Euphytica **90:** 31–37, 1996.

© 1996 *Kluwer Academic Publishers. Printed in the Netherlands.*

Smut reaction of pearl millet hybrids affected by fertility restoration and genetic resistance of parental lines *

K.N. Rai & R.P. Thakur
International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Andhra Pradesh 502 324, India

Received 8 November 1994; accepted 13 December 1995

Key words: pearl millet, *Pennisetum glaucum*, smut, *Tolyposporium penicillariae*, cytoplasmic-nuclear male sterility

Summary

Pearl millet (*Pennisetum glaucum* (L.) R. Br.) hybrids based on the A_1 cytoplasmic-nuclear male-sterile (CMS) lines are more susceptible to smut (*Tolyposporium penicillariae* Bref.) than open-pollinated varieties. Seventy eight pairs of hybrids, made onto male-sterile (A) lines and their counterpart maintainer (B) lines, were evaluated to examine the effects of male sterility and genetic resistance of parental lines on the smut severity of hybrids. The A-line hybrids had higher smut severity and lower selfed seedset than the counterpart B-line hybrids, indicating that it is the CMS-mediated male sterility rather than the A_1 cytoplasm *per se* that caused greater smut severity of A-line hybrids. However, with the use of resistant parental lines even male-sterile hybrids of A-lines, in several cases, were as resistant as some of the highly resistant male-fertile hybrids of B-lines. It would be possible to produce smut resistant hybrids (< 10% severity) on A-lines, albeit in low frequency, even if only one parent of a hybrid were resistant. However, the probability of producing such hybrids would be higher when both parents were resistant to smut. Thus, improvement in smut resistance of parental lines and fertility restoration ability of pollinators would provide the most effective genetic approach to smut disease management in hybrids.

Introduction

All commercial hybrids of pearl millet (*Pennisetum glaucum* (L.) R. Br.) are currently based on the A_1 cytoplasmic-nuclear male sterility (CMS). Soon after the southern corn leaf blight (*Bipolaris maydis* (Nisikado) Schoemaker) epidemic on maize (*Zea mays* L.) hybrids based on Texas CMS was reported in 1970 in the USA (Scheifele et al., 1970), a major epidemic of downy mildew (*Sclerospora graminicola* (Sacc.) Schroet.) (Safeeulla, 1977) and sporadic outbreaks of ergot (*Claviceps fusiformis* Loveless) (Natarajan et al., 1974) were reported on pearl millet hybrids in India. This led to concern about the possible association between the A_1 cytoplasm and susceptibility to these diseases. Studies show that the A_1 cytoplasm is not associated with susceptibility to downy mildew (Anand Kumar et al., 1983; Yadav et al., 1993). However, it has been shown that male sterility due to this cytoplasm increases the susceptibility to ergot (Thakur et al., 1989b).

Smut, caused by *Tolyposporium penicillariae* Bref., is a widespread and economically important disease of pearl millet (Rachie & Majmudar, 1980). Like ergot, it is a panicle disease that is more severe in CMS-based single-cross hybrids than in open-pollinated varieties (Thakur, 1989). The two major factors that determine the smut severity of hybrids are the availability of fertile pollen (Thakur et al., 1983; Wells et al., 1987) and genetic resistance of parental lines (Chavan et al., 1988). The objective of this study was to evaluate the relative role of CMS and genetic resistance of parental lines in influencing the smut severity of hybrids.

* Submitted as JA No 1737 by the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT).

Materials and methods

During 1985–91, four experiments involving different male-sterile (A) lines based on the A_1 cytoplasm, their corresponding maintainer (B) lines, and an array of diverse pollinators (P-lines), and hybrids (developed by crossing P-lines onto both A-lines and B-lines) were evaluated for smut susceptibility in smut nurseries during the rainy seasons (mean temperature 26–33° C) at ICRISAT Asia Center (IAC). Experiments 1–3 reported here, are parts of larger breeding trials that permitted comparisons of smut severities of A × P hybrids with B × P hybrids to evaluate the relationship between the CMS and smut susceptibility in hybrids developed from crosses between susceptible A/B lines and resistant pollinators. Based on observations in these experiments, experiment 4 was conducted to gain further insight into this relationship, and also to examine the relationship between the smut resistance of parental lines and their hybrids.

Experimental material

Experiments 1 and 2. A breeding trial conducted for smut evaluation in 1985 included 2 susceptible seed parents (843A and 843B), 43 smut-resistant source lines (P-lines) developed at IAC (Thakur et al., 1992), and 86 hybrids developed by crossing P-lines onto 843A and 843B. From this trial, a set of 15 random P-lines, 843A, 843B, and their 30 hybrids (experiment 1) were retested for smut severity in 1986. Based on the availability of remnant seed, 16 P-lines, 843A, 843B, and their 32 hybrids were retested in 1988 (experiment 2).

Experiment 3. This experiment consisted of 2 susceptible seed parents (81A and 81B), 7 smut-resistant B-lines used as pollinators (P-lines), and 14 hybrids derived by crossing these P-lines onto 81A and 81B. One smut-resistant P-line was developed from a cross between 843B and a smut-resistant inbred line derived from a cross between 81B and a smut-resistant source line (SRL 53). The remaining six smut-resistant P-lines were developed from a cross between BKM 2026 (bred at Fort Hays Branch Experiment Station, Kansas State University, USA) and a smut-resistant inbred line derived from a cross between 81B and SRL 50. This experiment was conducted in 1987 and 1988.

Experiment 4. Entries in this study consisted of 2 pairs of A/B lines, 20 P-lines, and 80 A × P and B × P hybrids. The A/B pairs consisted of 81A and 81B (susceptible), and ICMA 88006 and ICMB 88006 (resistant). The P-lines included 4 smut-resistant source lines, 6 smut-resistant B-lines, and 5 each of smut-susceptible B-lines and smut-susceptible restorer (R) lines. This experiment was conducted in 1989 and 1991.

Field layout

All experiments were conducted in a randomized complete block design. Experiments 1 and 2 were replicated twice, while experiments 3 and 4 were replicated three times. The entries were sown in single row plots, each 2–4 m long with 75 cm spacing between rows and 10 cm between plants within a row.

Evaluation for smut severity and seedset

Ten panicles were inoculated in each plot during 1987 and 1988 in experiment 3, and during 1989 in experiment 4. In the remaining tests, five panicles were inoculated per plot. Panicles inoculated at the boot stage were bagged and maintained at high relative humidity (> 80%) using a sprinkler irrigation system (Thakur et al., 1983). Inoculated panicles were scored for smut severity, using a standard rating scale of 0–100% (Thakur & King, 1988).

The seedset was also scored on a 0–100% scale for five non-inoculated selfed panicles in each plot of experiments 2 and 3 in 1988. Poor plant stand in 1991 permitted seedset data from only 4–8 plants from all three replications.

Statistical analysis

The mean smut severity of each plot was used for statistical analysis. The Genstat computer program (Genstat, 1986) was used for a mixed-model analysis of variance, assuming the CMS effect as fixed and pollinator effect as random. The CMS × pollinator interaction mean square, where significant, was used as an error term to test the statistical significance of CMS and pollinator effects. In experiment 4, four groups of pollinators, crossed on two pairs of A/B lines produced eight group of hybrids that had heterogeneous error variances. Thus, these eight groups of hybrids were analyzed as subsets (Finney, 1989) to estimate eight different standard errors to compare the differences between A-line hybrids and B-line hybrids. Missing

Table 1. Smut severity in hybrid parents of pearl millet, ICRISAT Asia Center, rainy seasons

Exp.	Hybrid parents			Year	Smut severity (%)			
					Seed parents		P-lines	
	A-line	B-line	P-lines		A-line	B-line[1]	Mean	Range
1	843A	843B	15	1985	70	32	0	–[2]
				1986	73	35	0	0–3
2	843A	843B	16	1985	70	32	0	–[2]
				1988	30	12	–[3]	–[3]
3	81A	81B	7	1987	93	72	5	0–34
				1988	47	18	1	0–5
4	81A	81B	20	1989	68	56	8	0–56
	ICMA 88006	ICMB 88006			1	0		
	81A	81B		1991	18	14	2	0–30
	ICMA 88006	ICMB 88006			0	0		

[1] Smut severity in 81B in trial 1: 65% in 1985 and 69% in 1986.
[2] All P-lines had < 0.5% smut severity.
[3] No data.

plot values within a hybrid group were estimated by using the observed values within that group only.

Results

Smut reaction of parental lines

The large variation in smut severities across years (14–72%) on a highly susceptible genotype 81B indicates that the experiments were conducted under varying disease pressures (Table 1). Under moderate to high disease pressures during 1985–89, the two susceptible A-lines (81A and 843A) had 30–93% smut severities, which were higher than the smut severities of their counterpart B-lines (12–72% severities). ICMA 88006 and ICMB 88006, bred for resistance to smut, were clearly highly resistant with no more than 1% smut severity. All the P-lines in experiments 1 and 2, and all but one in experiment 3 were highly resistant to smut (< 5% smut severity). In experiment 4, P-lines had a wide range of susceptibility levels under moderately high disease pressure in 1989. Fourteen lines had < 10% smut severity, while 3 lines had 10–20% and another 3 lines had 20–56% smut severity.

Relationship between smut severity of hybrids and parental lines

In experiments 1 and 2, hybrids made between susceptible seed parents (843A/843B) and resistant source lines used as pollinators, had low smut severity (2–13% for A-line hybrids and < 5% for the counterpart B-line hybrids) (Table 2). Rarely had an A-line hybrid > 20% smut severity, although both experiments had been conducted at moderately high disease pressure (65–69% severity in susceptible line 81B). There was no significant difference between mean smut severity of A-line hybrids and the counterpart B-line hybrids. In one instance (experiment 2 in 1988) where this difference was significant ($P < 0.05$), it has little practical significance as both A-line hybrids and B-line hybrids had no more than 5% smut severity.

In experiment 3, consisting of hybrids made between another pair of susceptible seed parents (81A/81B) and resistant B-lines, mean smut severities of A-line hybrids were higher than in experiments 1 and 2, with 54% severity in 1987 and 27% in 1988. In this experiment, a majority of A-line hybrids had > 20% smut severities. In comparison, a majority of the counterpart B-line hybrids had < 20% severity under the high disease pressure of 1987 and < 5% severity under the low disease pressure of 1988. In both years, mean smut severities of A-line hybrids were signifi-

Table 2. Smut severity in A-line hybrids and B-line hybrids produced by crossing with smut resistant pollinators of pearl millet

Exp.	Year	Hybrids			No. of hybrids in smut severity class								
		A/B line	No.	Smut severity (%)	0–5	6–10	11–20	21–30	31–40	41–50	51–60	61–80	81–100
1	1985	843A	15	4^a	12	1	1	1					
		843B	15	1^a	15	0	0	0					
	1986	843A	15	13^a	7	4	2	0	0	0	1	1	
		843B	15	4^a	11	2	2	0	0	0	0	0	
2	1985	843A	16	2^a	15	0	0	1					
		843B	16	0^a	16	0	0	0					
	1988	843A	16	5^a	11	1	3	1					
		843B	16	0^b	16	0	0	0					
3	1987	81A	7	54^a	0	1	0	1	1	0	1	1	2
		81B	7	11^b	3	1	2	0	0	1	0	0	0
	1988	81A	7	27^a	1	1	0	3	0	1	1		
		81B	7	1^b	7	0	0	0	0	0	0		

Mean smut severity of A-line hybrids and B-line hybrids followed by the same letter within a year for an experiment are not significantly different at P = 0.05.

Table 3. Smut severity in A-line hybrids and B-line hybrids in experiment 4 based on hybrids involving four groups of pearl millet pollinators, 1989 rainy season

Hybrids			No. of hybrids in smut severity class								
A/B line	No.	Smut severity (%)	0–5	6–10	11–20	21–30	31–40	41–50	51–60	61–80	81–100
Resistant source line (set I)											
81A	4	14^a	1	1	1	0	1				
81B	4	10^a	2	0	1	1	0				
ICMA 88006	4	1^a	4	0	0	0	0				
ICMB 88006	4	1^a	4	0	0	0	0				
Resistant B-line (set II)											
81A	6	36^a	1	0	0	1	2	1	0	1	
81B	6	10^b	2	2	1	1	0	0	0	0	
ICMA 88006	6	10^a	4	0	0	2	0	0	0	0	
ICMB 88006	6	1^a	6	0	0	0	0	0	0	0	
Susceptible R-line (set III)											
81A	5	60^a	0	0	0	0	0	0	2	3	
81B	5	39^b	0	0	1	0	2	0	2	0	
ICMA 88006	5	36^a	0	0	1	0	3	0	1	0	
ICMB 88006	5	6^b	2	3	0	0	0	0	0	0	
Susceptible B-line (set IV)											
81A	5	69^a	0	0	0	0	0	0	1	4	
81B	5	37^b	0	0	1	0	2	1	1	0	
ICMA 88006	5	39^a	0	1	0	1	0	1	1	1	
ICMB 88006	5	5^b	3	2	0	0	0	0	0	0	

Mean smut severity of A-line hybrids and B-line hybrids followed by the same letter within an A/B pair and a pollinator group are not significantly different at P = 0.05.

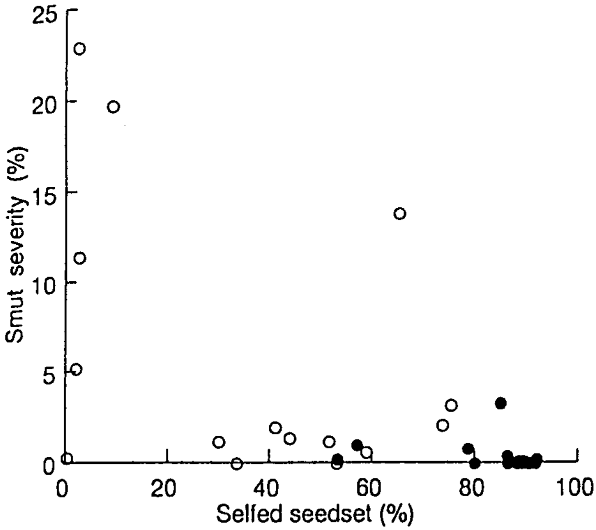

Figure 1. Relationship between selfed seedset and smut severity in hybrids of 843A (○) and 843B (●), ICRISAT Asia Center, 1988 rainy season.

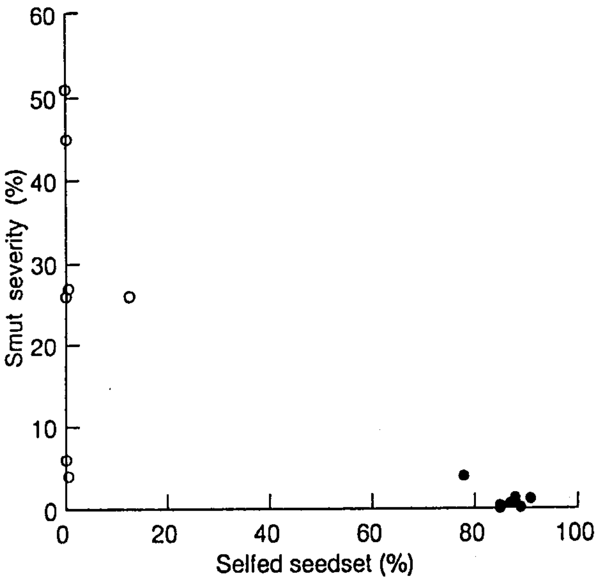

Figure 2. Relationship between selfed seedset and smut severity in hybrids of 81A (○) and 81B (●), ICRISAT Asia Center, 1988 rainy season.

cantly higher ($P < 0.01$) than the counterpart B-line hybrids.

Results of experiment 4 (Table 3) provided a greater insight into the relative role of CMS and genetic resistance of parental lines in determining smut severity of hybrids as both seed parents and pollinators had a wide

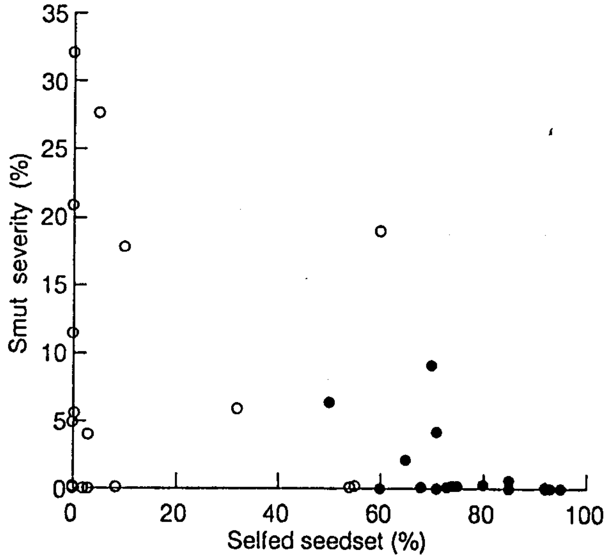

Figure 3. Relationship between selfed seedset and smut severity in hybrids of 81A (○) and 81B (●), ICRISAT Asia Center, 1991 rainy season.

range of resistance levels. In the hybrid set I, based on susceptible seed parents and resistant source lines as pollinators, mean smut severity of both A-line hybrids (14%) and B-line hybrids (10%) were higher than in experiment 1, but there was no significant difference between the smut severities of A-line hybrids and the counterpart B-line hybrids. In hybrid set II, produced from crosses between the same seed parents and resistant B-lines used as pollen parents, mean smut severity increased to 36% in A-line hybrids, but remained 10% in B-line hybrids, which were significantly different. In case of hybrids based on smut resistant male-sterile line (ICMA 88006), all hybrids in set I and 4 out of 6 hybrids in set II had no more than 5% smut severity, and there was no significant difference between A-line hybrids and the counterpart B-line hybrids. The smut severities were highest when both parents were susceptible, with 60–70% mean severity in A-line hybrids and 37% in B-line hybrids. With the involvement of susceptible pollen parents, even hybrids of ICMA 88006 had 36% mean severity and it was uncommon for a hybrid of this line to have < 20% severity. There were highly significant differences between the mean smut severity of A-line hybrids and the counterpart B-line hybrids, irrespective of the seed parents being resistant or susceptible and the pollen parents being B-lines or R-lines.

Relationship between selfed seedset and smut severity in hybrids

In experiment 2, almost all the hybrids made on 843B had very high smut resistance ($\leq$ 1% severity) and average to high selfed seedset, ranging from 54 to 92% (Figure 1). Hybrids of 843A, on the other hand, had a much wider range for both selfed seedset and smut severity. Hybrids with 30–91% selfed seedset had 0–3% smut severities, whereas three with < 1–10% selfed seedset had 11–23% smut severities. In experiment 3, all seven hybrids of 81B had 78–91% selfed seedset and < 4% smut severities (Figure 2). Six of the corresponding hybrids on 81A had < 1% selfed seedset, and four of these had higher smut severities (26–51%) than the corresponding B-line hybrids. In experiment 4, 16 hybrids of 81B (for which data were available) had 50–95% selfed seedset. Fourteen of these hybrids had 0–4% smut severity and none had > 9% smut severity (Figure 3). In the average smut severity range of 11–32%, five hybrids of 81A had 0–10% selfed seedset and one had 60% significant negative correlations between smut severity and seedset ($r = -0.580^{**}$ in experiment 1, -0.740^{**} in experiment 2 and -0.407^{*} in experiment 4). In all three experiments, however, there were a few hybrids of A-lines that had < 5% selfed seedset and < 5% smut severities. In general, hybrids of A-lines with higher smut severities were largely male-sterile and thus had no selfed seedset or it was very low.

Discussion

Under moderate to high disease pressure, smut-susceptible A-lines (81A and 843A) had 18–38% greater disease severity than their counterpart B-lines. The greater disease severity in A-lines could be due to unavailability of viable pollen in bagged panicles (Thakur et al., 1983; Wells et al., 1987). There were no viable pollen available in the bagged panicles of the resistant A-line ICMA 88006 as well. However, ICMA 88006 and its counterpart B-line had no more than 1% smut severity, indicating an overriding role of genetic resistance in providing a nearly complete protection from smut infection to even a male-sterile plant.

Hybrids between susceptible seed parents (81A and 843A) and resistant source lines had low mean disease severity (< 15%), which could be due to joint effects of dominance nature of resistance (Chavan et al., 1988) and lower degree of male sterility. Thus, as the male sterility of hybrids increased (e.g., hybrids of resistant B-lines made on 81A), smut severity also increased, with five hybrids having 26–51% smut, indicating that male sterility, resulting in unavailability of viable pollen, accentuated the susceptibility of these hybrids. However, when the same B-lines were crossed onto a resistant A-line (ICMA 88006), the mean smut severity of hybrids was no more than 10%, with 4 out of 6 hybrids having even < 5% severity. A few sterile hybrids with high degree of resistance (< 5% severity), albeit in low frequency, were produced even if one parent was resistant. More than 60% of the hybrids were highly resistant when both parents were resistant. On the other hand, no A-line hybrid, even if fertile, had < 40% smut severity when both parents were susceptible. Partitioning of sum of squares due to female parent also showed that contribution of genetic resistance to smut severity of hybrids was 20% more than that of the CMS, and both effects were highly significant. This again shows, as in case of male sterile lines, that genetic resistance played a greater role than the CMS in determining the smut severity of hybrids. However, the most effective genetic approach to smut disease management would involve development of resistant parental lines coupled with the improvement in fertility restoration ability of restorer lines.

Ergot, like smut, is a panicle disease of pearl millet. In both cases, pollination-based reduction in disease severity has been demonstrated (Thakur & Williams, 1980; Thakur et al., 1983). It has also been demonstrated that ergot severity levels of 20–30% under artificial inoculation can provide adequate levels of functional field resistance under natural ergot epidemic conditions (Thakur et al., 1989a). Irrespective of the male fertility level, at least 30% of the A-line hybrids produced with one resistant parent had < 30% smut severity under moderately high disease pressure created by artificial inoculation. It would be worthwhile testing the functional field resistance of such hybrids under natural smut epidemic conditions.

In all four experiments CMS × pollinator interactions for smut severity were highly significant ($P < 0.01$). Similar results were obtained by Yadav et al. (1992), but the nature of interaction and its implication in detection of CMS effect was different from that observed in our study. For instance, in our study there was not a single instance, as expected, where an A-line hybrid had less smut severity than a B-line hybrid, and mean smut severity of A-line hybrids (in cases where it was > 27%) was significantly higher than the counterpart B-line hybrids. Yadav et al. (1992) found that 12 out of the 35 A-line hybrids had significantly less smut severity than their counterpart B-line hybrids: the reverse was true in 6 comparisons. Thus, it is likely that internal cancellation of these differences in opposite directions finally led to non-significant difference between mean smut severity of A-line hybrids and B-line hybrids. The contribution of male fertility of A-line hybrids, as observed by good pollen shed, can partly account for this similar smut severity levels. However, visual assessment of pollen production in our study showed that, in several cases, A-line hybrids produced good amounts of pollen but had less selfed seedset than their counterpart B-line hybrids. For instance, hybrids of five restorers (R-lines) made onto 81A and ICMA 88006 in experiment 4 produced good amounts of pollen, but hybrids of two restorers on both A-lines had < 10% selfed seedset. Data from several other experiments also show that even profuse pollen-producing A-line hybrids have significantly less selfed seedset than the counterpart B-line hybrids (C.T. Hash & K.N. Rai, unpublished). This suggests that even an apparently prolific pollen-producing A-line hybrid may be less fertile than a B-line hybrid. Also, A-line hybrids may have longer protogyny (and hence poorer seedset) that may contribute to greater smut severity. Thus, selfed seedset may be a more relevant factor to be considered in relation to smut severity than a qualitative visual assessment of pollen shed from unbagged panicles.

References

Anand Kumar, K., R.P. Jain & S.D. Singh, 1983. Downy mildew reactions of pearl millet lines with and without cytoplasmic male sterility. Plant Dis. 67: 663–665.

Chavan, S.B., R.P. Thakur & K.P. Rao, 1988. Inheritance of smut resistance in pearl millet. Pl. Dis. Res. 3: 192–197.

Finney, D.J., 1989. Was this in your statistics textbook? V. Transformation of data. Expl. Agric. 25: 165–175.

Genstat Release 4.04, 1986. Lawes Agricultural Trust – Rothamsted Experimental Station, UK.

Natarajan, U.S., V.B. Guruswamy Raja, S. Selvaraj & C. Parambaramani, 1974. Grain loss due to ergot disease in bajra hybrids. Indian Phytopath. 27: 254–256.

Rachie, K.O. & J.V. Majmudar, 1980. Pearl Millet. The Pennsylvania State University Press, University Park and London. 307 pp.

Safeeulla, K.M., 1977. Genetic vulnerability. The basis of recent epidemics in India. Ann. New York Acad. Sci. 287: 72–85.

Scheifele, G.L., W. Whitehead & C. Rowe, 1970. Increased susceptibility to southern leaf spot (*Helminthosporium maydis*) in inbred lines and hybrids of maize with Texas male-sterile cytoplasm. Plant Dis. Rep. 54: 501–503.

Thakur, R.P., 1989. Flowering events in relation to smut susceptibility in pearl millet. Plant Pathol. 38: 557–563.

Thakur, R.P. & S.B. King, 1988. Smut disease of pearl millet. Information Bulletin no. 25. Patancheru, A.P. 502 324, India: International Crops Research Institute for the Semi-Arid Tropics.

Thakur, R.P., S.B. King, K.N. Rai & V.P. Rao, 1992. Identification and utilization of smut resistance in pearl millet. Research Bulletin no. 16. Patancheru, A.P. 502 324, India: International Crops Research Institute for the Semi-Arid Tropics.

Thakur, R.P., S.B. King & V.P. Rao, 1989a. Expression of ergot resistance in pearl millet under artificially induced epidemic conditions. Phytopathology 79: 1323–1326.

Thakur, R.P., V.P. Rao & S.B. King, 1989b. Ergot susceptibility in relation to cytoplasmic male sterility in pearl millet. Plant Dis. 73: 676–678.

Thakur, R.P., K.V. Subba Rao & R.J. Williams, 1983. Effects of pollination on smut development in pearl millet. Plant Pathol. 32: 141–144.

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

Wells, H.D., W.W. Hanna & G.W. Burton, 1987. Effects of inoculation and pollination on smut development in near-isogenic lines of pearl millet. Phytopathology 77: 293–296.

Yadav, O.P., I.S. Khairwal & S. Singh, 1992. Smut severity of pearl millet hybrids with male sterile and fertile cytoplasm. Euphytica 64: 139–142.

Yadav, O.P., V.K. Manga & G.K. Gupta, 1993. Influence of A_1 cytoplasmic substitution on the downy mildew incidence of pearl millet. Theor. Appl. Genet. 87: 558–560.