Fertility Restoration in Male Sterile × Maintainer Hybrids of Pearl Millet

K. N. Rai* and C. T. Hash

ABSTRACT

Effects of environment and parental nuclear genotype may cause considerable difficulties in the identification of maintainer lines (B-lines) and in the classification of different sources of cytoplasmic male-sterility (cms) in pearl millet [*Pennisetum glaucum* (L.) R. Br.]. We studied the pollen shedding pattern as a measure of fertility restoration in A × B hybrids of pearl millet by crossing seven cytoplasmic male-sterile lines (A-lines) with the B-lines of each of the A-lines at the ICRISAT Center in the dry and rainy seasons for 2 yr. Five of the A_1 system A-lines (i.e. A × B parental crosses) were stable for male-sterility, whereas one was sterile in the dry season but produced a low frequency of pollen shedders in the rainy season. The non-A_1 system A-line (PT 732A) produced a low frequency of pollen shedders in both dry and rainy seasons. In comparison, almost all the nonparental A × B hybrids were uniformly or predominantly fertile across all the tests. The seed set data under selfing were generally supportive of the pollen fertility pattern. These results indicate that isonuclear lines or hybrids should be used for the reliable classification of cms sources in pearl millet. Fertility restoration pattern of hybrids in our study does not provide adequate evidence for differentiating the cytoplasm of PT 732A from that of Tift 23A_1. A higher proportion of B-lines produced sterile hybrids on 81A than on other A-lines, indicating that 81A is a more efficient tester than other A-lines used in this study for identifying B-line sources.

FERTILITY RESTORATION PATTERN in F_1 hybrids derived from crosses between male-sterile lines (A-lines) and a set of maintainers (B-lines) or restorers (R-lines) has been widely used in maize (*Zea mays* L.) (Duvick, 1965; Gracen and Grogan, 1974), and sorghum [*Sorghum bicolor* (L.) Moench] (Schertz and Ritchey 1978; Rao et al., 1984) for the classification of cytoplasmic sources of male sterility. Burton and Athwal (1967) used this approach in pearl millet for the classification of three sources of cytoplasmic male-

K.N. Rai, Cereals Program, ICRISAT, Patancheru P.O., Andhra Pradesh, 502 324, India; and C.T. Hash, CIMMYT, Ado. Postal 6-641, Lisboa 27, Mexico 6, D.F. (formerly Cereals Program, ICRISAT), ICRISAT Journal Article no. 939. Received 7 Sept.1989. *Corresponding author.

Published in Crop Sci. 30:889–892 (1990).

sterility (cms). Later studies in pearl millet (Appadurai et al., 1982; Aken'Ova, 1985) have used this method to investigate the relationship of new cms sources with the existing ones. At the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) we observed that when a set of pearl millet inbred lines were crossed on different male-sterile lines, all possessing the cytoplasm of 'Tift $23A_1$', the frequency of sterile hybrids was influenced, to some extent, by the male-sterile line used as a female parent. The purpose of this research was to study the stability of A-lines as well as the pattern of fertility restoration in A × B hybrids that have an important bearing on the identification of new B-lines and the classification of cms sources.

MATERIALS AND METHODS

Seven male-sterile lines (A-lines) and their respective maintainers (B-lines) were used in this study. Male-sterile line PT 732A derives its cytoplasm from PT 819, an inbred line originating from Andhra Pradesh, India (Appadurai et al., 1982). The other six A-lines (Table 1) derive their cytoplasm from Tift 23A, an A-line bred at the Coastal Plain Experiment Station, Tifton, GA (Burton, 1965). In the 1985 dry season, we produced parental A × B combinations (actually A-lines) by crossing A-lines with their respective B-lines, and nonparental A × B hybrids by crossing A-lines with the B-lines of each of the other six A-lines.

Our experience through the years had shown that two male-sterile lines (834A and PT 732A) could register up to 10% pollen shedders under humid conditions in the rainy season. In comparison, the other five A-lines were highly stable with rare occurrence (<0.1%) of pollen shedders. This led us to assume the possible existence of some genetic variability for maintainer alleles, or modifiers in 834B and PT 732B, and near fixation of maintainer alleles and modifiers in other B-lines. Thus, to ensure that the production of nonparental A × B hybrids did not include fertile plants from A-line and restorer plants from B-lines, we resorted to plant × plant (P × P) crossing. All B-line plants that were involved in nonparental A × B crosses were also crossed on their own A-lines to ascertain if they were maintainers or restorers. At the time of harvesting, all the A-line plants used in crossing were examined for sterility (determined by the absence of seed set under the selfing bag) and any cross made on a fertile A-line plant was rejected.

A total of 173 P × P crosses were made to produce 7 parental A × B combinations and 42 nonparental A × B hybrids (Table 1). The number of P × P crosses varied from 10 to 14 for the parental A × B combinations and 1 to 4 (excluding reciprocals) for the nonparental A × B hybrids. All P × P progenies along with MBH 110 (a fertile check hybrid) were evaluated for pollen shedding in the rainy and dry seasons for 2 yr at ICRISAT Center, Patancheru. The two rainy season plantings were 20 June 1985 and 24 June 1986. The two dry season plantings were 24 Jan. 1986 and 10 Jan. 1987. All four tests were conducted in Alfisol (Udic Rhodustalf) fields, which received 40 kg N and 17.5 kg P ha^{-1} just before planting, with another 40 kg N ha^{-1} applied 12 to 15 d after the planting. During the 1985 rainy season, each entry was planted in an unreplicated plot consisting of 30 to 40 plants. In the other tests, there were 15 to 20 plants per entry in unreplicated plots.

At 75% anthesis, plants were scored for pollen shedding between 0830 to 1100 h by tapping the uncovered heads and observing for the pollen cloud. The plots were visually rated as 1 (shrunken anthers and complete lack of pollen shed in all plants), 2 (shrunken anthers and complete lack of pollen shed in most of the plants), 3 (plump anthers and pollen shed in most of the plants), and 4 (plump anthers and pollen shed in all the plants) In none of the plots having scores 2 or 3 were the frequencies of pollen shedders in the intermediate range. To examine the effect of B-lines on pollen shedding of A × B hybrids in nonparental crosses, we excluded the data on those P × P progenies that involved B-line plants having produced pollen-shedding parental A × B combinations. However, the data on all P × P progenies were presented for the parental A × B combinations to evaluate the extent of pollen shedders in A-lines.

Table 1. Number of plant × plant crosses made for different A × B F_1 hybrids.

A-line	B-line						
	5141B	Pb 111B	81B	842B	843B	834B	PT 732B
	no. of plant × plant crosses						
5141A	10	3	1	1	1	3	3
Pb 111A	3	10	3	2	2	1(1)†	2
81A	1	3	10	1	1	1(2)	1
842A	1	3	1	10	1	2	3
843A	–	3	1	1	11	3	1(1)
834A	3	2	3	3	4	14	2(1)
PT 732A	3	4	3	3	3	2(2)	10(3)

† Numbers in parentheses indicate the additional B-line plants that produced fertile hybrids on their own A-lines. Crosses involving these plants were included in the parental A × B data but not in the nonparental A × B data.

During the conversion of B-lines into A-lines in pearl millet, we occasionally encountered varying proportions of pollen shedders at early backcross stages. We have observed that all pollen shedders having any proportion of plump anthers invariably set seeds under selfing. Thus, the anther plumpness and pollen shedding in this study were taken as a measure of pollen fertility restoration. The degree of pollen shedding was not noticeably similar in all fertile hybrids. Such quantitative data, however, were not collected in this study.

During the 1986 rainy season, we selfed one head of 10 plants in each A × B hybrid to determine seed set as an additional measure of pollen fertility restoration. Seed set of each selfed head was scored following a rating scale of Thakur and Williams (1980) initially developed for scoring ergot (*Claviceps fusiformis* Loveless) in pearl millet. The average seed set score of 10 plants was calculated for each P × P progeny, which was further averaged over all P × P progenies (where more than one P × P progenies were studied) for each A × B combination.

Soil moisture, temperature, and relative humidity are assumed to be important environmental factors related to pollen shedding in A-lines. In our study, the earliest-flowering entry took 39 d and the latest-flowering entry took 65 d to flower in any of the four tests. There was 166-mm rain between planting to 39 d after planting (DAP) and 65-mm rain between 40 DAP to 65 DAP in the 1985 rainy season. During the 1986 rainy season, there was 160-mm rain between planting to 39 DAP and 210-mm rain between 40 DAP to 65 DAP. During the 1986 and 1987 dry seasons, tests were conducted with furrow irrigation provided at 7 to 10 d intervals from planting till the end of the crop season. Thus, discounting the imposition of any drought stress, we examined the temperature and relative humidity variations across tests during the 40 DAP to 65 DAP period.

RESULTS AND DISCUSSION

All the P × P progenies of parental A × B crosses involving five A_1-system male-sterile lines (5141A, 81A, Pb111A, 842A, 843A) were uniformly sterile in the rainy and dry seasons in both years (Table 2).

Table 2. Average pollen shedding scores of male sterile × maintainer pearl millet hybrids for two seasons in 2 yr at ICRISAT Center.

	B-Lines																											
	5141B				Pb 111B				81B				842B				843B				834B				PT 732B			
	Average pollen shedding scores																											
A-line	A†	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
5141A	1‡	1	1	1	4	4	4	4	1	1	1	1	4	4	4	4	3	3	4	3	4	4	4	4	4	4	4	4
Pb 111A	3	3	4	4	1	1	1	1	1	1	1	1	4	4	4	4	4	4	4	4	4	4	4	3	4	4	4	4
81A	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1	1	3	1	1	1	4	4	4	4
842A	3	4	4	4	4	3	4	1	1	1	1	1	1	1	1	1	4	4	4	4	3	4	3	3	4	4	4	4
843A	–§	–	–	–	4	4	4	4	1	1	1	1	2	2	1	1	1	1	1	1	4	4	4	4	4	4	4	4
834A	4	4	4	4	3	4	4	3	3	4	1	1	4	4	3	1	4	4	4	4	2	2	1	1	4	–	4	–
PT 732A	4	4	2	3	4	4	4	4	3	–	1	–	4	4	4	4	4	4	4	4	4	4	4	4	2	2	2	2

† A = Rainy season 1985; B = Rainy season 1986; C = Dry season 1986; D = Dry season 1987.
‡ 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.
§ = Not tested.

Shrunken anthers with complete lack of pollen shedding was taken as a measure of pollen sterility. The seed set score of 1 in these five male-sterile lines (A-lines) during 1986 rainy season test (Table 3) was supportive of the conclusion based on shrunken anthers and complete lack of pollen shedding. Male-sterile line 834A was unstable in the rainy season with 35 to 70% of the progenies in cross 834A × 834B producing a low frequency of plants having plump anthers and shedding pollen (pollen shedding score 2). In cross PT 732A × PT 732B, 65 to 75% of the progenies were uniformly sterile and the remainder had a low frequency of pollen shedders (pollen shedding score 2) in the four tests.

Such pollen shedders have been assumed to be fertile in this study as our experience with several A-lines and A × B hybrids in pearl millet shows that regardless of the frequency of plump anthers and the degree of pollen shedding, any plant having plump anthers that are shedding pollen invariably sets seed under selfing. The seed set scores of P × P progenies for 834A × 834B and PT 732A × PT 732B lend support to our application of plump anther morphology and pollen shedding as a valid measure of pollen fertility.

A pertinent question then is whether unstable sterility of some of the A-lines is due to their inherent property caused by the fixation of weak restorer genes and modifiers that show variable expression depending on environmental conditions or is it due to the variability for restorer and maintainer genes persisting in these lines. The latter explanation seems more probable as some of the progenies in both 834A × 834B and PT 732A × PT 732B crosses were uniformly sterile in all four tests. Under such a situation, it should be possible to select for stable male-sterility by P × P crossing within A/B pairs and evaluating the A × B progenies for sterility. Such an approach led to the development of an improved version of Pb-111A, free of pollen shedders, from an initial stock that had 10 to 15% pollen shedders (Gill et al., 1977). A similar approach led to the development of another stable male-sterile line that is the seed parent of a leading commercial hybrid, MBH 110 (R.R. Vohra, personal communication).

Complete sterility of 834A in the dry season in both years suggests that this male-sterile line can be effectively used for the increase of its own seed and its

Table 3. Average seed set scores of male sterile × maintainer pearl millet hybrids in 1986 rainy season at ICRISAT Center.

	B-line						
A-line	5141B	Pb111B	81B	842B	843B	834B	Pt732B
5141A	1†	4	1	2	1	2	3
Pb111B	4	1	1	3	2	2	8
81A	1	1	1	1	2	2	6
842A	2	3	1	1	2	2	6
843A	–‡	4	1	2	1	6	9
834A	2	6	2	4	6	2	–
PT 732A	7	8	–	9	8	10	4

† 1 = no seed set; 2 = 1% seed set; 3 = 5% seed set; 4 = 10% seed set; 5 = 20% seed set; 6 = 35% seed set; 7 = 50% seed set; 8 = 75% seed set; 9 = 90% seed set; and 10 = 100% seed set (adopted from Thakur and Williams, 1980).
‡ No tested.

hybrids in the dry season. On the other hand, the appearance of a low frequency of pollen shedders in both rainy season suggests that the rainy season provides a more favorable environment for the effective selection of male-sterile plants in 834A. In fact, several other A × B combinations (834A × 81B, 834A × 842B, 81A × 834B, 843A × 842B, PT 732A × 5141B, and PT 732A × 81B) displayed more fertility in the rainy season than in the dry season (Table 2). Considering no drought stress in the rainy season due to adequate rainfall, and in the dry season due to irrigation at 7 to 10 d intervals, temperature and relative humidity were considered as the two important environmental factors related to the effect of season on pollen shedding in some A-lines and A × B combinations.

During the 40 to 65 DAP period when most of the entries reached the anthesis stage, maximum daily temperatures were higher and minimum daily temperatures were lower in both years of the dry season than in the rainy season (Table 4). The relative humidity at both 0700 and 1400 h were lower in both years in the dry season than in the rainy season. Thus, either lower maximum temperatures or higher relative humidity or a combined effect of both factors might induce better expression of fertility restoration in some A-lines and A × B hybrids in the rainy season than in the dry season. Lower temperature and higher humidity have been found to represent a more favorable environment for fertility restoration in corn (Duvick, 1959).

The average pollen shedding scores, based on all the

Table 4. Temperature and relative humidity during 40 to 65 d after planting (DAP) period in four test environments at ICRISAT Center.

Weather variable		Environment			
		1985 rainy season	1986 rainy season	1986 dry season	1987 dry season
Max. daily temp., °C	Mean	29.5	28.1	36.4	32.1
	Range	25.6–31.9	23.5–30.6	33.2–39.2	29.6–34.0
Min. daily temp., °C	Mean	22.2	21.7	20.3	18.3
	Range	21.0–23.9	20.5–23.6	16.8–24.0	15.5–20.5
Relative humidity, %†	Mean	86	91	59	72
	Range	75–95	80–98	36–86	40–91
Relative humidity, %‡	Mean	63	73	22	25
	Range	50–84	53–95	15–34	16–36

† Relative humidity at 0700 h.
‡ Relative humidity at 1400 h.

P × P progenies for each of the parental and nonparental A × B crosses are given in Table 2. The largest proportion of nonparental A × B crosses that were uniformly or predominantly sterile (i.e., pollen shedding scores 1 to 2) across the tests involved 81A as a female parent or 81B as a male parent. Seed set data (Table 3) are also broadly supportive of the fertility pattern observed on the basis of pollen shedding data. That is, more B-lines produced sterile hybrids on 81A than on other A-lines. A low recovery of maintainers in the breeding materials has been a major constraint in the past in the genetic diversification of A-lines in pearl millet. The utilization of 81A as a tester will, therefore, increase the probability of identifying B-lines over that expected with the use of other A-lines.

Almost all nonparental A × B crosses, involving Tift $23A_1$ cytoplasm (and nuclear genome other than 81B), were uniformly or predominantly fertile, with the average pollen shedding scores higher than for the parental A × B crosses in all tests (Table 2). A similar pattern was evident with respect to the seed set for 1986 rainy season (Table 3). This suggests that more than one A_1 system A-lines should be used as a tester for the reliable classification of new cms sources in pearl millet. In fact, it may be appropriate to use isonuclear genotypes for classifying new cms sources (Beckett, 1971). These results also indicate that more than one major gene may be involved in the fertility restoration of A_1 system hybrids, and the role of modifiers in fertility restoration may be significant.

On the basis of pollen shedding, the most fertile nonparental A × B hybrids in this study were those involving PT 732 as a female or as a male parent. An interesting and equally fertile nonparental cross was 842A × 843B. Both 842A and 843A derive their cytoplasm from Tift $23A_1$. Also, both 842B and 843B have been developed from the same single cross population involving Tift $23D_2B$ and PI 185642 as parents (W.D. Stegmeier, unpublished data). All the nonparental A × B hybrids involving A_1-system cms lines and their maintainers that scored as fertile on the basis of pollen shed always set seed under selfing, ranging from 1 to 10% across hybrids (Table 3). However, some hybrids involving 834A or 834B (834A × Pb111B, 834A × 843B, and 843A × 834B) had 20 to 35% seed set under selfing.

In comparison, the seed set under selfing in almost all the nonparental A × B hybrids involving PT 732A or PT 732B varied from 35 to 100%, representing the other extreme of the continuum. Thus, fertility restoration patterns based on pollen shedding data and preliminary data on seed set under selfing indicate that further studies are required to reassess the conclusion (Appadurai et al., 1982) that PT 732A cytoplasm represents a cms system different from that of Tift $23A_1$. The occurrence of a high frequency of fertile nonparental A × B hybrids presents a rather discouraging scenario for the breeding of F_1 seed parents for utilization in the development of three-way hybrids. Also, the supposedly complex genetic mechanism of sterility maintenance would require that recurrent selection in B-composites include a routine testcross evaluation of progenies for ascertaining their sterility maintaining ability.

ACKNOWLEDGMENTS

We wish to thank Mr. A.S. Rao for his assistance in conducting field studies and to Dr. J.R. Witcombe for his valuable suggestions during the preparation of this article.

REFERENCES

Aken'Ova, M.E. 1985. Confirmation of a new source of cytoplasmic-genic male-sterility in bulrush millet (*Pennisetum americanum* (L.) Leeke). Euphytica 34:669–672.

Appadurai, R., T.S. Raveendran, and C. Nagarajan. 1982. A new male-sterility system in pearl millet. Indian J. Agric. Sci. 52:832–834.

Beckett, J.B. 1971. Classification of male-sterile cytoplasms in maize (*Zea mays* L.). Crop Sci. 11:724–727.

Burton, G.W. 1965. Pearl millet Tift 23A released. Crops Soils 17:19.

Burton, G.W., and D.S. Athwal. 1967. Two additional sources of cytoplasmic male-sterility in pearl millet and their relationship to Tift 23A. Crop Sci. 7:209–211.

Duvick, D.N. 1959. Genetic and environmental interactions with cytoplasmic pollen sterility in corn. p. 42–52. *In* Proc. 14th Annu. Hybrid Corn Industry Res. Conf. Am. Seed Trade Assoc. Chicago, IL.

Duvick, D.N. 1965. Cytoplasmic pollen sterility in corn. Adv. Genet. 13:1–52.

Gill, K.S., P.S. Phul, and L.N. Jindla. 1977. Improved bajra male-sterile line Pb-111A. Seed Techno. News. 7:3,6.

Gracen, B.E., and C.O. Grogan. 1974. Diversity and suitability for hybrid production of different sources of cytoplasmic male sterility in maize. Agron. J. 66:654–657.

Rao, N.G.P., D.P. Tripathi, and B.S. Rana. 1984. Genetic analysis of cytoplasmic systems in sorghum. Indian J. Genet. 44:480–496.

Schertz, K.F., and J.M. Ritchey. 1978. Cytoplasmic-genic male-sterility systems in sorghum. Crop Sci. 18:890–893.

Thakur, R.P., and R.J. Williams. 1980. Pollination effects on pearl millet ergot. Phytopathology 70:80–84.