An Improved Field Screening Technique for Downy Mildew Resistance in Pearl Millet

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ABSTRACT

Williams, R. J., Singh, S. D., and Pawar, M. N. 1981. An improved field screening technique for downy mildew resistance in pearl millet. Plant Disease 65:239-241.

An effective, large-scale field screening technique has been developed to identify sources of resistance to downy mildew (*Sclerospora graminicola*) in pearl millet (*Pennisetum americanum*). The technique, based on preplanted infector rows that provide sporangial inoculum, has been successfully used to identify and improve downy mildew resistance in composites, cultivars, and hybrid parents. The major advantages of the technique are reliability, uniformity of inoculum distribution, flexibility in location and size of screening plots, effectiveness throughout the year (including the dry winter [postrainy] season), and independence from rainfall.

Additional key words: oospores, zoospores

Downy mildew, caused by *Sclerospora graminicola* (Sacc.) Schroet., became a major production constraint in the Indian hybrid pearl millet (*Pennisetum americanum* (L.) Leeke) crop in the early 1970s and is still the greatest threat to improved pearl millet cultivars in Africa and India. The absence of effective, economically feasible chemical control methods necessitated the development of resistant cultivars.

Early attempts to screen for sources of resistance to pearl millet downy mildew depended on "sick plots;" ie, plots into which infected, oospore-bearing pearl millet plants had been plowed for several years (4). The test materials were planted in these plots, and infection was initiated by the oospores in the soil.

This screening system had several drawbacks: 1) several seasons were needed to incorporate enough oospores into the soil; 2) producing a uniform inoculum distribution throughout the plots was difficult, particularly for large-scale screening; 3) unless soil moisture conditions were optimal for oospore germination and infection throughout the plots during the first 3–4 wk after seeding, nonuniform or no infection occurred; and 4) size and location of the screening area were fixed. The use of sick plots slowed progress in resistance breeding, because lines that had escaped infection were often selected as resistant. The demonstration of the significant role of sporangia (and their zoospores) in the epidemiology of this disease (6) led to the development of the improved field-inoculation technique described here.

Journal Article 144 of the International Crops Research Institute for the Semi-Arid Tropics.

0191-2917/81/03023903/$03.00/0

MATERIALS AND METHODS

Inoculum. Oosporic inoculum, obtained by pulverizing dried, mature, infected leaves, was maintained as a dry powder in glass bottles in the laboratory and was used as a seed dressing (5 g of oospore powder per kg of seed) to produce the initial infected plants. Sporangial inoculum was subsequently maintained continuously on susceptible pearl millet hybrids HB-3 and NHB-3 by sequential plantings in a small disease garden area adjacent to the laboratory. Mist irrigation for 30 min in the late evening hours three times a week assured the development of high levels of downy mildew in all seasons.

We found that infected leaves that had received more than 6 hr of sunlight produced sporangia profusely in 7–8 hr when maintained in a moist chamber in the dark at 20 C. We used this information to produce sporangia for inoculations in the mornings (0500–0600 hours) and in the evenings (1700–1800 hours).

For morning inoculations, leaves were detached from infected plants in the field at about 1630 hours the previous day. Leaf surfaces were gently rubbed with moist cotton wool to remove old sporangiophores. Leaves were placed in plastic chambers lined with moist blotting paper and incubated at 20 C for 7 hr. The incubators were programmed to lower the temperature to 5 C at this time; the next day at 0400 hours, the temperature was returned to 20 C for 30–60 min. The mature sporangia were harvested in tap water by gently brushing the leaf surfaces with a camel's-hair brush.

For evening inoculations, the leaves were detached from infected plants in the field at 1630 hours and maintained overnight with cut ends in tap water under fluorescent light in the laboratory. The next day at 0900 hours, the leaves were cleaned, placed in a moist chamber as for morning inoculation, and incubated at 20 C for 8 hr. Sporangia were harvested in tap water at about 1700 hours.

Primary infector plants. Seeds of susceptible hybrid NHB-3 and cultivar 7042 were planted in plastic buckets 25 cm in diameter in red soil (Alfisol) mixed with farmyard manure (3:1). The stem bases of the seedlings were injected with sporangial (zoospore) suspensions 10–15 days after planting. Within 7 days of sporangial inoculation, high levels of downy mildew occurred, and the pots were taken to the screening plots.

Field infector rows. A 1:1 mixture of NHB-3 and 7042 seed was planted in every third row throughout the screening field in 1976 and 1977, and subsequently in every fifth row (rows 0.75 m apart), as "infector rows." When the seedlings were emerging, the buckets of primary infector plants were placed along these infector rows at 10-m intervals. Mist irrigation was used in the late evenings for 30 min three to four times a week to promote sporulation on the primary infector plants. The sporangia (and their zoospores) so produced infected the infector-row plants. By about 21 days after planting, the field infector rows had developed at least 40% downy mildew infection. Uninfected main shoots and tillers that escaped infection were removed from the infector-row plants to promote further production of infected tillers.

Test and indicator rows. The test rows (materials to be screened for resistance to downy mildew) were planted when 40–50% of infector-row plants had developed the disease. At the same time, rows of NHB-3 were planted after every 10 rows of test material to act as an indicator of disease pressure.

Humidity. Throughout crop development, a "perfospray" irrigation system provided high humidity in the screening field (Fig. 1). The perfospray was operated at a pressure of 0.7–1.4 kg/cm^2

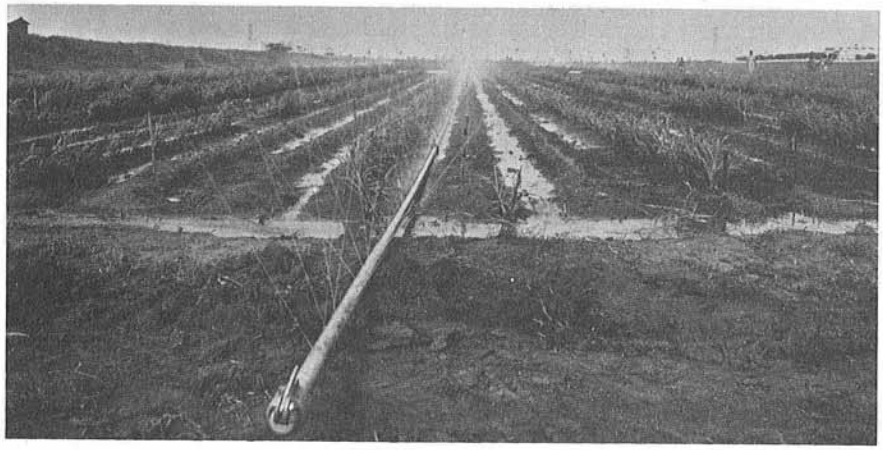

Fig. 1. The "perfospray" mist irrigation system used to promote sporulation and infection by *Sclerospora graminicola* in the large-scale, infector-row screening system for resistance to downy mildew in pearl millet. Note the preplanted infector rows (every third row).

Table 1. Incidence of downy mildew in six pearl millet composites planted in the screening nursery at ICRISAT Center and in nearby breeding plots during the 1976 rainy season

		Downy mildew incidence			
		Screening nursery		Breeding plots	
Composite	Number of entries	Mean (%)	Range (%)	Mean (%)	Range (%)
WC-FS	8	51	33–94	< 1	0–36
MC-FS	6	53	39–73	5	0–13
GAM-75-S_1	22	69	49–92	6	0–38
GAM-73-S_2	3	56	49–59	17	5–47
GAM-73-FS	2	56	53–64	1	0–3
RF Synthetic	26	67	44–100	27	0–97

Table 2. Incidence of downy mildew in four pearl millet entries grown as standard checks in the ICRISAT pearl millet downy mildew field-screening nurseries during the 1977 and 1978 rainy seasons

	1977		1978	
	Incidence[a]		Incidence[a]	
Entry	(%)	SD[b]	(%)	SD[b]
HB-7	28	± 4.8	26	± 3.0
BJ-104	44	± 8.5	61	± 7.3
NHB-3	93	± 4.9	...[c]	...
7042	...[c]	...	83	± 3.0

[a] Each value is the mean of five trials with two replicates per trial.
[b] Standard deviation of the sample.
[c] Entry not included.

(7–21 m head). At the recommended pressure of 1.2 kg/cm^2 (12-m head), each meter length of pipe (5-cm diameter) sprayed 52 L/min. The application rate was approximately 13 mm/hr. The perfospray system, operated on alternate evenings after sunset for 30 min, was ideally suited for the promotion of downy mildew in pearl millet.

Disease incidence. Incidence of downy mildew in test materials was recorded 20–25, 40–45, and 60–70 days after planting. At the first two ratings, small, slim, red-topped bamboo pegs were placed beside infected plants, so that plants that died and were washed away during heavy rains could still be counted at later ratings.

RESULTS

The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) Center has used this screening technique on a large scale (4–6 ha) in two seasons a year (rainy and postrainy) since the 1976 rainy season.

During the rainy seasons of 1976 and 1977, four types of materials were screened—inbreds, hybrids, male-sterile lines, and composites and composite products (S_1, S_2, full-sib, and half-sib lines). Composites and composite products constituted 85% and 83% of entries during the rainy seasons of 1976 and 1977, respectively, and were the only materials screened in the 1976–1977 postrainy season. Since then, germ plasm, hybrids, inbreds, and synthetics have also been widely screened, and in the 1977 and 1978 rainy seasons, we screened all the breeding trials of the All-India Coordinated Millet Improvement Project (AICMIP).

The mean incidence of downy mildew in the susceptible indicator rows was 84% during the 1976–1977 postrainy season and ranged from 66–79% and 60–80% during the 1976 and 1977 rainy seasons, respectively.

In the 1976 rainy season, the first season the technique was used, 3,470 breeding lines were screened in four groups of materials, and a wide range of downy mildew reactions was obtained. Similar ranges of reactions were observed in 2,197 lines screened in the 1976–1977 postrainy season and in 6,021 lines tested in the 1977 rainy season. The proportion of lines with less than 10% downy mildew in the composites, which were selected (by selfing resistant plants in all lines) and recombined (by making full-sibs with resistant plants in selected lines) under downy mildew pressure, increased from 55% in the 1976 rainy season to 79% in the 1977 rainy season. The proportion of susceptible lines (more than 26% infection) decreased from 22% to 5% in the three seasons.

The effectiveness of the screening technique is further indicated in Table 1, which compares downy mildew levels in selected lines from several populations growing in the screening nursery and growing in nearby breeding plots with natural inoculum levels. Selection of "resistant" lines from the breeding plots would obviously include many escapes.

The reactions of the standard checks in the 1977 and 1978 AICMIP trials provide a valuable measure of the reliability of the screening system (Table 2). The standard checks were hybrids HB-7, BJ-104, and NHB-3 in 1977; HB-7, BJ-104, and cultivar 7042 in 1978. BJ-104 was consistently more susceptible than HB-7 and less susceptible than NHB-3 and 7042. The HB-7 reactions were similar in both years, but BJ-104 appeared more susceptible in 1978 than in 1977. Because the seed source can vary from one year to another, within-year comparisons are more valuable in evaluating the screening system than between-year comparisons.

DISCUSSION

The technique we have described allows effective, large-scale field screening with flexible size and location of the screening nursery. The effectiveness of the technique in both rainy and postrainy seasons permits rapid advance of breeding material under downy mildew pressure. The major advantages of the technique over the traditional sick-plot technique, in addition to the added flexibility, are 1) a more uniform inoculum distribution over a large area; 2) an extended period of inoculation, which lessens opportunities for escape, because sporangia are produced every night for several weeks and can infect young tillers that are produced continually during pearl millet plant growth; 3) independence from rainfall; 4) much greater efficiency at less cost than techniques that involve spraying or injecting plants manually (1,5); and 5) the ability to do resistance screening on a large scale, unlike a laboratory-based system (2).

The success of the technique depends

on the timing of the various operations and the availability of an irrigation system to promote sporulation and infection. Plans must be made far enough in advance to make the components of the system available at the appropriate time, and the mist irrigation system must be used in the late evenings often enough to promote epidemic development. The practice of marking the position of early-infected plants that may die and be washed away is also vital to the collection of accurate data on disease incidence.

We have used this system two seasons per year since 1976 and now operate it on 6 ha per season to screen 7,000–10,000 entries. Breeding operations carried out in the downy mildew nursery have resulted in the rapid development of resistant hybrids and cultivars (3).

ACKNOWLEDGMENT

We are grateful to P. Malla Reddy for technical assistance.

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