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Stability of male-sterile sources and fertility restoration of their hybrids in pearl millet

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With 7 tables

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Abstract

Genetic background has a significant effect on the expression of cytoplasmic-nuclear male sterility (CMS) in pearl millet (*Pennisetum glaucum* (L.) R. Br.). Therefore, a reliable characterization of CMS sources requires the use of near-isonuclear lines and their hybrids. We used this approach to characterize five CMS sources (A_1, A_2, A_3, A_v, and A_4). Male sterility of 81A_4 was the most stable, followed by 81A_1, and 81A_v, indicating the relative merits of these CMS sources in breeding stable male-sterile lines. Male-sterile lines 81A_2 and 81A_3 were highly unstable, indicating their minimal value. Differential male fertility restoration patterns of hybrids made on 81A_v and 81A_4 suggest that the A_v and A_4 cytoplasms represent CMS systems that are different from each other and from A_1, A_2 and A_3. An evaluation of topcross hybrids of 15 diverse populations made on 81A_1 and 81A_4 showed that each population had restorers and maintainers of both cytoplasms and that the frequency of maintainers of A_4 was as high as, or higher than, that of the A_1 cytoplasm. Thus, use of the A_4 cytoplasm can substantially increase the probability of breeding stable male-sterile lines based on inbreds developed from diverse genetic backgrounds, and also provide the opportunity for breeding restorers from each of these diverse genetic sources.

Key words: *Pennisetum glaucum* — pearl millet — CMS sources — fertility restoration — male sterility

The entire pearl millet (*Pennisetum glaucum* (L.) R. Br.) hybrid seed industry today is based on the A_1 system of cytoplasmic-nuclear male sterility (CMS), made available through the release of male-sterile line Tift 23A_1 (Burton 1965). Considering the likely risk of disease or insect pest epidemic associated with cytoplasmic uniformity, as witnessed in case of the southern leaf blight, *Bipolaris maydis* (Nisikado & Miyake) Shoemaker, epidemic on the Texas CMS-based hybrids of corn (*Zea mays* L.) in the USA (Scheifele et al. 1970), the search for new CMS sources became an integral part of several pearl millet breeding programmes. In addition to the A_1, A_2 and A_3 CMS sources (Burton and Athwal 1967) used for breeding hybrids, two other sources (A_v and A_4), derived from different accessions of *P. glaucum* ssp. *violaceum* = *monodii* (Marchais and Pernes 1985, Hanna 1989), a wild relative of cultivated pearl millet, are now available. However, these have not yet been compared with each other for the stability of their male sterility and the fertility restoration of their hybrids.

Fertility restoration patterns of F_1 hybrids developed from crosses between male-sterile lines possessing different CMS sources and a set of inbreds has been routinely used for the classification of CMS sources in maize (Duvick 1965, Gracen and Grogan 1974), sorghum (*Sorghum bicolor* (L.) Moench) (Schertz and Ritchey 1978, Rao et al. 1984) and pearl millet (Burton and Athwal 1967, Hanna 1989). Considering a significant effect of nuclear genetic background on fertility restoration of hybrids, studies in maize (Beckett 1971) and pearl millet (Rai and Hash 1990) have emphasized the need for using hybrids made on isonuclear A-lines for fertility restoration pattern analysis and CMS classification. We developed near-isonuclear lines of the A_1, A_2, A_3, A_v and A_4 CMS sources in the nuclear genetic background of 81B, which is the maintainer line of a commercial male-sterile line (81A) used widely in India.

The objectives of this research were (1) to evaluate the stability of male sterility of these five CMS sources in a common nuclear genetic background, (2) to study male fertility restoration patterns of single-cross hybrids made on these near-isonuclear lines for CMS classification, and (3) to evaluate fertility/sterility reactions of hybrids made on to the A_4 CMS source with a diverse range of breeding populations.

Materials and Methods

Isonuclear A-lines: The six near-isonuclear A-lines (hereafter referred to as isonuclear lines) used in this study were developed by more than seven generations of backcrossing of the nuclear genome of 81B into five different CMS sources. These A-lines were: 81A_1 (ICMA 1) with Tift 23A_1 cytoplasm (Anand Kumar et al. 1984); 81A_v (= ICMA 88001) with cytoplasm from a *P. glaucum* ssp. *violaceum* accession from Senegal (Marchais and Pernes 1985); 81A_4 with cytoplasm from a different *violaceum* accession from Senegal (Hanna 1989); two versions of 81A_2 (Pb 310A_2 and Pb 311A_2) with A_2 cytoplasm via different initial A-lines developed at Punjab Agricultural University (PAU), Ludhiana (Virk and Brar 1993); and 81A_3 (= Pb 406A_3) with A_3 cytoplasm, again developed at PAU (Virk and Brar 1993). Our field experience had shown that A-lines of the A_2-CMS and A_3-CMS sources display varying degrees of pollen shedding, including whole-plant pollen shedding, whole-panicle pollen shedding, and sectorial pollen shedding within panicles. It has also been observed that A-lines of these two CMS sources visually rated as pollen sterile (i.e., no pollen shedding) may have varying degrees of selfed seedset. Therefore, male sterility was

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evaluated on the basis of both pollen shedder frequency and selfed seedset.

A-lines were evaluated during the 1992 rainy season (June–September) and the 1993 warm-dry season (January–April) (hereafter referred to as the dry season) at ICRISAT Asia Center (IAC). More than 150 plants of each line from a single plot in each season were evaluated for frequency of pollen shedders. These lines were also evaluated for selfed seedset during the 1990–92 rainy seasons and 1991–93 dry seasons at IAC (18°N). For this, usually 50–100 plants that were found shedding no pollen in the main panicle were selected for each A-line in each environment. A tiller panicle of these plants was selfed and covered with a parchment paper bag at the beginning of panicle emergence. Two weeks before maturity, the bags were pushed down the panicles and the standard ergot (*Claviceps fusiformis* Loveless) rating scale (Thakur and Williams 1980) was used to score selfed seedset. Selfed seeds of panicles in two seedset classes (1–5% and 41–60%) from the 1992 rainy season and the 1993 dry season evaluations at IAC were bulk-threshed for each A-line and plants from these seeds were evaluated at IAC during the 1993 rainy season for the percentage of off-type plants to determine the degree to which this could perhaps arise from contamination with pollen from other plants.

Single-cross hybrids: Each of the six isonuclear lines was crossed with a common set of 18 diverse inbred lines to produce 108 hybrids. These inbred lines included four maintainer lines of the A_1 CMS system (Nos. 1–4), one maintainer line each of the A_2 (No. 5) and the A_3 (No. 6), and 12 lines from the ICRISAT Pearl Millet Pollinator Collection as potential restorers of the A_1 CMS system (Nos. 7–18) (Table 4). Bulk pollen collected from more than 10 bagged panicles of a pollen parent was used for crossing with all six A-lines at the same time. Crosses were made on the tiller panicles of only those plants whose main panicles had been observed to be shedding no pollen. Hybrids were grown in unreplicated nurseries of single-row plots of 4 m length (about 30 plants per plot) during the dry and rainy seasons of 1991 at IAC, and during the 1991 dry season at Pune (18°N). Following the procedure of Rai and Hash (1990), each plot at 75% anthesis was visually scored as: 1 (all plants having shrunken anthers and shedding no pollen), 2 (most of the plants having shrunken anthers and shedding no pollen), 3 (most of the plants having plump anthers and shedding pollen) or 4 (all plants having plump anthers and shedding pollen). None of the plots scored as 2 or 3 had frequencies of both pollen-shedding and non-shedding plants anywhere in the intermediate range. Ten plants in each plot were selfed and scored for selfed seedset in the same way as the isonuclear lines.

Topcross hybrids: Two open-pollinated varieties (OPVs) and 13 diverse composites (Table 7) were assayed for the frequencies of maintainers and restorers of the A_4 and A_1 (control) cytoplasms. The well-mixed bulk pollen collected from several bagged panicles of a composite or OPV was crossed on to tiller panicles of those plants of $81A_1$ and $81A_4$ whose main panicles did not shed pollen. Pollen collection from any one plant was done only once. Pollen collection from a composite or OPV continued for 5–6 days to ensure that bulk pollen from a total of more than 100 plants had been used in crossing on to both A-lines to produce 15 pairs of topcross hybrids. The selfed seedset of generally more than 40 plants of each topcross hybrid was evaluated in unreplicated observation nurseries during the 1991 rainy season and the 1992 dry season at IAC, following the same procedure as described earlier for the isonuclear lines. This resulted in the selfed seedset evaluation of 750–800 plants of topcross hybrids on each of the two A-lines in each season.

From the viewpoint of breeding A-lines, it is essential that the hybrid between an A-line and its potential maintainer line does not set seed when selfed. However, from the viewpoint of breeding a good restorer line, it is desirable that its hybrid sets more than 50% selfed seed. Therefore, in this paper we present frequencies of topcross hybrid plants in 0% and more than 50% selfed seedset classes only.

Crop management: All the field tests were conducted under optimum management conditions, characterized by high fertility (80 kg N/ha and 40 kg P/ha). Periodic irrigation during the dry season and protective irrigation during the rainy season were given to avoid moisture stress. There was no incidence of any disease or insect pest.

Table 1: Frequency of pollen shedders in six isonuclear A-lines of pearl millet, ICRISAT Asia Center, rainy season 1992 and dry season 1993

Isonuclear A-line	Season	Number of plants	Per cent pollen shedders
$81A_1$	Rainy 1992	147	0
	Dry 1993	239	0
$81A_4$	Rainy 1992	139	0
	Dry 1993	128	0
$81A_v$	Rainy 1992	222	0
	Dry 1993	195	0
$81A_2$ (Pb $310A_2$)	Rainy 1992	178	23**
	Dry 1993	219	12
$81A_2$ (Pb $311A_2$)	Rainy 1992	158	18**
	Dry 1993	219	4
$81A_3$	Rainy 1992	171	5
	Dry 1993	247	7

**Significant at P = 0.01

Statistical analysis: The difference between the two seasons for percentage of plants with an attribute of a male-sterile line or between the percentage of plants of two male-sterile lines for an attribute in a season was tested by a Z test (Snedecor and Cochran, 1967).

Results

Isonuclear A-lines

Three isonuclear lines ($81A_1$, $81A_v$ and $81A_4$) had no pollen shedders during the 1992 rainy season and 1993 dry season at IAC (Table 1). However, these lines differed with respect to selfed seedset. During the 1991–93 dry seasons, an average of 97% plants of $81A_1$ and 99% plants of $81A_4$ did not set any seeds under selfing bags, and no plants exceeded the 1–5% seedset class (Table 2). In comparison, 92% plants of $81A_v$ had no seedset, 6% plants had 1–5% seedset, and very few plants (<1%) had even >20% seedset. During the 1990–92 rainy seasons, there was a significant reduction in the percentage of plants in all A-lines that did not set seed under selfing. Thus, an average of 82% plants of $81A_4$ had no seedset and the remaining 18% had 1–5% seedset. In comparison, 74% plants of $81A_1$ and only 55% plants of $81A_v$ had no seedset. There were 24% plants in $81A_1$ and 42% plants in $81A_v$ that had 1–5% seedset, with an additional 3% plants of $81A_v$ having 6–10% seedset: there were but rare instances of plants in both $81A_1$ and $81A_v$ that even had 11–20% seedset in the rainy season.

The level of sterility was much less in $81A_2$ (Pb $310A_2$ and Pb $311A_2$) and $81A_3$ (Pb $406A_3$) compared with the above three A-lines, with significantly higher sterility levels in the dry than the rainy seasons. For instance, the two A_2-system A-lines had 18–23% pollen shedders in the rainy season and 4–12% in the dry season (Table 1). The $81A_3$ had 5–7% pollen shedders. Much fewer plants of these A-lines, ranging from 22 to 27% in the dry season and 5 to 11% in the rainy season, had no selfed seedset (Table 2). Also 8–13% plants in the dry as well as rainy seasons even had greater than 20% seedset.

A grow-out test of seeds harvested from selfed panicles of the 1–5% selfed seed class of the 1992 rainy season and the 1993 dry season seed sources showed that 82–100% of such seeds from the $81A_4$ produced off-type plants (Table 3). Such plants were much fewer in $81A_1$ (67%) and $81A_v$ (26–40%). For

Table 2: Selfed seedset (%) in isonuclear A-lines of pearl millet, ICRISAT Asia Center

Isonuclear A-line	Season[1]	Number of plants[2]	Per cent plants[3] in seedset class: 0	1–5	6–10	11–20	>20
$81A_1$	Dry	432	97**	3	0	0	0
	Rainy	342	74	24	1	<1	0
$81A_4$	Dry	327	99**	1	0	0	0
	Rainy	318	82	18	0	0	0
$81A_v$	Dry	385	92**	6	1	0	<1
	Rainy	477	55	42	3	<1	0
$81A_2$ (Pb $310A_2$)	Dry	299	25**	31	22	14	8
	Rainy	274	5	50	20	16	9
$81A_2$ (Pb $311A_2$)	Dry	339	27**	40	14	6	13
	Rainy	301	11	44	16	15	14
$81A_3$	Dry	373	22**	35	18	13	12
	Rainy	382	10	41	23	17	9

[1]Dry seasons 1991–93; Rainy seasons 1990–92
[2]Total number of plants over 3 years
[3]Mean per cent of plants over 3 years
**Significant at P = 0.01

Table 3: Per cent off-type plants from seeds harvested from selfed panicles of two extreme selfed seedset classes of six isonuclear A-lines of pearl millet, ICRISAT Asia Center, rainy season 1993

Isonuclear A-line	Seed source	1–5% Seedset class: Total plants	Per cent plants: Off type	True type	41–60 % Seedset class: Total plants	Per cent plants: Off type	True type
$81A_1$	Rainy 1992	36	67	33	0	—	—
	Dry 1993	59	66	34	0	—	—
$81A_4$	Rainy 1992	38	82**	18	0	—	—
	Dry 1993	59	100	0	0	—	—
$81A_v$	Rainy 1992	62	26	74	0	—	—
	Dry 1993	68	40	60	0	—	—
$81A_2$ (Pb $310A_2$)	Rainy 1992	101	4	96	86	0	100
	Dry 1993	69	7	93	0	—	—
$81A_2$ (Pb $311A_2$)	Rainy 1992	86	5	95	57	3	97
	Dry 1993	101	3	97	164	0	100
$81A_3$	Rainy 1992	74	3	97	0	—	—
	Dry 1993	54	11	81	185	1	99

**Significant at P = 0.01

$81A_2$ and $81A_3$, generally less than 10% of the seeds harvested from the selfed panicles of the 1–5% seedset class and less than 4% of those from 41–60% seedset class produced off-type plants in grow-out tests (Table 3). The frequency of off-type plants was independent of the seed source, except for the $81A_4$ where selfed seeds harvested from the dry season produced significantly higher percentages of off-type plants than the seeds from the rainy season.

Single-cross hybrids

Except for the $81A_1$-hybrid with 843B in the rainy season (R), hybrids of $81A_1$ and $81A_v$ with four pollen parents (81B, 843B, ICMB 89111 and Pb $408B_3$) had a pollen-shedding score of 1 (Table 4). Hybrids of these two A-lines with six pollen parents (Pb $302B_2$, ICMP 501, ICMP 83506, ICMR 85410, ICMR 356 and H 77/833-2) had a pollen-shedding score of 4. There were seven pollen parents whose hybrids on to these A-lines had clear differential pollen-shedding patterns. Hybrids of four of these (K 560-230, IPC 98, IPC 390 and ICMP 82601) produced on to $81A_1$ had a score of 4 and those produced on to $81A_v$ had a score of 1. Conversely, hybrids produced with three other pollen parents (ICMB 90111, IPC 501 and ICMP 85303) on to $81A_1$ had a score of 1 or 2 and those produced on to $81A_v$ had scores of 3 or 4. Among the hybrids of $81A_4$, 16 had a score of 1, and two had a score of 4. Hybrids of each of these three male-sterile lines had as good as, or greater, pollen shedding in the rainy season than in the dry season.

The pollen-shedding patterns for hybrids of the A_2- and A_3-system A-lines were not as clear-cut as for those based on A_1, A_v and A_4 cytoplasms, with a large proportion of hybrids having scores of 2 or 3 in one or both seasons (Table 4). Among the hybrids whose pollen-shedding scores were consistent across the two seasons, there were two pollinators (ICMR 356 and H 77/833-2) whose hybrids on Pb $406A_3$ had pollen-shedding scores that were different from those made on either or both of Pb $310A_2$ and Pb $311A_2$. However, hybrids of two pollinators (H 77/833-2 and ICMP 82601) on to Pb $310A_2$ also had pollen-shedding scores that were different from those made on to Pb $311A_2$, although both A-lines purportedly had the same cytoplasm and the same nuclear genotype.

Seedset patterns of $81A_4$-hybrids were identical to their pollen-shedding patterns. Thus, hybrids of 16 pollen parents with a score of 1 for pollen shedding had no or less than 1% selfed seedset, while hybrids of two pollen parents (ICMR 85410 and ICMP 85303) with a pollen-shedding score of 4 had 88–93% seedset in both dry and rainy seasons at IAC and 68–76% seedset in the dry season at Pune (Table 5).

For hybrids having pollen-shedding scores of 3 and 4, the relationship between pollen shedding and selfed seedset was not as good for the hybrids of $81A_1$ and $81A_v$ as for the hybrids of

Table 4: Pollen shedding patterns in single-cross hybrids of isonuclear A-lines in pearl millet, ICRISAT Asia Center, dry (D) and rainy (R) seasons 1991

Pollen parent	Pollen shedding score of hybrid on male-sterile line											
	$81A_1$		$81A_4$		$81A_v$		$81A_2$ ($Pb\ 310A_2$)		$81A_2$ ($Pb\ 311A_2$)		$81A_3$	
	D	R	D	R	D	R	D	R	D	R	D	R
81B	1	1	1	1	1	1	2	4	2[1]	2	1	1
843B	1	2	1	1	1	1	4[1]	4	4	4	4[1]	4
ICMB 89111	1	1	1	1	1	1	4[1]	4	3[1]	4	4[1]	4
ICMB 90111	1	2	1	1	3[1]	3	1	1	1	1	1	1
Pb $302B_2$	4	4	1	1	4	4	1	1	1	2	1	1
Pb $408B_3$	1	1	1	1	1	1	1	2	2	2	2	2
ICMP 501	4	4	1	1	4	4	3	2	1	1	2	2
ICMP 83506	4[1]	4	1	1	4	4	3	4	3	3	3	3
ICMR 85410	4	4	4	4	3[1]	4	4	4	4	4	4	4
ICMR 356	4	4	1	1	4[1]	4	2	2	2	2	3[1]	3
J 104	4	4	1	1	2[1]	3	1	3	2	2	2	2
K 560-230	4	4	1	1	1	1	4[1]	4	4[1]	4	4[1]	4
H 77/833-2	4	4	1	1	4	4	4[1]	4	2	2	4[1]	4
IPC 98	4	4	1	2	1	1	4	4	4	4	4	4
IPC 390	4	4	1	1	1	1	1	1	2[1]	2	2[1]	2
IPC 501	1	1	1	1	3	3	2	2	2	2	2	2
ICMP 82601	4	4	1	1	1	2	1	1	4[1]	3	3[1]	2
ICMP 85303	1	2	4	4	4[1]	4	4	4	4[1]	4	4	4

Pollen-shedding score: 1 = All plants having shrunken anthers and shedding no pollen; 2 = Most of the plants having shrunken anthers and shedding no pollen; 3 = Most of the plants having plump anthers and shedding pollen; 4 = All plants having plump anthers and shedding pollen
[1]Shy pollen shedding

Table 5: Per cent selfed seedset in single-cross hybrids of three relatively stable isonuclear A-lines of pearl millet

Pollen parent	Per cent selfed seedset in hybrids on male-sterile line							
	$81A_1$			$81A_v$		$81A_4$		
	IC(D)	IC(R)	P	IC(D)	IC(R)	IC(D)	IC(R)	P
81B	<1	<1	0	<1	1	0	1	0
843B	0	2	<1	<1	2	<1	0	0
ICMB 89111	<1	<1	0	3	1	0	<1	0
ICMB 90111	<1	2	0	4	4	0	<1	0
Pb $302B_2$	64	83	74	36	51	0	0	0
Pb $408B_3$	<1	1	<1	1	1	<1	0	<1
ICMP 501	92	94	88	89	94	<1	0	<1
ICMP 83506	27	57	15	54	79	<1	0	0
ICMR 85410	44	64	57	26	58	89	93	76
ICMR 356	32	71	49	10	5	<1	<1	0
J 104	4	9	12	<1	<1	0	<1	0
K 560-230	51	63	45	1	<1	<1	1	0
H 77/833-2	35	70	72	5	31	<1	<1	<1
IPC 98	87	87	88	4	12	<1	1	0
IPC 390	93	94	95	1	2	0	0	0
IPC 501	0	2	0	8	28	<1	0	0
ICMP 82601	47	68	58	5	2	0	<1	0
ICMP 85303	5	3	0	1	31	88	90	68

IC = ICRISAT Asia Center; D = dry season 1991; R = rainy season 1991; P = Pune, dry season 1991

$81A_4$. For instance, among 11 hybrids of the $81A_1$ that had a pollen-shedding score of 4, one had 4% seedset and three additionally had 27–35% seedset in the dry season at IAC (Table 4). Similarly, among six hybrids of $81A_v$ that had a score of 4, three had 1–10% seedset in the dry season and 5–31% seedset in the rainy season at IAC. There were only four pollen parents (Pb $302B_2$, ICMP 501, ICMP 83506 and ICMR 85410) whose hybrids on $81A_v$ had good seedset (51–94%) in the rainy season at IAC. Apart from these, there were six more pollen parents whose hybrids on $81A_1$ had 63–94% seedset. Nevertheless, the differential seedset pattern of $81A_1$-hybrids and the counterpart $81A_v$-hybrids, developed from crosses with three pollinators (K 560-230, IPC 390 and ICMP 82601) was similar to the pollen-shedding pattern. During the dry season at both IAC and Pune, the seedset remained at similar levels as in the rainy season for some hybrids and was lower for the others.

There was not a single hybrid either on $81A_2$ (Pb $310A_2$ and Pb $311A_2$) or Pb $406A_3$ that had consistently <10% selfed seedset across all the test environments (Table 6). In this group of hybrids also, selfed seedset was generally higher in the rainy season than in the dry season. Hybrids with pollen-shedding scores of 3 or 4 had 48–97% selfed seedset in the dry season at both IAC and Pune. Almost all the hybrids of these A-lines with pollen-shedding scores of 1 or 2 had moderate (25–50%) to high (>50%) selfed seedset. The 81B, ICMB 90111, Pb $302B_2$ and Pb $408B_3$ were the only pollinators whose hybrids on Pb $310A_2$ and Pb $406A_3$ had low selfed seedset (<20%) in the dry season at both IAC and Pune.

Table 6: Per cent selfed seedset on single-cross hybrids of three relatively unstable isonuclear A-lines of pearl millet

Pollen parent	Per cent selfed seedset in hybrids on male-sterile lines: $81A_2$ = Pb $310A_2$ IC(D)	IC(R)	P	$81A_2$ = Pb $311A_2$ IC(D)	IC(R)	P	$81A_3$ = Pb $406A_3$ IC(D)	IC(R)	P
81B	16	36	13	23	71	8	19	54	15
843B	48	94	53	62	90	64	48	93	63
ICMB 89111	57	96	41	58	90	42	68	87	65
ICMB 90111	14	31	17	14	17	15	3	9	3
Pb $302B_2$	9	28	8	26	50	25	7	50	14
Pb $408B_3$	4	8	3	15	38	2	16	33	7
ICMP 501	66	86	41	37	63	32	65	71	34
ICMP 83506	76	92	88	73	88	59	82	88	52
ICMR 85410	87	93	78	82	94	72	76	91	78
ICMR 356	47	67	15	43	55	26	60	75	21
J 104	15	34	17	12	29	10	16	69	26
K 560-230	79	83	31	67	89	47	66	90	52
H 77/833-2	42	75	50	23	60	46	64	55	65
IPC 98	94	95	91	97	94	88	97	93	96
IPC 390	31	23	16	36	47	36	58	51	32
IPC 501	24	52	38	45	47	33	61	58	59
ICMP 82601	62	82	50	60	72	67	61	73	53
ICMP 85303	95	92	91	92	92	83	83	90	78

IC = ICRISAT Asia Center; D = dry season 1991; R = rainy season 1991; P = Pune, dry season 1991

Table 7: Relative frequency of male-sterile (0 % selfed seedset) and highly male-fertile (> 50 % selfed seedset) plants in topcross hybrids of male-sterile lines $81A_1$ and $81A_4$, ICRISAT Asia Center

Pollinator population	1991 Rainy season: Number of hybrid plants on $81A_1$	$81A_4$	Per cent of hybrid plants with 0 % SS[1] $81A_1$	$81A_4$	> 50% SS[1] $81A_1$	$81A_4$	1992 Dry season: Number of hybrid plants on $81A_1$	$81A_4$	Per cent of hybrid plants with 0 % SS[1] $81A_1$	$81A_4$	> 50% SS[1] $81A_1$	$81A_4$	Z test for difference between 1991 rainy season and 1992 dry season: $81A_1$ 0 % SS	$81A_1$ > 50% SS	$81A_4$ 0 % SS	$81A_4$ > 50% SS
Open-pollinated variety																
ICTP 8203	72	50	50	34	6**	24	67	67	87	76	2	7	**		**	**
ICMV 87901	56	59	52	53	13	24	21	51	95	76	0*	20	**		*	
Composite																
SSC C6	42	44	21	25	48	39	47	22	34*	59	36	36			**	
D_2C C_1	54	37	30*	51	20	14	54	69	59**	87	17	12	**		**	
D_1C C_2	58	68	10**	59	64**	7	64	70	28**	90	45**	8	*	*	**	
ICRC II	49	62	33*	53	37**	15	35	62	57**	87	23*	8	*		**	
HiTiP 88	64	35	11**	80	47**	3	41	51	39**	92	39**	6	**			
NCD_2	37	66	3**	50	35	18	39	64	64	69	10	17	**	**	*	
EBD_2	55	44	22	39	42	41	61	56	49*	70	20	23	**	*	**	
AfPop 87	31	43	16*	40	26	21	10	42	40*	74	30	24			**	
SRC C_3	52	56	17*	39	40	30	63	44	52	59	27	39	**		*	
EC II C_1	58	43	10**	35	59**	26	65	48	29**	75	40	25	**	*	**	
MC C_{10}	60	43	20*	44	38**	14	75	55	60**	84	29	15	**		**	
IVC C_7	15	44	7**	43	73**	32	39	55	28**	78	49**	20			**	
NELC C_6	48	58	13**	48	48**	16	58	56	50**	96	40**	0	**		**	**

*, **Significant at P = 0.05 and P = 0.01, respectively
[1]Selfed seedset

Topcross hybrids

Fertility restoration results of single-cross hybrids (Hanna 1989) had shown that more than 80% of the hybrids of the A_4 CMS lines were male sterile. This raised a concern regarding the possible dearth of restorers of this CMS source for use in breeding male-fertile grain hybrids. An evaluation of topcross hybrids of two open-pollinated varieties (ICTP 8203 and ICMV 87901) and 13 diverse composites showed that hybrids of all populations, except perhaps NELC C_6, had varying frequencies of plants in more than 50% of selfed seedset class in rainy and in dry seasons (Table 7). Six composites (SSC C_6, EBD_2, AfPop 87, SRC C_3, EC II C_1 and IVC C_7) and ICMV 87901 had as many as 20–39% plants in the > 50% selfed seedset class. No composite produced significantly higher percentages of hybrids on $81A_4$ in this seedset class across the two seasons. On the other hand, nine composites produced significantly higher percentage plants in $81A_4$-hybrids than $81A_1$-hybrids that did not set seed under selfing. Interestingly, ICRC II and HiTiP 88, which had been developed by random mating of restorer lines of the A_1 CMS system, produced hybrids on to $81A_4$ that had only 6–8% plants setting over 50% seed in the dry season. Hybrids of these two composites on to $81A_1$ had 23–39% plants in the over 50% selfed seedset class. Hybrids of both A-lines with most of the composites, in general, had significantly higher percentage of male-sterile plants (0% seedset) in the dry season than in the rainy season.

Discussion

In the nuclear genetic background of inbred line 81B, three isonuclear lines ($81A_1$, $81A_v$, and $81A_4$) had no pollen shedders.

These could apparently be considered as equally stable for their male sterility. Our inability to detect differences between these lines for pollen shedders could be due to the relatively small number of plants (128–239 plants per line) scored in our study. Hanna (1989) scored >400 000 panicles and found 0.095% revertants in Tift $23A_1$ compared with none in its isonuclear line Tift $23A_4$. Based on selfed seedset, we found $81A_4$ to be the most stable, followed by $81A_1$ and $81A_v$. Some degree of self-pollination (a measure of the lack of stability of male sterility) was evident in all three A-lines, as reflected in the 'true-to-type' plants observed in the grow-out test of seeds from the selfed panicles of the 1–5% seedset class. Such plants were minimal in $81A_4$, with 18% in the rainy season seed source and none in the dry season seed source. These frequencies were higher (33%) for $81A_1$ and even higher (>60%) for $81A_v$. Thus, the A_4 cytoplasm appears to provide a better alternative to the A_1 cytoplasm for breeding stable male-sterile lines. The economic viability and practical utility of the A_v cytoplasm as a superior alternative to the A_1 cytoplasm remains to be determined by studying the stability of male sterility of A-lines based on other maintainer lines. The A_2- and A_3-CMS systems were highly unstable in the genetic background of 81B. Almost all the male-sterile lines of these two CMS systems developed at the Punjab Agricultural University in diverse genetic backgrounds and evaluated multilocationally in the All-India Coordinated Pearl Millet Improvement Project trials have been found to have far higher frequencies of pollen shedders than those developed on the A_1 CMS source. This indicates a serious limitation to the utilization of the A_2 and A_3 CMS sources in breeding stable male-sterile lines.

In the study of single-cross hybrids, pollen-shedding pattern of $81A_4$-hybrids was in accordance with selfed seedset pattern in both rainy and dry seasons. This relationship, however, was not as good for the hybrids of $81A_1$ and $81A_v$ where some hybrids with pollen-shedding scores of 3 and 4 had less than 10% selfed seedset (one hybrid of $81A_1$ and four hybrids of $81A_v$). Thus, selfed seedset is a more reliable criterion for the evaluation of fertility/sterility of hybrids. Based on this criterion, 10 of the 18 pollen parents produced fertile hybrids on $81A_1$. Of these 10 pollen parents, four produced fertile hybrids on $81A_v$ while only one (ICMR 85410) produced a fertile hybrid on $81A_4$. One pollen parent (ICMP 85303) that produced a fertile hybrid on $81A_4$, produced a sterile hybrid on $81A_1$. Its hybrid on $81A_v$ was fully sterile in the dry season and partially so in the rainy season. These differential fertility restoration patterns of hybrids support the results of Hanna (1989) that A_1 and A_4 CMS systems are different from each other and further show that the A_v source represents a CMS system different from both A_1 and A_4.

There were no hybrids of the two A_2-systems and one A_3-system male-sterile lines that had greater than 5% selfed seedset in both the seasons, and almost all of them had greater than 30% seedset in at least any one season. It is difficult to get as good a maintainer for the A_2 and A_3 CMS sources as for the other three sources. This in itself is evidence that A_2 and A_3 CMS sources are different from the other three sources. Also, hybrids of 843B and ICMB 89111 made on to $81A_1$, $81A_4$, and $81A_v$ were sterile with 0–3% selfed seedset in the dry season at IAC. Hybrids of these two pollen parents made on to $81A_2$ and $81A_3$ had 48–68% selfed seedset in the same environments. Similarly, hybrids of ICMP 85303 made on to $81A_1$ and $81A_v$ had 1–5% selfed seedset in the dry season at IAC while hybrids of this pollinator made on to $81A_2$ and $81A_3$ had 83–95% selfed seedset. Hanna (1989) also observed high frequencies of partially fertile hybrids made on the A_2- and A_3-system male sterile lines. Since both $81A_2$ and $81A_3$ produce pollen shedders in the forms of whole plant, whole panicle, and sector(s) of a panicle, a plant visually rated as a non-shedder on the basis of its main panicle might have had other panicles fertile. Crosses made on these panicles are likely to produce hybrids with a high frequency of fertile plants. However, the levels of seedset of hybrids made with these pollinators, for example, are far higher than can be expected on the basis of pollen shedders and fertile sectors in $81A_2$ and $81A_3$. This shows that both the A_2 and the A_3 CMS systems are clearly different from the A_1, A_v and A_4 CMS systems. Our inability to distinguish between the A_2 and the A_3 CMS sources might have resulted from the lack of complete sterility of A-lines that included these cytoplasms in the 81B genetic background. Several A-lines based on these cytoplasms and diverse maintainer lines have been evaluated for pollen shedders and selfed seedset in the All-India Coordinated Pearl Millet Improvement Project (AICPMIP) parental trials. Results show that it may be extremely difficult to find complete maintainers of these CMS sources. Also, considering the availability of other stable CMS sources, a search for perhaps rare good maintainers of the A_2- and A_3-CMS sources is not warranted.

The most consistent patterns across seasons, both for pollen-shedding and seedset, were obtained for hybrids of $81A_4$, followed by those of $81A_1$ and $81A_v$. Therefore, in breeding maintainer and restorer lines of the A_4-CMS system, one can rely greatly on the visual pollen-shedding scores of plots to decide if a line or hybrid is fertile. This would make the collection of selfed seedset data to evaluate male-fertility of hybrids unnecessary. For the hybrids of the A_1-CMS system, pollen-shedding score as a measure of their fertility/sterility is relatively less reliable, and it is even less so for the hybrids of $81A_v$. Irrespective of the evaluation criteria, identification of a maintainer line for breeding stable male-sterile line will be more effective in the rainy season as the level of fertility was higher in the rainy season than the dry season. In contrast, identification of a restorer line for breeding hybrids with good male fertility will be more effective in the dry season. This is in agreement with observations of Rai and Hash (1990).

The frequency of male-sterile hybrids was about 40% when made on $81A_1$ and considerably higher when made on $81A_v$ (60%) and $81A_4$ (90%), indicating that cytoplasms of the last two lines apparently increase the probability of identifying maintainer lines and hence provide greater scope for the genetic diversification of male-sterile lines. However, taking into account the stability of male-sterility also, only the A_4 CMS system appears to provide a better alternative to the A_1 CMS system. The main disadvantage with utilization of the A_4-system male-sterile lines for breeding grain hybrids is that the majority of the A_1-system restorers currently in use are non-restorers of the A_4 CMS system. This conclusion is further supported by the fertility reaction of hybrids of two composites (ICRC II and HiTiP 88), developed predominantly from restorer lines of the A_1 CMS system (Table 7). These composites produced very low frequencies of well-restored plants (6–8%) in topcross hybrids on $81A_4$ in the dry season that had >50% selfed seedset (i.e., well-restored).

A survey of the above two composites and 13 other diverse breeding populations showed that all had very high frequencies of non-restorers that can be utilized in breeding A_4-system male-sterile lines. At the same time, all composites had low to average frequencies of good restorers of this CMS system as well that could be used to breed pollen parents of A_4-system hybrids.

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