Evaluation of a New Field Screening Technique for Smut Resistance in Pearl Millet

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ABSTRACT

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An effective field screening technique was developed to identify resistance to smut in pearl millet. The technique involved inoculation of tillers at the boot-leaf stage by injecting a sporidial suspension of *Tolyposporium penicillariae* into the space around the inflorescence within the flag leaf sheath, followed by covering the tiller "boots" with parchment bags. High humidity was maintained with frequent overhead sprinkler irrigation throughout the period of inoculation, flowering, and grain development. Inflorescences were scored for smut reaction 20–25 days after inoculation with the aid of a standard key developed to estimate percent severity. This technique was used successfully in the field during the 1981 rainy season to screen more than 200 pearl millet lines. Resistance was confirmed in a few lines and many resistant plants with selfed seeds were selected.

Additional key words: *Pennisetum americanum*.

Smut, caused by *Tolyposporium penicillariae* Bref., has been recognized as an important or potentially important disease of pearl millet [*Pennisetum americanum* (L.) Leeke] in Africa and Asia since the early part of this century (3,4,10) and more recently in North America (14). The disease has become important in northern India on F_1 hybrid cultivars in large commercial plantings (9).

Airborne inoculum of the smut pathogen infects pearl millet florets directly at flowering (1,2,13). In the tropics pearl millet is grown primarily by subsistence farmers, which makes chemical control of this disease economically unlikely. The development and use of resistant cultivars offers the most technically and economically feasible means of control; however, progress depends primarily on the availability of an effective technique to identify resistant germ plasm and progenies. Research workers (2,5,7,8,11) have tried several methods of inoculation with variations in time of inoculation during flowering, different types of inoculum, different types of bags to cover inoculated tillers, and the use of various rating scales. However, no technique has been reported that is sufficiently reliable to ensure the identification of various levels of smut resistance in pearl millet.

Since 1980, efforts have been made at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) to develop an effective, reliable, field-based screening technique for the identification of sources of resistance to smut in pearl millet.

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MATERIALS AND METHODS

Sporeball longevity in vitro. Sporeballs of *T. penicillariae* were collected from infected panicles in different years (1976–1981) and stored in small polythene bags in a refrigerator (~10 C). The viability of these sporeballs stored for six different time periods (ranging from 1 mo to 61 mo) was tested by incubation for 18–20 hr at 30 C in cavity slides containing sterile distilled water. One hundred sporeballs in five microscopic fields were examined for germination in each of two cavity slides and mean percent germination was calculated for each batch.

Sources of inoculum and inoculation method. Inocula of *T. penicillariae* from two sources were: sporidia produced on potato agar (extract from 200 g of peeled potato and 20 g of agar in 1 L of water) grown at 35 C for 3 days to >60 days (Table 1), and sporidia from germinated sporeballs were collected from matured sori on the inflorescences and soaked in water for 24 hr. The concentration of the aqueous sporidial suspensions was adjusted to about 10^6 sporidia per milliliter based upon counting with a hemocytometer. Five treatments were compared (Table 1). With each suspension, 10–20 tillers of two pearl millet male-sterile lines and two F_1 hybrids were inoculated by injecting inoculum into the "boot" (the flag-leaf sheath enclosing the inflorescence just prior to inflorescence emergence) so that the suspension filled the spaces between the leaf sheath and the inflorescences. Boots injected with water were used as checks. The boots were bagged immediately after inoculation. The experiment was conducted both in a screenhouse and in the field at the ICRISAT Center. High relative humidity (RH) was maintained by providing sprinkler irrigation in the field. Smut severity was estimated as percent florets transformed into smut sori 20–25 days after inoculation with the aid of a standard key similar to the one used for estimating ergot severity (12).

Flowering stage at inoculation. Sporidial suspensions were prepared in sterile distilled water from 10-day-old cultures of *T. penicillariae* on potato-dextrose agar, adjusted to 10^6 sporidia per milliliter and used to inoculate a pearl millet hybrid and a male-sterile line at four stages of flowering. These included: the boot-leaf (inflorescence enclosed in the flag leaf sheath), early stigma emergence, full stigma emergence, and anthesis stages (Table 2). Inoculation was by injection at the boot stage and by spraying to run-off at the three other flowering stages. The experiment was conducted in a screenhouse at ICRISAT Center and was repeated with the same hybrid in the field at the ICRISAT Sub-Center at Hissar in northern India where there was no provision for sprinkler irrigation. In each experiment, 10–15 inflorescences were inoculated per treatment per test genotype. The degree of smut development was estimated as described above.

Inoculum concentration. Aqueous sporidial suspensions were prepared from 10-day-old cultures from potato agar and adjusted to four concentrations (Table 3) with a hemocytometer. The suspensions were used to inoculate inflorescences of two pearl millet hybrids by injection at the boot stage as described above. Hybrid BJ-104 was inoculated in the field (20 inflorescences per treatment), and hybrid ICH-220 was inoculated in the screenhouse (10 inflorescences per treatment). Inoculated tillers were bagged immediately after inoculation. Smut severity was estimated 20–25 days after inoculation with the rating system described above.

Type of selfing bag. An aqueous sporidial suspension (~10^6 sporidia per milliliter) prepared from 10-day-old cultures from potato agar was used to inject-inoculate two pearl millet hybrids and two male-sterile lines at boot stage. The inoculated boots were covered with polythene or white parchment selfing bags. Four treatments (Table 4) with 10–15 inflorescences per treatment in each test genotype were maintained. Smut severity was assessed 20–25 days after inoculation with the above rating system.

TABLE 1. Effect of different sources of *Tolyposporium penicillariae* inoculum on percent smutted florets in pearl millet male-sterile lines and hybrids

	Experiment location and pearl millet line[b]					
	Screenhouse			Field		
				BJ-104		
Inoculum source[a]	5054-A	5141-A	BJ-104	I	II	ICH-105
Sterile distilled water (check)	<1	0	0	0	<1	0
3–5 day culture[c]	73	74	86	54	–[d]	68
15–20 day culture[c]	80	73	75	39	29	56
>60 day culture[c]	71	43	41	–[d]	–	–
Sporeballs soaked in water for 24 hr	35	10	15	18	13	16
L.S.D. ($P<0.05$)	13.3	12.3	14.5	20.1	5.3	14.7

[a]Sporidial suspension (~10^6 sporidia per milliliter) was used to inoculate the tillers at the 'boot' stage.
[b]Mean of 10–20 inflorescences per treatment.
[c]Growth at 35 C on potato agar.
[d]Treatment not included.

TABLE 2. Effect of flowering stage at time of inoculation on percent smutted florets in a pearl millet hybrid and male-sterile line

		Expt. location and pearl millet line[a]		
		Screenhouse[b]		Field[c]
Flowering stage at inoculation	Inoculation method	BJ-104	5054-A	BJ-104
Boot leaf	Injection	63	95	29
Early stigma-emergence	Spray	41	55	<1
Full stigma-emergence	Spray	6	30	<1
Anthesis	Spray	0	0	0
L.S.D.($P<0.05$)		14	3	10

[a]Mean of 10–15 inflorescences per treatment.
[b]Polythene bags were used to cover the inoculated boots.
[c]Conducted at Hissar without sprinkler irrigation.

TABLE 3. Effect of concentration of *Tolyposporium penicillariae* sporidial suspensions on smut development in two pearl millet hybrids

	Smutted florets (%)	
	Field[b]	Screenhouse[c]
Sporidial conc.[a] (sporidia/ml)	BJ-104	ICH-220
Sterile distilled water (check)	<1	2
0.7×10^5	78	54
0.5×10^6	79	55
3.2×10^6	87	54
2.4×10^7	87	57
L.S.D. ($P<0.05$)	7	25

[a]Obtained as water dilution from 10-day growth on potato agar at 35 C.
[b]Mean of 20 inflorescences per treatment.
[c]Mean of 10 inflorescences per treatment.

TABLE 4. Effect of type of bags on smut development in a pearl millet hybrid and two male-sterile lines

	Smutted florets (%)[a]					
	Screenhouse experiment			Field experiment		
				BJ-104		
Treatment	BJ-104	5141-A	5054-A	I[b]	II	5141-A
Uninoculated check	0	0	0	0	1	0
Inoculated[c]						
No bag	<1	<1	0	0	3	<1
Parchment bag	26	34	2	54	78	79
Polythene bag	82	83	73	23	57	11
L.S.D. ($P<0.05$)	11	13	7	14	6	6

[a]Mean of 10–15 inflorescences per treatment.
[b]Conducted during summer (March) 1980 without sprinklers.
[c]Sporidial suspensions of *T. penicillariae* (~10^6 sporidia per milliliter) from 10-day growth on potato/carrot agar was used for inoculating tillers at the boot leaf-stage.

The screening technique and its use. Based on the results of the experiments reported above, a technique was utilized to screen more than 200 pearl millet genotypes for resistance to smut in the field during the 1981 rainy season. Sporidia of *T. penicillariae* were harvested in sterile-distilled water from 3- to 10-day-old cultures maintained in potato agar at 30–35 C and adjusted to a concentration of about 10^6 sporidia per milliliter. Fresh sporidial suspensions were injected into the space between the inside of the flag leaf sheath and the inflorescence when tillers were at the boot-leaf stage and covered immediately after inoculation with white parchment bags. High RH was maintained by operating overhead sprinklers two or three times a day, 30 min each time. Inoculated inflorescences were scored for percent smut severity 20–25 days after inoculation using the standard rating key and inflorescences with little or no smut were selected to provide selfed seed for further evaluation and utilization.

Data were subjected to analysis of variance, in which the number of inoculated/uninoculated inflorescences were considered as replications in each treatment, and the LSD ($P<0.05$) between treatment means was determined.

RESULTS

Sporeball longevity in vitro. There was a sharp decline in viability of sporeballs with an increase in storage time, and after 61 mo of storage, less than 5% of the sporeballs germinated (Fig. 1).

Effect of inoculum sources. Sporidia from potato agar cultures produced significantly more smut than did sporidia from sporeballs soaked in water for 24 hr on hybrids BJ-104 and ICH-105 in the field experiment. In the screenhouse experiment, however, both 3- to 5-day-old and 15- to 20-day-old cultures produced significantly more smut than did 60-day-old cultures on two of the three genotypes tested (Table 1). There was less than 1% smut in the uninoculated check treatment.

Effect of flowering stage at inoculation. Inoculations made at the boot-leaf stage resulted in signficantly more smut than inoculations at the other three stages of flowering in the screenhouse and field (Table 2). In the screenhouse, inoculation at the "early stigma-emergence" stage produced significantly more smut than inoculation at the "full stigma-emergence" stage. No infection occurred when inoculation was made at anthesis. More smut developed in the screenhouse than in the field at Hissar.

Effect of inoculum concentrations. All four sporidial concentrations produced higher levels of smut in field at ICRISAT Center than in the screenhouse. However, concentrations of 3.2×10^6 sporidia per milliliter and above produced significantly more smut than lower concentrations in the field experiment, but this was not observed in the screenhouse (Table 3).

Effect of types of bags. Covering the inoculated "boot" with polythene bags produced significantly more smut than with parchment bags in the screenhouse, but opposite results were obtained in the field on all the test genotypes (Table 4).

Effectiveness of the screening technique. More than 200 pearl millet lines were screened during the 1981 rainy season (Table 5). All the hybrids but one, which had mean severity of only 10%, were highly susceptible. Pearl millet cultivars and local collections were generally less susceptible than hybrids. Lines identified as susceptible and resistant in earlier screenings at Hissar (PMSN and IPMSN entries in Table 5) and in the multilocational testing (6) did not show much variation for smut reactions and, thus, their resistance (low susceptibility) was confirmed. Seed from self-fertilization of more than 500 smut-free single plants was collected.

Attempts to screen about 300 lines during the January–April 1982 crop season were not successful. The period from inoculation to disease development remained relatively dry compared to the 1981 rainy season. The susceptible check cultivars developed only 19% smut severity compared to 92% in the rainy season (Table 6).

DISCUSSION

Results reported here clearly indicate that factors important for the development of an effective field screening technique for smut

TABLE 5. Screening of pearl millet lines for smut resistance at ICRISAT Center during the 1981 rainy season

Nursery or plant material evaluated	Entries (no.)	Smut severity (%)[a] Mean	Range	Plants inoculated (no.)	Smut-free plants selected (%)
F_1 hybrids[b]	38	72	10–92	760	0
Populations[b]	36	46	8–82	720	0
Local collections[b]	17	23	8–47	340	0
PMSN[c]	39	4	0–20	1560	11
IPMSN[d]	29	4	0–28	1160	9.5
F_3 Smut res. bulks[e]	43	1	0–22	1720	15
Susc. check (ICH-220)	1	85	80–91	40	0

[a] Overall mean and range of entry means based on 20–40 inoculated plants per entry.
[b] Entries from All India Coordinated Millet Improvement Projects (AICMIP).
[c] Pearl Millet Smut Nursery.
[d] International Pearl Millet Smut Nursery.
[e] Smut resistant F_3 lines developed at the ICRISAT-Hissar sub-center.

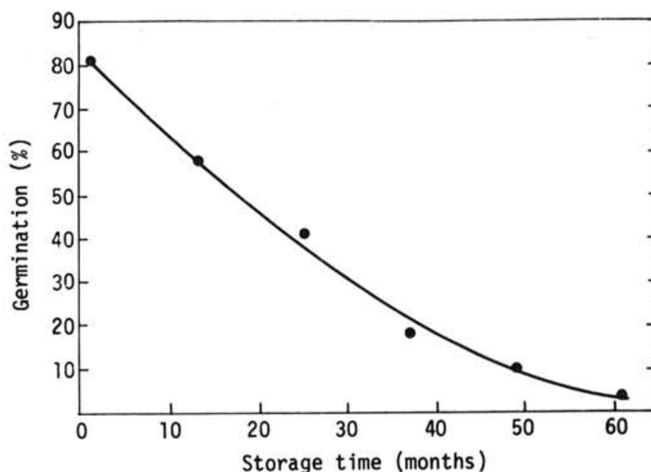

Fig. 1. Germination of sporeballs of *Tolyposporium penicillariae* after storage for different time periods at 10 C.

TABLE 6. Relative humidity and temperature during the period from inoculation to smut assessment, and smut development in a pearl millet hybrid and a male-sterile line

Season	(Time inoc. to disease assessment)	Average temp. (C) Min.	Average temp. (C) Max.	Average RH (%) Min.	Average RH (%) Max.	Hours RH > 80% (no.)	Smut sev. (%) ICH-220	Smut sev. (%) 5054-A
1981 Rainy	21 Aug–9 Sept	20.4	24.5	73.0	87.4	16.2	92[a]	92[b]
1981–1982 Post-rainy	3–22 Feb	19.8	26.7	52.1	85.5	7.6	19[b]	17[b]

[a] Mean of 40 inflorescences.
[b] Mean of 10 inflorescences.

resistance are inoculum source and concentration, plant growth stage at inoculation, type of selfing bag used to cover inoculated tillers, and the RH during the inoculation and disease development period. Significant changes in any of these factors might lead to large variation in smut reactions of cultivars or failure of the screening technique.

RH during the period from inoculation to disease assessment appears to be a critical factor for smut development (Table 6). In our field studies during the 1981 rainy season high RH (>80%) was maintained for about 16 hr per day throughout the disease development period, and 92% smut severity developed on susceptible check cultivars compared to only 19% severity when the same RH was maintained only 7 hr per day during February 1982. There was no large variation in temperature during the two seasons suggeting it was not a significant factor in smut development in our studies. Accurate smut screening in the field can probably be done only in the rainy season at Hyderabad. However, more critical experiments are needed to clearly understand the role of RH in relation to smut infection and development.

Bagging the "boot" immediately after inoculation helps promote smut development in two ways, by maintaining high RH in and around the boot, and by protecting the inoculated inflorescences from cross pollination. Our observations support Bhatt's observation (2) that cross pollination greatly reduces smut development in pearl millet. Patel and Desai (7) and Husain and Thakur (5) used polythene bags while Pathak and Sharma (8) and Sangwan and Thakur (11) used parchment bags in their screening programs. In our studies, however, parchment bags were more effective in the field and polythene bags were more effective in the screenhouse in promoting smut development. This was probably because the screenhouse provided enough shading to prevent heat buildup in polythene bags, whereas direct sunlight in the field resulted in too much heat accumulation for optimum infection. Parchment bags, in addition to promoting more smut in the field, are more porous than polythene bags and dry out once sprinkler irrigation is stopped. This allows normal pollen shedding, and selfed seed is produced which permits direct selection of smut resistant plants. In a highly cross-pollinated crop like pearl millet, it is important to obtain the selfed seed from a plant which has shown resistance, as it is not possible to obtain the same genotype from the remnant seed stock (unless the line is inbred and thus uniformly homozygous).

In any resistance screening program, the major objective is to identify resistant plants to use in the breeding program. The screening technique described here can be scaled up to handle larger numbers. Several lines with confirmed resistance are being utilized in the ICRISAT Center breeding program and Indian national program to develop smut-resistant hybrids and cultivars of pearl millet.

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