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## Inheritance of Male Fertility Restoration in Pearl Millet

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The A<sub>4</sub> system of cytoplasmic-nuclear male sterility (CMS) in pearl millet [Pennisetum glaueum (L.) R. Br.] is more stable in its male sterility and produces a higher frequency of male sterile hybrids than the commercially used A<sub>1</sub> CMS system (Hanna 1989). This greatly increases the utility of the A4 CMS system in breeding genetically diverse and stable male-sterile lines (A-lines). However, it also means that greater efforts will be required to breed restorers of the A4 CMS system than that of the A<sub>1</sub> CMS system. An understanding of the inheritance of male fertility restoration will be useful in breeding restorer parents of this new CMS system. In a testcross nursery grown during the 1994 dry season at the ICRISAT Asia Center, we observed that a hybrid between 81A<sub>4</sub> and 834B was male-fertile, with excellent pollen shedding and selfed seedset. 834B is a maintainer line of an early-maturing A<sub>1</sub>-system A-line (834A). It is of medium height, has high seedling vigor, large seeds (12 g  $1000\text{-seed}^{-1}$ ), a high level of resistance to downy mildew [Sclerospora graminicola (Sacc.) Schroet], large and bristled but loose panicles, and good general combining ability. Therefore, this line can be directly used as a restorer parent of the  $A_4$ -system A-lines and an elite source of restorer gene(s).

We studied the inheritance of fertility restoration in a cross between  $81A_4$  and 834B. Six  $F_1$  plants in this cross were selfed to produce  $F_2$  progenies, and backcrossed on  $81A_4$  to produce  $BC_1$  progenies during the 1995 rainy season. About 200-240 plants of each  $F_2$  progeny and 100-150 plants of each BC, progeny were visually evaluated for pollen shedding during the 1996 dry season. Plants shedding pollen were classified as fertile (F), and those not shedding pollen as sterile (S).

The aggregate segregation ratio in the  $F_2$  generation did not fit a 3F:1S ratio, expected of a monogenic inheritance, and the segregation pattern across the six progenies was heterogeneous (Table 1). Three progenies gave a good  $X^2$  fit to 3:1 ratio (P=0.10-0.75). The other three progenies did not fit a 3:1 ratio, and had an excess of sterile plants. Male sterility in pearl millet has been shown to be more pronounced in the dry season than in the rainy season (Rai and Hash 1990). The aggregate segregation ratio in the  $BC_1$  generation gave an excellent fit to a 1:1 ratio (P=0.75-0.90) and the segregation pattern across the six progenies was homogeneous (P=0.05-0.10). These preliminary results indicate that 834B carries a single dominant gene for male fertility restoration of the  $A_4$  CMS system.

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Table 1. Segregation for pollen fertile (F) and sterile (S) plants in  $F_2$  and backcross (BC<sub>1</sub>) generations of a pearl millet cross (81A<sub>4</sub> x 834B), ICRISAT Asia Center, dry season 1996.

Generation	Progenies	Plants		Expected			Heterogeneity	
		F	S	F:S ratio	X <sup>2</sup>	P	X <sup>2</sup>	Р
F <sub>2</sub>	6	915	391	3:1	17.0	<0.01	23.0	<0.01
BC <sub>1</sub>		354	360	1:1	0.1	0.75-0.90	9.6	0.05-0.10

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## Recurrent Selection Does Not Increase Pathogenicity of *Moesziomyces*penicillariae to Trichomeless Pearl Millet

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Isolates of the smut fungus *Moesziomyces penicillariae* (Bref.) Vanky differ in their pathogenicity to pearl millet (Wilson and Bondari 1990). The potential of *M. penicillariae* to adapt to pearl millet with resistance conferred by the *tr* allele (Wilson 1995) was evaluated.

Two bulk cultures of the pathogen were established. Sori were collected from naturally infected *TrTr* and *trtr* pearl millet in the field in 1992. Teliospores from 15 sori collected from either genotype were cultured on individual plates of V8®-juice agar. Twelve uncontaminated cultures were bulked according to whether they were collected from *TrTr* or *trtr* plants. These bulk cultures were designated TR-0 and tr-0.

Five cycles of inoculation with Tr-0 and tr-0 were performed. Greenhouse-grown Tift 23DA (*TrTr*) and Tift 23DAS (*trtr*) were inoculated by misting five panicles with either TR-0 or tr-0 (1.4 x 10<sup>7</sup> sporidia mL<sup>-1</sup>). Fifteen mature sori were collected from each cultivar x inoculum combination, and teliospores were cultured. After bulking 12 cultures from each cultivar x inoculum combination, the bulk culture collected from an inbred was inoculated back onto that inbred. The process was repeated for five cycles of inoculation, resulting in four cultures designated tr-5DAS, tr-5DA, Tr-5DAS, and Tr-5DA. Designation for the first isolate indicated the original culture was isolated from a *trtr* cultivar, and then cycled for five generations on Tift 23DAS. Cultures were preserved in 20% aqueous glycerine and stored at -80°C.

The virulence of cultures tr-0, Tr-0, tr-5DAS, tr-5DA, Tr-5DAS, and Tr-5DA was evaluated in three experiments in 1993. In Experiment 1, bagged panicles of Tift 23DAS and Tift 23DA were inoculated when stigmas were 50% emerged. Ten panicles of each inbred were

misted with sporidial suspensions of the six cultures or a deionized water control on 18 Aug. Inoculated panicles were covered with pre-wetted plastic bags and a brown paper selfing bag. Plastic bags were removed after 18 h and selfing bags were replaced. Smut severities were estimated as the percentage of infected florets 3 weeks after inoculation. In Experiment 2, 5 mL of inoculum of the six cultures or deionized water was injected into the tips of ten boot leaves of the two inbreds as the panicles were just emerging on 23 Sep. Boot leaves were bagged after inoculation, and smut severities were estimated as above. Four cultivars were inoculated on 8 Sep in Experiment 3; the recurrent parents Tift 8677, Tift 90DBE (Hanna 1993), and corresponding trichomeless backcross<sub>2</sub>, F<sub>3</sub> progeny. Eight panicles of each genotype were inoculated and evaluated as in Experiment 2. Severities were analyzed within each inbred x inoculation date combination, and separated by Fisher's LSD.

Smut severities of panicles inoculated with tr-0 did not differ from the control (Table 1 and 2). When retrieved from cryogenic storage, this culture resembled a single sporidial isolate with low pathogenicity (Wilson and Bondari 1990). Although virulence changes of the cultures from cycle 0 to cycle 5 could not be compared because of the anomalous characteristics of tr-0, there were no differences in final virulence among cultures repeatedly inoculated on, and isolated from, either the *TrTr* or *trtr* genotype. Selection for increased virulence to *trtr* pearl millet did not occur in these experiments. Sample sizes and genetic diversity of the original and

Table 1. Smut severities of Tift 23DAS (*trtr*) and Tift 23DA (*TrTr*) inoculated in the field with six cultures of *Moesziomyces penicillariae*.

	Experi	ment 1	Experiment 2			
	Tift	Tift	Tift	Tift		
	23DAS	23 D A	23DAS	23DA		
Tr-0	37.8 b <sup>1</sup>	72.4 ab	5.7 ab	29.4 a		
tr-O	9.7 c	56.2 bc	0.3 c	4.3 b		
Tr-5DAS	50.4 a	66.8 ab	4.2 ab	25.7 a		
tr-5DAS	49.6 ab	78.1 a	4.0 ab	35.7 a		
Tr-5DA	55.0 a	78.8 a	6.7 a	29.0 a		
tr-5DA	45.4 ab	78.3 a	2.8 bc	33.6 a		
Control	17.1c	46.2 c	0.0 c	0.1 b		
LSD (0.05)	15.6	19.5	9.8	13.4		

Numbers within a column followed by the same letter do not differ significantly.