B	3.02	3.32	3.20	1.69	2.81
	5054B	2.86	3.47	2.63	1.55	2.63
	ICMB 88004	2.93	3.68	3.27	1.98	2.97
A_5	81B	2.98	3.53	3.10	1.83	2.86
	5054B	2.82	3.49	2.62	1.60	2.63
	ICMB 88004	2.97	3.53	3.07	1.89	2.87
Fertile	81B	2.97	3.28	3.09	1.89	2.81
	5054B	2.82	3.48	2.19	1.34	2.46
	ICMB 88004	3.04	3.59	2.99	1.96	2.90
CMS background	A_1	2.94	3.49	3.03	1.74	2.80
	A_5	2.92	3.52	2.93	1.77	2.79
	Fertile	2.94	3.45	2.76	1.73	2.72
	Trial mean	2.93	3.49	2.91	1.75	2.77
	SE±[c]	0.04	0.04	0.05	0.04	0.02

[a] Mean of five hybrids

[b] E1 = Patancheru 2004; E2 = Patancheru 2005; E3 = Jamnagar 2004; E4 = Jamnagar 2005

[c] SE for comparing the effect of three CMS backgrounds averaged over nuclear backgrounds of the male and female parents

and ICMB 88004 in E2, and in the genetic background of 5054B in E4.

Experiment 2 was also conducted at varying productivity levels, with the mean grain yield of hybrids in the trial ranging from 1.75 t ha^{-1} at Jamnagar in 2005 (E4) to 3.49 t ha^{-1} at Patancheru in 2005 (E2) (Table 8). The CMS effect on grain yield was significant ($P < 0.05$) in this trial also (Table 7), but averaged over all the environments and the genetic backgrounds of the A-lines and R-lines, the mean grain yield of the A_1-system hybrids was 2.80 t ha^{-1}, which was very similar to the mean grain yield of the A_5-system hybrids and 3% more than the mean grain of the hybrids made on B-lines (Table 8). Interaction of CMS effect with the genetic backgrounds of the A-lines and the environment was also significant ($P < 0.05$). The A_1-based hybrids with the mean grain yield of 2.63 t ha^{-1} in the genetic background of ICMB 88004 and in environment E3 registered the largest yield advantage of 7% over the A_5-based hybrids. But in environment E4 and in the genetic background of 81B, A_5-based hybrids, on an average, had 8% more grain yield than the A_1-based hybrids.

The CMS effects on plant height and time to 50% flower were significant in both experiments (data not presented). However, averaged over the environments and genetic backgrounds of R-lines, the difference between the A_1-system hybrids and those based on the A_4- and A_5-CMS systems varied from none to 2 days for flowering and from none to 3 cm for plant height. The CMS effect was significant for panicle length in experiment 2 (though no more than 1 cm of changes were observed), and non-significant for the number of panicles per plant in both experiments.

Discussion

The A_1 male sterility system discovered and disseminated more than 40 years ago for use in hybrid parent development continues to be the only CMS system under commercial use till today. Pearl millet research programs at ICRISAT, and in the National Agricultural Research System and the private sector in India, and in the US, have developed a number of A-lines based on this cytoplasm that are seed parents of commercial hybrids, with a highly diversified hybrid cultivar base in India (Mula et al. 2007). This CMS system, however, has an inherent problem of unstable male sterility, resulting in varying frequency of pollen shedders, depending on the genetic backgrounds of the A-lines and the environments. The frequency of pollen shedders in these A-lines is generally very low (<5% in most of the genetic backgrounds and in most of the environments), but none of the A-lines produced so far has been found to be free of pollen shedders. Since the frequency of pollen shedders in the A lines based on A_1-system is low, and none of the several CMS sources identified subsequently was better than this (Rai et al. 1996, 2001), the widespread use of the A_1 CMS system in hybrid development continued. Search, however, also continued to identify more stable CMS sources. A preliminary study had shown that while $81A_1$, the most widely used commercial A-line in India, had 0–0.6% pollen shedders, $81A_4$ and $81A_5$ had none (Rai et al. 2001). This comparison of the three CMS systems was, however, based on a single genetic background of the A-lines. Results of the present study across three genetic backgrounds confirmed the earlier findings of $81A_1$ having pollen shedders up to 0.6%. There was no discernible pattern in the pollen shedder frequency across the environments that varied for the mean maximum temperatures ranging from 28.9°C in the rainy season to 39.9°C during the late-dry season, and relative humidity ranging from 57% during the dry summer to 91% during the rainy season. In $5054A_1$, however, the frequency of pollen shedders increased to 1.2–2.5% across the rainy season but was comparable to that in $81A_1$ during the dry seasons having higher temperatures and lower relative humidity. The frequency of pollen shedders in A-lines with the A_4 cytoplasm was very rare, while there were no pollen shedders in A-lines with the A_5 cytoplasm, regardless of the genetic backgrounds and the test environments.

Visual assessment of pollen shedding fails to detect those partially fertile plants that produce traces of viable pollens. A low frequency of such plants (<4%) had 1–5% selfed seed set in A-lines with the A_1 cytoplasm, indicating low fertility levels that could arise from reversion to male fertility. The frequency of such plants was largely influenced by the genetic backgrounds of A-lines (highest in $81A_1$ and lowest in ICMA$_1$ 88004) rather than by the environments, as there was no clear patterns of pollen shedding across seasons. A very low frequency of plants (<1%) in A-lines with this cytoplasm had even >10% selfed seed set in all the three genetic backgrounds in some environments. It has been shown that these seeds produce relatively higher frequency of pollen-shedding plants, with 22–24% pollen shedders in $81A_1$ and up to 63% pollen shedders in $5054A_1$ (Rai et al. 2006). The few selfed seeds in these few plants could have arisen due to cytoplasmic mutations, leading to reversion to male fertility as reported earlier in pearl millet (Burton 1977). Further, there are reports of genetic backgrounds of A-lines influencing the reversions to male fertility in pearl millet (Clement 1975) and maize (Singh and Laughnan 1972). A-lines with the A_4 cytoplasm had much fewer plants in 1–5% seed set class and had no plant exceeding 5% seed set level, while the A-lines with the A_5 cytoplasm had rarely any plants that had even 1–5% seed set. Thus, the selfed seed set patterns in non-shedding plants of A-lines were in broad conformity to the pollen shedding patterns, indicating that the A_5 CMS system imparted complete and the most stable male sterility, followed by the A_4 CMS system that confers nearly complete sterility in most of the genetic backgrounds and environments. The sterility of the A_1 CMS system is least stable, and is greatly influenced by the genetic backgrounds but influence of environmental factors such as temperatures and relative humidity did not show any definite pattern.

Based on the average frequency of male-sterile plants in topcross hybrids of a wide range of populations of diverse origin and genetic base, it appeared that about 98% of the inbred lines derived from them can be expected to be maintainers of the A_5 CMS system, 59% of the A_4 system and 33% of the A_1 system. Hence the A_5 CMS system provides the greatest opportunity for genetic diversification of A-lines, which would enhance the probability of producing genetically diverse hybrids, especially of forage types as sterile hybrids are likely to be preferred to fertile hybrids. However, use of A-lines with the A_5 cytoplasm for grain hybrid development would require much greater efforts for the breeding of restorer lines than required for the A_1 and the A_4 CMS systems. A backcross breeding scheme devised and efficiently used for converting 48 breeding lines into their A_5-restorer versions at ICRISAT showed that the restorer breeding efficiency of this (and the A_4) CMS system was a rather simpler task. This approach keeps the restorer genes in the sterile cytoplasmic background all through the backcross generations, and hence allows for the selection of plants with high levels of male fertility in the main panicles before using their tiller panicles for backcrossing (Rai et al. 2006). Further, the highest possible levels of male fertility restoration ability in the restorer lines is not a necessary requirement in pollinators of grain hybrids of this wind-pollinated and prolific pollen-producing species. Conversely, the highest level of stable male sterility is a necessary requirement in A-lines for economical seed production (cost savings from overcoming the roguing operations) and maintenance of seed quality standards (a seed certification requirement). The frequency of A_4-system maintainers from a diverse range of populations is likely to be twice as high as that of the A_1-system maintainers, which would double the effectiveness of genetic diversification of A-lines with the A_4 CMS system, although this would be only half as efficient as the A-line breeding with the A_5 CMS system.

The highly male-sterilizing abilities of the A_4 and A_5 CMS systems would enhance the breeding efficiency of not only male-sterile inbred lines, but also of the male-sterile populations for use in breeding inter-population hybrids, and male-sterile F_1s for use in breeding three-way hybrids. The feasibility of producing a male-sterile population based on the A_4 CMS system has been demonstrated (Rai et al. 2000). The breeding of male-sterile populations with the A_5 CMS system is likely to be even more efficient. It has been observed that the A_1-system A-lines when crossed with non-parental B-lines, do not necessarily produce fully male-sterile F_1s (Rai and Hash 1990), reducing the prospects of breeding male-sterile F_1s. This difficulty can be substantially reduced with the use of the A_4 CMS system, and more so with the use of the A_5 CMS system. Both types of seed parents would increase the cost effectiveness of seed production due to their higher seed yield potential on account of varying degrees of heterozygosity, and the stability of resistance to downy mildew in hybrids due to genetic heterogeneity and more effective resistance gene deployment strategies in parental lines of three-way hybrids (Witcombe and Hash 2000). These types of seed parents would be specifically suitable for breeding forage hybrids as the within-cultivar variability in forage hybrids would be of much less concern than in the grain hybrids. However, these could also be useful for breeding grain hybrids for the arid and semi-arid African agricultural situations where the concept of uniform hybrids has not yet established a foothold.

The difference between mean grain yield of the A_1- and the A_5-system hybrids (<1%) was non-significant, with significant interaction with the genetic backgrounds and the environments. Thus, the difference between the A_1- and A_5-system hybrids varied from none to no more than 9%, depending on the genetic backgrounds and the environments, and it was not consistent in the direction of one cytoplasm or the other. This showed that the A_5 CMS system has no adverse effect on grain yield. The grain yield differences between the A_1- and A_4-system hybrids were of relatively larger magnitude, with the A_1-system hybrids having 5% overall grain yield advantage over the A_4-system hybrids when averaged over the genetic backgrounds of both A-lines and R-lines and the environments. In none of the genetic backgrounds of the A-lines and the environments, the A_4-system hybrids had any yield advantage over the A_1-system hybrids. Rather the A_1-system hybrids had significantly higher mean grain yield than the A_4-system hybrids especially in the genetic backgrounds of both 5054B and ICMB 88004. In some of the environments grain yield advantage in A_1 hybrids was up to 17%. In another study that compared isonuclear hybrids with A_1 and A_4 cytoplasm in a single environment, 8% higher yield of A_4-hybrids was observed (Yadav 1996). On the other hand, Chandra-Shekara et al. (2007) reported negative effect of the A_4 CMS system and positive effect of the A_5 CMS system on grain yield in pearl millet, though these differences were significant in only one of the two environments. This study, however, was not based on isonuclear A-lines and involved a set of common pollen parents, all of which not necessarily produced fertile hybrids on the A_4-system A-lines and none produced fertile hybrids on the A_5-system A-lines. Even though all hybrids in the trial would have had good seed set under open pollination, lack of pollen production per se may have positive effect on grain yield as reported in maize (Duvick 1957). Thus, the yield differences of the A_1- and A_4-system hybrids are highly variable depending on the genetic backgrounds and the environments. In determining the relative usefulness of the A_1 and A_4 CMS systems, hybrids need to be viewed in relation to level and stability of male-sterility, and higher levels of maintainer frequency of the A_4 CMS system.

The above results of this study demonstrate that A_5 CMS system provides a greater opportunity for genetic diversification of seed parents with complete and most stable male sterility and without any adverse effects on grain yield. However, the use of A_5 CMS system for grain hybrid development would require greater efforts than those required for the A_1- and A_4-system for restorer parents development, but this can be rapidly achieved by using highly effective restorer stocks and backcross breeding procedure. The A_4 CMS system is likely to be relatively lesser efficient than the A_5 CMS system in breeding seed parents, but is clearly more efficient than the A_1 CMS system.

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