Plant Breeding **119**, 335—339 (2000)

ISSN 0179-9541

Feasibility of breeding male-sterile populations for use in developing inter-population hybrids of pearl millet

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With 5 tables

Received March 30, 1999/Accepted February 4, 2000
Communicated by H. H. Geiger

Abstract

Inter-population hybrids of pearl millet, *Pennisetum glaucum* (L.) R. Br., have a substantial grain yield advantage over open-pollinated varieties that makes them an appropriate and economically viable proposition for many African agricultural situations, provided that stable male-sterile populations can be developed for use as seed parents. The objective of this research was to examine the feasibility of breeding stable male-sterile populations, using the d_2 dwarf version of Nigerian Composite NCD_2 and the A_4 cytoplasmic–nuclear male sterility system as a test case. Results showed that two cycles of recurrent selection for sterility maintenance ability led to the development of a fully effective maintainer version of NCD_2. There was no significant difference between the original C_0 cycle bulk and the C_3 cycle bulk (developed from the third and final cycle of recurrent selection) for grain yield and other agronomic traits. The male-sterile population at the third backcross stage, developed from the maintainer version of NCD_2, had as high a level of stable male sterility as the A_1 system commercial inbred male-sterile line $841A_1$. Thus, it is concluded that with the use of the A_4 cytoplasmic male-sterile system, it would be possible rapidly to develop a maintainer version of any population without detrimental effects on grain yield and agronomic traits. Male sterility of populations developed from these maintainers will be highly stable, paving the way for their effective utilization as seed parents in breeding inter-population hybrids.

Key words: *Pennisetum glaucum* — inter-population hybrid — male-sterile population — recurrent selection

Pearl millet is a highly cross-pollinated crop with 75–80% natural outcrossing (Burton 1974). An extensive survey of pearl millet literature showed 40% average better-parent heterosis for grain yield, ranging from −57 to 424% (Virk 1988). These two features of the crop satisfy some of the basic requirements for the exploitation of heterosis in cultivar development. Commercial exploitation of heterosis in pearl millet only became possible with the development of a cytoplasmic–nuclear male-sterile line Tift 23A in the USA (Burton 1965). The first single-cross grain hybrid (HB-1) based on this male-sterile line, released in India in 1965, outyielded improved local open-pollinated varieties (OPVs) by as much as 100% (Athwal 1965). The extent of grain yield advantage and release of HB-1 stimulated substantial pearl millet grain hybrid research and development activity in India. As a result, more than 18 hybrids are now grown on varying scales, covering more than half of the 10×10^6 ha area under pearl millet in India, and doubling the grain yield over the pre-hybrid era (Rai et al. 1997).

Single-cross hybrids may not provide a good starting point for the exploitation of heterosis in hybrid cultivars in much of Africa, especially western Africa. There are three main reasons for this contention. First, genetic uniformity of single-cross hybrids makes them more vulnerable to downy mildew, *Sclerospora graminicola* (Sacc.) J. Schröt., epidemics, as has repeatedly happened in India (Hash 1997; Hash et al. 1997; Rai et al. 1997). This problem is likely to be more challenging in western Africa where downy mildew pathotypes have been shown to be relatively more virulent than those in much of India (Singh et al. 1997). Single-cross hybrids, especially those based on cytoplasmic male-sterile (CMS) lines, will face additional challenges from smut, *Moesziomyces penicillariae* (Bref.) Vànky, and ergot, *Claviceps fusiformis* Loveless, which are more severe in parts of Africa than in India. Second, pearl millet displays a high degree of inbreeding depression. Thus, if seed production is undertaken in the main crop season, characterized by relatively harsher environmental conditions, inbred lines of single-cross hybrids would have poor emergence and vigour, leading to economically unacceptable seed yields in the hybrid seed production plots. Third, inbred parents of single-cross hybrids take considerable time and research investment for development and testing, and the material and manpower resources in the African regions are inadequate to undertake such activities. Thus, the immediate alternative could be to use existing improved OPVs as male parents to breed inter-population hybrids on male-sterile populations, which would address all three issues mentioned above. Because of their adaptation and acceptance by farmers, successful OPVs would make good hybrid parents for interpopulation hybrids, thus further capitalizing on OPV breeding. Improved OPVs are already being used as pollen parents to breed topcross hybrids of pearl millet for African agricultural situations (Anand Kumar et al. 1999).

Commercial success of inter-population hybrids would depend on the extent of their grain yield advantage over OPVs, and the feasibility of breeding male-sterile populations. Preliminary results from western Africa have shown inter-population hybrids outyielding their improved OPV parents by 27–59% (Lambert 1983) and 32–45% (Ouendeba et al. 1993). This level of grain yield advantage is of significant value for commercial viability of inter-population hybrids. The feasibility of breeding a male-sterile population would depend on selection efficiency for sterility maintenance ability in the seed parent population. Recurrent selection has not been found to be effec-

tive in rapid development of maintainer versions of populations with respect to the A_1 CMS system. A composite developed from diverse maintainers of the A_1 system A-lines produced $<30\%$ fully sterile testcrosses when tested on the male-sterile line $5141A_1$. Less than 60% of the testcross progenies derived even from the C_2 bulk of this population were sterile when crossed on the same tester line (K. N. Rai, unpublished data). Aken'Ova (1982) conducted six cycles of recurrent selection in a *maiwa* population, using Tift $23A_1$ as a tester, but did not report on the extent of genetic improvement in sterility maintenance ability of the improved cycle bulks. An alternative CMS system, designated as A_4 (Hanna 1989), has more stable male sterility and this sterility is less influenced by environmental factors than that of the A_1 system (Andrews and Rajewski 1994, Rai et al. 1996). The objective of this research was to examine the feasibility of breeding stable male-sterile populations, using the d_2 dwarf version of Nigerian Composite NCD_2 (Rai et al. 1995a) and the A_4 CMS system as a test case.

Materials and Methods

Population and CMS tester: The dwarf Nigerian Composite NCD_2 of *Pennisetum glaucum* L. R.B.V., used in this study is a d_2 dwarf version of a tall Nigerian composite (NC) developed by a sidecar method of limited backcrossing in which successive cycle bulks of the tall NC undergoing recurrent selection were used as a recurrent parent and GAM 73 (a dwarf synthetic from Senegal) as the d_2 gene donor (Rai 1990). Forty-one dwarf BC_3F_5 progenies derived from this backcross programme were random-mated three times to develop NCD_2. In yield trials conducted for 2 years at two locations in southern India, NCD_2 was the highest yielding population among the seven tall composites and their respective dwarf versions. NCD_2 has a high level of downy mildew resistance and the long panicles typical of many African landraces. It has also been found to have a high level of seedling thermotolerance (Peacock et al. 1993). These traits made NCD_2 an ideal population, not only for the assessment of the recurrent selection efficiency to convert it into a maintainer version, but also for developing it into a male-sterile population that may have a direct applied value in breeding inter-population hybrids for African conditions, provided that its downy mildew resistance level is comparable to the original Nigerian composite. A dwarf male-sterile line, $81A_4$, based on the A_4 CMS system was used as a tester to identify maintainer plants in NCD_2 and its selected versions. During the mid-1990s, when the present study was initiated, line $81A_4$ had been found to have the most stable male sterility among several isonuclear A-lines with diverse CMS sources, and the largest proportion of plants and progenies from diverse genetic backgrounds were maintainers of this A-line (Rai et al. 1996).

Recurrent selection: Three cycles of recurrent selection were conducted for the improvement of sterility maintenance ability of NCD_2. The procedure involved selfing individual plants of NCD_2 (to produce S_1 seed), as well as crossing those plants onto line $81A_4$ (to produce testcrosses), evaluation of testcrosses for their sterility/fertility reaction, and recombining the S_1 progenies of those NCD_2 plants that produced fully sterile testcrosses. For recombination, pollen collected from all S_1 progenies flowering at a given time was bulked and then used for crossing on two or three plants of each of the selected S_1 progenies to simulate random mating. This crossing work was deliberately spread over 7–8 days until all the progenies had flowered. The testcrosses, grown in 1-row plots (25–35 plants) during the rainy season, were visually evaluated at 75% anthesis for male fertility/sterility on the basis of pollen shedding. Testcrosses that had no pollen-shedding (PS) plants were classified as sterile (S), those having predominantly non-PS plants as S/F, those having predominantly PS plants as F/S, and those having all plants PS as fertile (F). Segregation for fertility would occur if a testcross involved a NCD_2 plant that was heterozygous for a fertility restoration gene. The number of testcrosses evaluated and S_1 lines recombined in various selection cycles is given in Table 1. The number of S_1 progenies recombined in the cycles C_0 and C_2 were slightly less than the number of sterile testcrosses. In the C_1 cycle, only about 50% of those S_1 progenies that corresponded to sterile testcrosses were recombined.

Composite bulks: The four cycle bulks (C_0–C_3) of NCD_2 were used for two purposes. Their topcross hybrids with $81A_4$ were used to evaluate the progress in sterility maintenance ability and the bulks *per se* were evaluated in a yield trial to examine if recurrent selection for sterility maintenance caused any changes in grain yield and other agronomic traits.

Topcrosses were made by crossing the male-sterile line $81A_4$ with bulked pollen from 100–120 random plants of each composite bulk. The number of topcross plants used for PS assessment during the 1996 and 1997 rainy seasons at Patancheru is given in Table 2. Prior to this evaluation, progress in sterility maintenance ability was monitored while recurrent selection continued. For this, equal amounts of seed from all the testcrosses (used as test units for recurrent selection) from each of the C_0, C_1 and C_2 bulks were pooled to produce 'topcross equivalents', which were evaluated for frequency of male-sterile plants during the course of recurrent selection under various environmental conditions. The number of hybrid plants and the year/season of evaluation are given in Table 3.

The yield trial of the four cycle bulks was conducted in four-row plots of 4 m length, replicated eight times in a randomized complete block design during the rainy and post-rainy seasons of 1996 at Patancheru. Grain yield and time to 50% flowering were recorded on a whole-plot basis. Plant height and panicle length were recorded from the main shoot of 10 random plants, and 1000-seed mass was estimated from a single 200-seed sample for each plot. The number of panicles per plant was determined from plant and panicle count data for each plot. Plot means were used for a fixed model analysis of variance using the Genstat 5 statistical package (Genstat 5 Committee 1993).

Male-sterile population: During the course of recurrent selection, the latest available cycle bulk of NCD_2 at the time was crossed and backcrossed into the cytoplasm of $81A_4$, following a sidecar method (Rai 1990), to develop a male-sterile version of NCD_2. During backcrossing, crosses were made on random male-sterile plants. A BC_3 version of this population (hereafter referred to as NCD_2A_4-BC_3), was developed by three backcrosses into the A_4 cytoplasmic background. This male sterile population was evaluated for stability of male sterility using two A_1-system commercial inbred male-sterile lines, $81A_1$ (Anand Kumar et al. 1984) and $841A_1$ (Singh et al. 1990), as controls.

The trial was conducted in isolated plots at Patancheru during two rainy-season environments and two post-rainy-season environments. The number of plants evaluated for pollen shedding and seedset under selfing bags is given in Table 4. Seedset was scored following the standard rating procedure developed for the fungal disease ergot, *C. fusiformis* Loveless (Thakur and Williams 1980).

Results

Recurrent selection was highly effective in improving male sterility maintenance ability of the NCD_2 population. The frequency of male-sterile testcross progenies increased from 36% for the C_0 bulk to 88% for the C_1 bulk and 100% for the C_2 bulk (Table 1). The testcrosses that segregated for fertile and sterile plants decreased rapidly from 42% for the C_0 bulk to 7% for the C_1 bulk and 0% for the C_2 bulk. A comparison of topcross hybrids made with four cycle bulks (C_0 to C_3) and evaluated during the 1996 and 1997 rainy seasons confirmed the above improvement in the sterility maintenance ability of NCD_2 (Table 2). However, the topcross produced from the C_0 bulk had 75.3–77.3% PS plants, which was significantly higher than expected on the assumption of control of sterility by single recessive gene, as reflected in the frequencies of sterile and

Table 1: Number of testcrosses ($81A_4 \times S_0$ plants) of three selection cycle bulks of NCD_2 in pearl millet and their frequencies in male-sterile (S), segregating (S/F and F/S), and fertile (F) classes, and number of selected S_1 lines in the rainy season at Patancheru

Cycle bulk	Year	Number of testcrosses sown	Testcrosses: frequency (%) in class				Number of S_1 lines selected[1]
			S	S/F	F/S	F	
C_0	1992	392	36	14	28	22	131
C_1	1994	152	88	5	2	5	76
C_2	1995	123	100	0	0	0	116

[1] Number of S_1 lines corresponding to testcross progenies of the S class that were recombined to produce the succeeding cycle bulk.

Table 2: Number of plants evaluated and frequency of male-sterile plants of topcross hybrids of four NCD_2 selection cycle bulks in pearl millet during the 1996 and 1997 rainy season at Patancheru

Topcross hybrid	Total plants (n)		Male-sterile plants (%)	
	1996	1997	1996	1997
$81A_4 \times NCD_2\ C_0$	328	941	75.3	77.3
$81A_4 \times NCD_2\ C_1$	310	1231	95.8	99.6
$81A_4 \times NCD_2\ C_2$	567	855	97.7	99.8
$81A_4 \times NCD_2\ C_3$	310	637	98.1	99.8

Table 3: Frequency of pollen-sterile plants in 'topcross equivalents' of three cycle bulks of NCD_2 at Patancheru

Topcross equivalent	Year	Season	Total number of plants	Male-sterile plants (%)
$81A_4 \times NCD_2\ C_0$	1993	Cool dry	229	62
	1994	Rainy	214	50
	1995	Rainy	214	62
	1996	Hot dry	170	59
$81A_4 \times NCD_2\ C_1$	1994	Rainy	430	91
	1995	Rainy	190	93
	1996	Hot dry	1058	99
$81A_4 \times NCD_2\ C_2$	1995	Rainy	220	100
	1996	Hot dry	933	100

Table 4: Grain yield and other agronomic traits of four cycle bulks of NCD_2 in pearl millet; means across the 1996 rainy and dry seasons at Patancheru

Cycle bulk	Grain yield (t/ha)	Days to 50% flowering	Plant height (m)	Panicle length (cm)	Number of tillers per plant	1000-seed mass (g)
C_0	1.96	52.8	1.51	34.1	1.13	7.33
C_1	1.45	58.1	1.43	33.6	1.13	6.67
C_2	1.80	55.7	1.50	33.8	1.13	7.10
C_3	1.83	53.4	1.54	33.3	1.18	7.08
$LSD_{0.05}$	± 0.16	± 0.6	± 0.06	± 1.1	± 0.06	± 0.28

segregating testcross progenies observed during recurrent selection. The frequency of PS plants in the 'topcross equivalent' made with the C_0 bulk ranged from 50% in the 1994 rainy season to 62% in the 1993 dry season (Table 3), giving an average of 58%, which is similar to 57% PS plants that could be expected from the testcross data (all sterile and 50% of the segregating testcrosses), assuming control of sterility by a single recessive gene. A similar close correspondence was observed for the C_1 and C_2 cycles.

Recurrent selection for sterility maintenance ability in NCD_2 had no significant effect on grain yield and other agronomic traits of C_2 and C_3 cycle bulks. The C_1 cycle bulk, however, had significantly lower grain yield and 1000-seed mass, shorter plants and delayed flowering compared with the C_0 bulk (Table 4).

A male-sterile population at the BC_3 stage of its development should be identical to its recurrent parent with respect to nuclear gene(s) responsible for male sterility, assuming simple inheritance and no complication by modifiers. Thus, sterility evaluation in a male-sterile backcross population at BC_3 should permit assessment of the sterility level expected in the final male-sterile population. In the absence of a commercial A_4-system A-line, the commercial viability of such a would-be male-sterile population was tested by comparing the sterility of NCD_2A_4–BC_3 with two widely used commercial A_1-system male-sterile lines ($81A_1$ and $841A_1$). Male sterility of NCD_2A_4–

Table 5: Frequency of pollen shedders and distribution of seedset in the BC_3 population of NCD_2 with A_4 cytoplasm (NCD_2A_4–BC_3) and in CMS control lines $81A_1$ and $841A_1$ during the 1996 and 1997 rainy and dry seasons at Patancheru

		Male sterility		Seedset under selfing bags: per cent plants in seedset class					
Population/line	Season[1]	Total plants (n)	Pollen shedders (%)	Total plants (n)	0	1–5	6–20	21–50	51–100
NCD_2A_4–BC_3	Rainy 96	1431	1.0	539	91.3	5.6	1.3	0.7	1.1
	Hot Dry 97F	1067	0.2	398	97.7	1.5	0.5	0.3	0.0
	Hot Dry 97M	1033	0.1	349	100.0	0.0	0.0	0.0	0.0
	Rainy 97	568	0.5	419	97.9	1.7	0.2	0.2	0.0
	Overall	4099	0.5	1705	96.7	2.2	0.5	0.3	0.3
$81A_1$ (control)	Rainy 96	1618	0.6	599	97.8	2.2	0.0	0.0	0.0
	Hot Dry 97F	1200	0.3	483	95.7	3.9	0.4	0.0	0.0
	Hot Dry 97M	1066	0.0	393	98.7	1.0	0.3	0.0	0.0
	Rainy 97	785	0.1	487	98.8	1.2	0.0	0.0	0.0
	Overall	4669	0.3	1962	97.8	2.1	0.2	0.0	0.0
$841A_1$ (control)	Rainy 96	1119	1.0	513	93.4	4.9	1.5	0.2	0.0
	Hot Dry 97F	1333	0.2	714	97.7	2.0	0.3	0.0	0.0
	Hot Dry 97M	1216	0.2	318	98.7	0.6	0.6	0.0	0.0
	Rainy 97	985	0.4	622	96.6	2.7	0.5	0.2	0.0
	Overall	4653	0.5	2167	96.6	2.6	0.7	0.1	0.0

[1] F = February planting; M = March planting.

BC_3 was as high and stable as that of $841A_1$. Line $81A_1$ was only marginally better, especially in the 6–20% and 21–50% seedset classes (Table 5). Generally, all three had $<1\%$ PS plants, and $>96\%$ of the non-PS plants set no seed. In each of the three seedset classes (1–5%, 6–20% and 21–50%), sterility of NCD_2A_4–BC_3 was similar to that of $841A_1$, except that NCD_2A_4–BC_3 had 1.1% plants in the 51–100% seedset class while $81A_1$ and $841A_1$ had none. In the 1997 rainy season, 591 NCD_2A_4–BC_6 plants were also evaluated for PS and seedset. There were no PS plants, 91.7% of the selfed plants had no seedset, 7.6% of the plants were in the 1–5% seedset class and the remaining 0.7% were in the 6–20% class (data not presented). This indicated that there was a marginal improvement in the sterility of NCD_2A_4–BC_6 compared with that of the NCD_2A_4–BC_3.

Discussion

A rapid change in the frequency of male-sterile testcrosses from 36% for the C_0 bulk to 100% for the C_2 bulk of NCD_2 population indicated that two cycles of recurrent selection were effective in developing its completely maintainer version. It should be noted that most of this progress was made in the first selection cycle. Since S_1 progenies of only those plants from the C_0 bulk that had produced sterile testcrosses were recombined into the next cycle, a low frequency of fertile testcrosses (5%) and segregating testcrosses (7%) for the C_1 bulk was unexpected, assuming single recessive gene control of the A_4 system of male sterility (Du et al. 1996). This implies that either the genetic control of A_4 CMS system involves more than one gene, including modifiers, or that environmental effects caused some of the potentially segregating testcrosses to express as fully sterile. Another possibility is that for testcross units of 25–35 plants with the pollen-shedding data taken at 75% anthesis, it is likely that a low frequency of potentially segregating testcrosses consisted of only male-sterile plants, and hence such testcrosses were misclassified as fully sterile. Multi-environment evaluation of testcrosses would minimize such misclassification if the environmental effects were significant and using a larger size of testcross units would minimize this problem if the number of plants in testcross units were inadequate.

While conducting selection for male sterility maintenance ability, it is important to ensure that no undesirable changes take place in the population during the course of recurrent selection. Although the final two-cycle bulks (C_2 and C_3) of the NCD_2 did not differ significantly from the C_0 bulk for grain yield and other agronomic traits evaluated in this study, the C_1 cycle bulk was significantly different from the C_0 bulk with respect to grain yield, time to flowering, plant height and 1000-seed mass. This suggests the possibility of genetic changes in the populations for non-target traits, which should be carefully monitored during the course of recurrent selection.

The maximum certifiable pollen shedders permitted in pearl millet is 0.05% in the foundation seed production plots of A-lines and 0.1% in the certified seed production plots of hybrids (Chopra et al. 1999). An A_4-system male-sterile line $81A_4$ has been found not to produce any pollen shedders (i.e. complete sterility) across a range of environments (unpublished data). The NC_2A_4–BC_3 population (i.e. NCD_2 at the third backcross stage), however, was found to be less sterile than $81A_4$, but as high and stable for male sterility (0.1–1.0% pollen shedders) as an A_1-system commercial A-line, $841A_1$ (0.2–1.0% pollen shedders). Thus, it may not be possible to achieve as high levels of sterility in male-sterile populations as in male-sterile A-lines with the A_4 cytoplasm. However, developing stable male-sterile populations with the A_4 system is at least as feasible as developing commercial male-sterile A-lines with the A_1 CMS system. Further studies with populations of different genetic backgrounds will be required to validate the efficacy of this approach in breeding stable male-sterile populations.

In addition to developing a male-sterile population, there is an important point related to the maintenance of male sterility in populations that must be resolved. The morphological uniformity of inbred male-sterile lines permits easy detection of genetic contaminants and seed admixture in seed production plots. This would be rather difficult to achieve in heterogeneous (morphologically variable) male-sterile populations. Breeding

dwarf male-sterile populations (< 1.3 m height) can help manage this problem to some extent. Since pollinator populations will need to be medium to tall (1.5–2.0 m) to produce hybrids of the desired height for African agricultural situations, any contamination or mechanical admixtures from such populations can easily be detected in dwarf populations during the preflowering stage, to permit timely and effective roguing. Management of this problem can be made even more efficient by introducing easily identifiable recessively inherited seedling markers in male-sterile populations. One such recessively inherited marker gene that produces yellowish seedlings, which later change to green foliage colour during the post-flowering period (Rai et al. 1995b), is currently being introduced into the NCD_2 male-sterile population.

Acknowledgements

Part of this work was done while KNR was a visiting scientist at the University of Nebraska, Lincoln, USA. Partial funding support from Maharashtra Hybrid Seeds Co. Pvt. Ltd. and INTSORMIL is gratefully acknowledged.

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