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A Seedling Inoculation Technique for Detecting Downy Mildew Resistance in Pearl Millet

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ABSTRACT

Singh, S. D., and Gopinath, R. 1985. A seedling inoculation technique for detecting downy mildew resistance in pearl millet. Plant Disease 69:582-584.

A seedling inoculation technique that produces consistently heavy downy mildew (*Sclerospora graminicola*) infection (≥85%) on susceptible pearl millet cultivars is described. It involves inoculation of seedlings in pots with an aqueous suspension of sporangia applied by a microsyringe. The greatest downy mildew infection occurred in seedlings inoculated before the first leaf unfolded and sharply decreased when the seedlings were inoculated at a later stage. The technique, which is more effective than field inoculation in producing downy mildew, resembles natural inoculation but allows inoculum uniformity and does not affect normal host activity. It is valuable for checking the reliability of field-identified resistance and for detecting high levels of resistance in key material.

Many sources of resistance to downy mildew (*Sclerospora graminicola* (Sacc.) Schroet.) in pearl millet (*Pennisetum americanum* (L.) Leeke) have been identified (1), employing a field screening technique that uses naturally disseminated sporangial inoculum supplied by earlier planted infector rows (4). The resistance in many entries identified by this method has been stable across locations and over years (1). Some entries, however, that initially gave resistant reactions were subsequently found to be susceptible. One of the reasons for such a phenomenon may be "escapes" under field conditions. To minimize escapes, it is obviously desirable to have a stringent greenhouse/laboratory inoculation technique to check the reliability of field reactions. Such a method could also be used to select for high levels of resistance in specialized materials.

In seeking such a method, we have tested several greenhouse/laboratory inoculation techniques during the past few years (3) and have found only one, described in this paper, that produced consistently high levels of downy mildew infection in repeated tests.

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MATERIALS AND METHODS

Host cultivars. Several pearl millet cultivars with known responses to field inoculation with *S. graminicola* were used: NHB-3 (F_1 hybrid) and 7042 (land race cultivar from Chad), both highly downy mildew-susceptible (>80% infection under heavy inoculum pressure); ICP-220, a highly downy mildew-susceptible male hybrid parent (initially classified as resistant); and WC-C75 (ICRISAT-released, open-pollinated variety), 700516, and 700651 (germ plasm accessions from Nigeria), all highly resistant to downy mildew.

Seedling production and growth stage classification. Seedlings were grown in 12-cm-diameter plastic pots that were three-quarters filled with a potting mixture (Alfisol plus farmyard manure in the ratio of 3:1, v/v). Twenty seeds were planted a uniform distance apart on a well-leveled soil surface and covered with a 2-cm layer of potting mixture, irrigated, and maintained at 25–30 C in the greenhouse.

The seedlings were classified into six growth stages (GS) on the basis of development at the time of inoculation: GS 1 = seedlings < 5 mm high (above soil surface) and still covered with the coleoptile; GS 2 = seedlings > 5 but ≤ 10 mm high with the first leaf still folded; GS 3 = seedlings > 10 mm high with the first leaf still folded; and GS 4, GS 5, and GS 6 = seedlings whose first, second, and third leaves, respectively, were unfolding. Staggered planting made all the growth stages available for simultaneous inoculation.

Inoculum production and inoculation. Methods of inoculum production and collection were described earlier (4). An isolate of *S. graminicola* collected at ICRISAT Center and maintained on 7042 in the greenhouse was used.

To determine the optimum growth stage for inoculation, individual NHB-3 seedlings were inoculated using an Agla micrometer syringe (Wellcome Reagents Limited, Beckenham, England). A 25-μl drop of inoculum was deposited on the tip of each seedling and allowed to flow down the plant surface to the base (Fig. 1). The aboveground surfaces of GS 1 plants were generally completely covered by the inoculum, whereas the surfaces of

GS 2 and GS 3 plants were often not covered entirely. GS 4, GS 5, and GS 6 plants were inoculated by placing the inoculum drop in the leaf whorl. Inoculated seedlings were marked with toothpicks to distinguish them from those that might emerge later. The pots were covered with moistened polythene bags and placed in an incubator at 20 C. After 12 hr, the bags were removed and pots were maintained at 20–25 C on benches in a greenhouse. The pots were randomized among the treatments. The number of pots per treatment varied between 12 and 15 (except ICP-220, which had 84 pots), and the number of seedlings per pot ranged from six to 19. Downy mildew incidence was recorded 7 and 14 days after inoculation.

To compare downy mildew infection developed by drop inoculation with that developed by field inoculation, all cultivars that were inoculated by drop inoculation in a greenhouse as described previously were also planted in the field downy mildew nursery that uses sporangial inoculum supplied by earlier planted infector rows and can be successfully run in both the rainy and the postrainy seasons (4). The entries were planted in two 4-m row plots in two replicates. High levels of humidity, necessary for sporulation, dissemination, and infection processes, are maintained by a perfo-spray mist irrigation system operated every day during the postrainy season and when needed during the rainy season. This helps build up high disease levels that have been consistently high (>80%) on susceptible checks across years and seasons.

RESULTS

Seedling growth stage and percent downy mildew incidence. The highest incidence of downy mildew (85–90%) was observed on NHB-3 plants in GS 1 (Table 1), and incidence declined progressively when seedlings were inoculated after GS 3. The experiment was repeated twice with the first three stages (GS 1–3) with similar results.

Development of downy mildew by field and drop inoculation. The cultivars showed differential responses to the two inoculation methods (Table 2). Three susceptible cultivars (ICP-220, NHB-3, and 7042) showed no significant (P = 0.05) differences between their downy mildew infection levels produced by the two inoculation methods, whereas the three resistant cultivars, while still showing resistance, developed significantly (P = 0.05) more downy mildew by drop inoculation than by field inoculation.

DISCUSSION

For a greenhouse/laboratory screening procedure to be reliable, it is essential that it 1) allows inoculation in a natural fashion, 2) does not disturb the normal host activities (does not underemphasize the host and overemphasize the pathogen), and 3) allows consistent inoculum uniformity (both by volume and spore number) on an individual plant basis. The inoculation method described here combines all these requirements and is therefore superior to the previously described inoculation procedures including whole seedling dip in sporangial suspension, spraying uprooted 2-day-old seedlings and/or root and shoot dips (3), and putting germinating seedlings in contact with the freshly developed sporangia on infected leaves (2). These

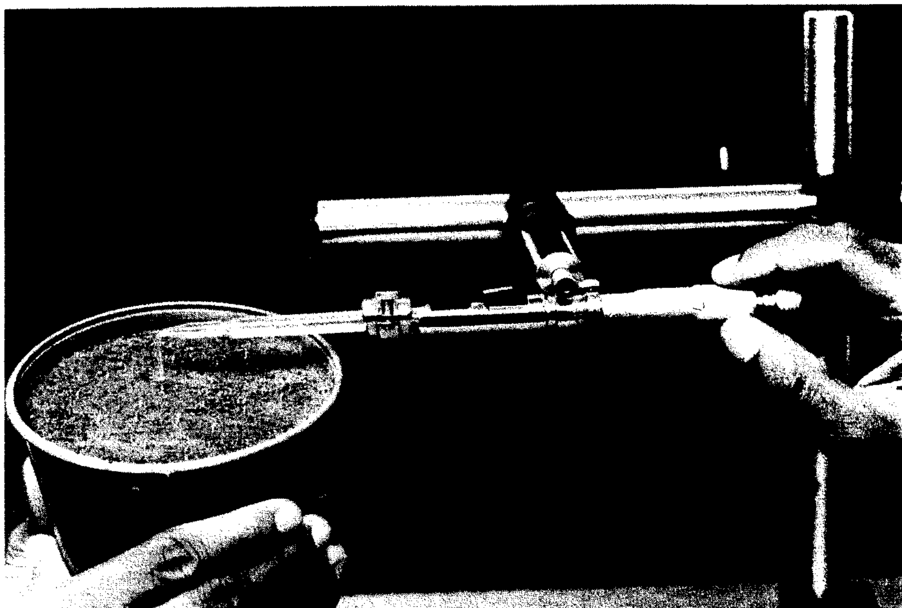

Fig. 1. Inoculation of pearl millet seedlings with sporangia of downy mildew (*Sclerospora graminicola*) by microsyringe.

Table 1. Final downy mildew incidence[y] on susceptible NHB-3 pearl millet seedlings inoculated with sporangia at six stages of growth

Growth stage	Description	No. of plants	Downy mildew (%)
1	≤5 mm long	283	90.0 a[z]
2	>5, ≤10 mm long	251	85.6 a
3	>10 mm long, first leaf still folded	213	84.9 a
4	First leaf unfolded	276	64.1 b
5	Second leaf unfolded	233	33.4 c
6	Third leaf unfolded	76	6.1 d
	Uninoculated check	441	0.0 e

[y] Mean of two tests except in the case of third leaf unfolded, which involved only one test.
[z] Figures followed by the same letter are not significantly (P = 0.05) different based on statistics z (standard normal deviate) used for testing the equality of two binomial proportions ($z = \hat{P}_1 - \hat{P}_2 / \sqrt{\hat{p}\hat{q}(1/n_1 + 1/n_2)}$), where $\hat{P}_1$ and $\hat{P}_2$ are observed proportions in two cases based on samples of sizes of n_1 and n_2, respectively.

Table 2. Reactions of six pearl millet cultivars to downy mildew developed in field downy mildew nursery and by drop inoculation at growth stages 1 and 2

Method of inoculation	Percent downy mildew incidence on cultivars					
	WC-C75	700516	700651	ICP-220	NHB-3	7042
Drop[x]	19.0 a[y] (143)	13.0 a (176)	17.0 a (160)	79.8 a (1,246)	95.0 a (121)	87.5 a (136)
Field[z]	4.0 b (174)	2.0 b (242)	3.0 b (197)	77.0 a (313)	93.0 a (531)	87.0 a (504)

[x] Inoculum concentration was 4×10^5 sporangia per milliliter for WC-C75 and 6×10^5 for the others.
[y] Differences between the two inoculation methods are significant (P = 0.05) if the two figures in a column are not followed by the same letter, based on statistics z (standard normal deviate) used for testing the equality of two binomial proportions ($z = \hat{P}_1 - \hat{P}_2 / \sqrt{\hat{p}\hat{q}(1/n_1 + 1/n_2)}$), where $\hat{P}_1$ and $\hat{P}_2$ are observed proportions in two cases based on samples of sizes of n_1 and n_2, respectively. Figures in parentheses are total number of plants evaluated.
[z] Data based on mean of three tests (1981–1983 rainy seasons) for WC-C75, 700516, and 700651; for the others, data pertain to 1982–1983 postrainy and 1983 rainy seasons.

techniques either disturb normal host activity or do not ensure inoculum uniformity, although they may provide the most congenial conditions for the development of the pathogen. Such techniques may give variable results or, by an artifact of enforced inoculation, make an otherwise field-resistant genotype appear highly susceptible.

The results of the technique described show that, given the availability of viable sporangial inoculum, the most important factor governing the expression of susceptibility is the seedling age at inoculation. Inoculation of seedlings just at emergence will allow the expression of the maximum level of susceptibility, which can rapidly be lost by age. This highlights the necessity of challenging the emerging seedlings with a heavy sporangial inoculum coupled with high humidity if the field-located downy mildew nursery is to be consistently effective. To achieve this, the test material must be planted after heavy sporulation has been observed in the infector rows.

The failure of inoculum supply from infector rows at the time of emergence, caused by either insufficient humidity or high temperature, will allow escapes in test material that are only detected later. Alternatively, when inoculum pressure is low, it is likely that some single plant selections made within families being tested may include susceptible plants, and thus resistance will not be recovered in the progeny. Either of these possibilities could explain the apparent loss of resistance in material under selection such as ICP-220. The seedling inoculation technique described here will be of use in detecting such escapes as well as increasing the levels of resistance in population where resistance genes are present in low frequency.

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An In Vitro Test for Evaluating Efficacy of Mycoparasites on Sclerotia of *Sclerotinia sclerotiorum*

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ABSTRACT

Mueller, J. D., Cline, M. N., Sinclair, J. B., and Jacobsen, B. J. 1985. An in vitro test for evaluating efficacy of mycoparasites on sclerotia of *Sclerotinia sclerotiorum*. Plant Disease 69:584-587.

A modification of Kohn's technique for apothecial induction in the Sclerotiniaceae was developed to test the efficacy of mycoparasites on sclerotia of *Sclerotinia sclerotiorum*. The modification is an incubation period at 25 C prior to Kohn's initial cold treatment. The new method was compared with the previously used method of pairing the fungi on an agar medium (such as potato-dextrose agar) and determining the effects on growth of *S. sclerotiorum* mycelium. This method tested the effects of *Gliocladium roseum*, *G. virens*, *Trichoderma harzianum*, and *T. viride* on carpogenic germination of *S. sclerotiorum*. Numbers of carpogenically germinating sclerotia and apothecia produced per sclerotium were used to measure the effects of the mycoparasites. Mycoparasites were also evaluated on the basis of their percentage of reisolation from sclerotia. A good separation of the fungi was obtained using this technique. *G. virens* was the mycoparasite most frequently recovered from *S. sclerotiorum* sclerotia and significantly reduced all measurements of carpogenic germination.

Sclerotinia sclerotiorum (Lib.) de Bary is a ubiquitous plant pathogen with a host range of more than 360 species distributed in 64 families (9). Crop rotation, sanitation, reduced irrigation, resistant varieties, and the use of protectant chemicals often cannot economically and consistently control diseases caused by *S. sclerotiorum* (13,14).

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Since 1979, more than 30 species of fungi and bacteria as well as insects and other organisms have been reported to be antagonists of *Sclerotinia* spp. (13). *Coniothyrium minitans* Campbell (4), *Gliocladium roseum* (Link.: Fr.) Bain. (8), *G. virens* Miller & Foster (15), and *Trichoderma viride* Pers.: Fr. (3,5,8) parasitize sclerotia of *S. sclerotiorum* in soil. *G. virens* prevented the formation of *S. sclerotiorum* sclerotia and parasitized preformed sclerotia in an in vitro study (15). *C. minitans* and *T. viride* destroyed *S. sclerotiorum* sclerotia after 100 days of incubation in soil in vitro at an ambient air temperature of 20–22 C (4). Neither of these studies reported the frequency of myceliogenic or carpogenic germination of surviving sclerotia (7).

In this study, we examined the effect of the length of incubation period with a mycoparasite prior to a cold treatment on the ability of *S. sclerotiorum* sclerotia to germinate carpogenically. The technique used was adapted from Kohn (6) and differs from other in vitro methods in that the mycoparasite contacts mature sclerotia instead of mycelia.

MATERIALS AND METHODS

We evaluated Kohn's (6) technique using *G. roseum*, *G. virens*, and *T. viride*, all reported as antagonists of *S. sclerotiorum*, and *T. harzianum* Rifai, an antagonist of other sclerotium-forming fungi (8). The isolate of *S. sclerotiorum* used was obtained from naturally infected soybean (2). The designations for the mycoparasites (and their sources) are as follows: *G. roseum*-12 (Illinois soybean), *G. roseum*-40 (West Virginia University culture collection), *G. virens* (J. C. Tu, Agriculture Canada, Harrow, Ontario), *T. harzianum* (Kalo Laboratories, Kansas City, MO), and *T. viride* (West Virginia University culture collection). Stock cultures of *S. sclerotiorum* and the mycoparasites were maintained on potato-dextrose agar (PDA).

A spore suspension ($1-4 \times 10^6$ conidia per milliliter of each mycoparasite) was prepared in sterile water from 7- to 10-day-old PDA cultures incubated in