ISSN 0014-2336

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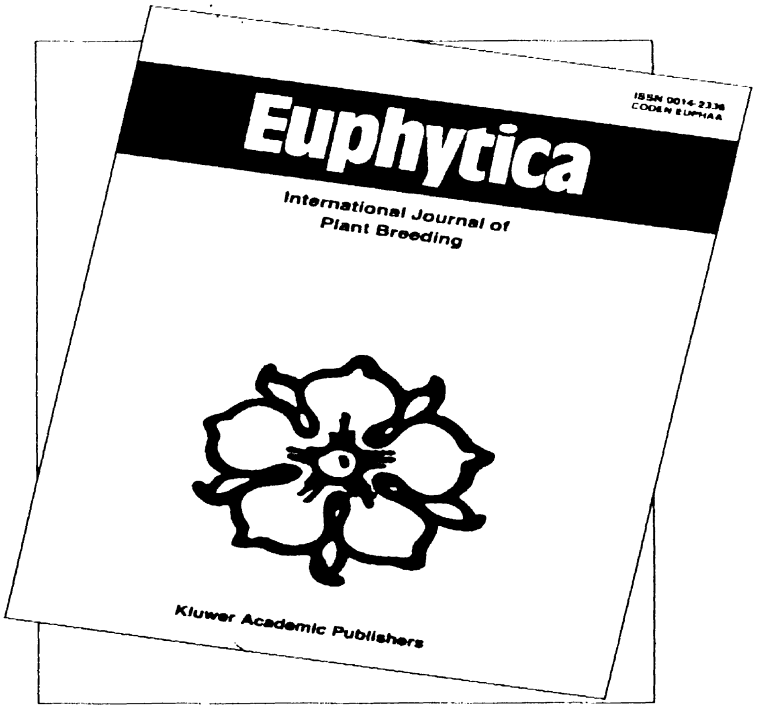

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Euphytica **86**: 49–53, 1995.

JA 1667

Evaluation of greenhouse inoculation techniques to screen sorghum for resistance to downy mildew *

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Received 20 June 1994; accepted 21 May 1995

Key words: Sorghum bicolor, Peronosclerospora sorghi, downy mildew, inoculation technique, resistance, sorghum

Summary

Six inoculation techniques were compared for the artificial promotion of downy mildew (*Peronosclerospora sorghi*) in sorghum. These were (1) sprouted seeds incubated between sporulating infected leaves, (2) sprouted seeds dipped in conidial suspension, (3) sprouted seeds sprayed with conidial suspension, (4) seedlings at plumule stage inoculated with drops of a conidial suspension, (5) seedlings at plumule stage sprayed with a conidial suspension, and (6) seedling showered with conidia falling from infected leaves. Seedlings at the one-leaf stage sprayed with a conidial suspension (6×10^5 ml^{-1}) showed the highest systemic infection (100%) in the susceptible lines IS 643 and IS 18433. This technique is effective, repeatable, and allows the deposition of a conidial suspension as a fine mist on the entire seedling surface. In the greenhouse, the technique was used to test the downy mildew reaction of genotypes previously reported as resistant (< 5% incidence) in 3–4 years of field screenings. Of the 61 genotypes tested, 21 were free from downy mildew, 14 had less than 5% incidence, and the rest showed variable susceptible reactions. Therefore, the technique can be reliably and effectively used in the greenhouse to detect disease escapes and to indentify resistance.

Introduction

Downy mildew, caused by *Peronosclerospora sorghi* (Weston & Uppal) Shaw, is a destructive disease of sorghum (*Sorghum bicolor* (L.) Moench) and maize (*Zea mays* L.) in cool, humid areas of the world (Frederiksen & Renfro, 1977; Frederiksen, 1980). The pathogen infects the roots primarily by oospores and the leaves by conidia, and reaches the meristem causing systemic infection. Conidia are produced as a white downy growth on the abaxial surface of infected leaves. Airborne conidia are the major infective propagules for secondary infection and to a limited extent for primary infection. Soilborne oospores are responsible for most primary infections of seedlings in the field.

There are several options for downy mildew management such as host plant resistance, chemical control, and cultural methods (Williams, 1984). Screening for resistance to the disease has been carried out in the field and in the greenhouse. Anahosur & Hegde (1979) compared several inoculation techniques in the field and found the infector row method most effective. Similarly, different inoculation techniques have been used in the greenhouse to test the resistance of sorghum genotypes to downy mildew by different workers (Craig, 1976; Jones, 1970; Schmitt & Freytag, 1974; Williams et al., 1982). These techniques differed with respect to age of plants at inoculation, plant parts inoculated, method of inoculum placement, and post-inoculation incubation conditions. The relative efficacy of these techniques is not known. A greenhouse inoculation technique that can induce 100% infection in susceptible controls would be useful to check the reliability of field reactions by detecting disease escapes, and in studies dealing with the genetics of resistance and pathogen variability.

* **Approved as Journal Article No. 1667** by the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Andhar Pradesh, 502 324, India

The purpose of this study was to compare six inouclation techniques in the greenhouse to select a technique that is simple, reliable, and repeatable, and that ensured infection in all inherently susceptible plants. The selected technique was used to screen 'field-resistant' genotypes for downy mildew resistance in the greenhouse.

Materials and methods

Inoculum

The source of initial inoculum was collected from downy mildew infected plants at Dharwar, Karnataka State, India. The inoculum was multiplied and maintained on downy mildew susceptible sorghum cultivar DMS 652 at Patancheru where the greenhouse experiments were conducted. Conidia were obtained from 3-week-old systemically infected sorghum plants maintained in 25.4 cm-diameter pots in the greenhouse at 25 ± 4° C. The plants were exposed to bright sunlight during day to allow photosynthate accumulation necessary for abundant sporulation (Schmitt & Freytag, 1974). Chlorotic leaves were excised, wiped with wet absorbent cotton to remove old downy mildew conidia produced previously, and wiped again using tissue paper to remove moisture from the leaf surface. The leaves were then cut into 4-5 cm lengths, and placed with their abaxial surface facing up in 9 cm-diameter Petri plates lined with moist blotting paper on both sides. The plates were incubated at 20° C in the dark for 6–7 hr for sporulation. Conidia were harvested by washing the sporulated leaves in chilled (5° C) distilled water using a camel hair brush. The suspension was filtered through a double layered of muslin cloth to remove conidiophores and other particles. The concentration of conidia was adjusted to 6×10^5 ml^{-1} using a hemacytometer. The wetting agent, Tween 20 (1 drop l^{-1}) was added to the conidial suspension before inoculation.

Inoculation techniques

Sprouted seeds and emerged seedlings of the downy mildew susceptible sorghum cultivars IS 643 and IS 18433 (DMS 652) were inoculated at a time. Sprouted seeds were inoculated prior to sowing and seedlings were inoculated after emergence using various techniques.

Inoculation of sprouted seeds

Seeds were soaked in water for 3 hr, and placed in moist chamber for 24 hr at 28° C to sprout. Three methods were used to inoculate sprouted seeds: dip inoculation, spray inoculation and sandwich inoculation. For the dip inoculation methods, the sprouted seeds were immersed in the conidial suspension for 5 min and the suspension was drained off. For spray inoculation method, a single layer of sprouted seeds was spread in Petri plates lined with moist filter paper, and the seeds were sprayed with a conidial suspension using an atomiser. The sandwich inoculation method described by Safeeulla (1976) was followed. In Petri plates lined with wet filter paper, sprouted seeds were placed in between two layers of infected leaf pieces in such a way that the abaxial leaf surface of both layers faced the sprouted seeds. Sprouted seeds inoculated by these three methods were incubated for 16 hr at 20° C in the dark, then sown in 10 cm-diameter pots containing sterilized soil (Vertisol), and maintained in the greenhouse at 25 ± 4° C with 70–90% relative humidity for disease expression.

Seedlings inoculation

Seeds were sown in 10 cm-diameter pots containing sterilized soil (Vertisol), in a greenhouse at 25 ± 4° C. At the plumule emergence stage when the first leaf was in the whorl, pots were transferred to an inoculation chamber maintained at 20 ± 1° C with high relative humidity (100%) 1 hour prior to inoculation. Three methods of inoculation were used: drop inoculation, conidial spray, and conidial showering. Seedlings were drop-inoculated by placing a drop of inoculum in the whorl using a syringe (Singh & Gopinath, 1985). Spray inoculation was done with an atomiser until the entire surface of the seedlings were covered with fine droplets of inoculum. For conidial showering, the outer rim of each pot with seedlings was covered with a layer of moist muslin cloth on which a layer of detached downy mildew infected leaves was placed with abaxial surface facing the seedlings. On top of the infected leaves were placed 2–3 layers of wet blotting paper. All the pots were kept in a tray containing about 1.5 cm water and covered with another tray lined with moist blotting paper. The pots were incubated overnight at 20° C to allow the infected leaves to sporulate and the conidia to drop onto the emerged seedlings. The following morning, the muslin cloth, blotting papers, and infected leaves were removed. All the pots were trans-

Table 1. Evaluation of downy mildew inoculation techniques on two sorghum genotypes in the greenhouse at ICRISAT Center

Growth stage	Inoculation method	No. of seedlings inoculated		Downy mildew incidence (%)[a]		
		IS 643	IS 18433	IS 643	IS 18433	Mean
Sprouted seeds	Sandwich	71	73	94	96	95.0
	Dip	75	48	99	100	99.7
	Spray	48	48	89	91	90.1
Emerged seedlings	Drop	48	48	81	82	81.6
	Spray	48	47	100	100	100.0
	Showering	48	41	97	98	97.8
	Control	65	62	0	0	0
	SE(±)			2.4	2.1	3.2
	CV(%)			4	4	4

[a] Mean of three replications of one test.

ferred to a greenhouse and maintained at 25 ± 4° C and 70–90% relative humidity for the establishment of seedlings and disease development. Seedlings in which water drops and water spray were used instead of the downy mildew inoculum were maintained as controls.

The six inoculation treatments and the two controls were arranged in a randomized block design with three replications, each with three pots containing about 41–75 seedlings. The number of systemically infected plants and total plants were recorded up to 21 days after inoculation and percent downy mildew incidence was calculated. Percent data were used to perform analysis of variance. The experiment was conducted thrice.

Evaluation of sorghum cultivars for resistance to downy mildew

Sixty-one genotypes, previously reported as resistant, (< 5% downy mildew incidence) for 3–4 years in field screening at Dharwar using infector row method (Anahosur & Hegde, 1979) were selected to test their reactions in the greenhouse. Seeds were surface sterilized in a solution of 0.1% mercuric chloride for 5 min, thoroughly washed in distilled water, and planted in 10.5 cm-diameter pots containing sterilized soil (Vertisol) mixed with sand and compost (2 : 1 : 1). Two pots, each with 50 seedlings, were maintained for each genotype. When the first leaf emerged in the whorl, the seedlings were spray-inoculated with conidial suspension (6×10^5 ml^{-1}), and incubated for 18 hr at 20° C and high relative humidity (90–100%) in the dark. Inoculated seedlings were then moved to a greenhouse maintained at 25 ± 4° C and 70–90% relative humidity for 3 weeks for disease development. The sorghum genotype DMS 652 was maintained as susceptible control. The experiment was conducted in a randomized block design with two replications, each containing one pot. The greenhouse test was conducted twice. The numbers of total and diseased plants were recorded, and the percent disease incidence was calculated. Data from the two greenhouse tests were compared by analysis of variance to determine the repeatability of the technique.

Results

Inoculation techniques

Downy mildew incidence varied from 82 to 100% in the six inoculation methods (Table 1). Maximum downy mildew incidence (100%) occurred when seedlings at the first leaf stage were spray-inoculated, followed by dip inoculation of sprouted seeds (99.7%), conidial showering (97.8%), sandwich method (95%), spray-inoculation of sprouted seeds (90%) and drop inoculation (82%). Downy mildew incidences in the latter two treatments were significantly less than in the seedling spray method. Both genotypes had similar levels of downy mildew in each inoculation treatment. There was no difference in the latent period among the six inoculation methods since the symptoms appeared

Table 2. Number of sorghum genotypes in different class intervals for percent downy mildew incidence in greenhouse and field inoculation tests

Greenhouse[a]	Field[b]			Total
	0	0.1–3.0	3.1–5.0	
0	21	0	0	21
0.1– 3.0	12	0	0	12
3.1–10	8	4	1	13
10.1–20	4	1	0	5
20.1–30	1	1	0	2
30.1–40	0	0	2	2
40.1–50	0	1	2	3
50.1–60	0	1	2	3
Total	46	8	7	61

[a] Seedlings (first leaf stage) sprayed with conidial suspension (6×10^5 ml^{-1}).
[b] Field screening using infector row method. Based on maximum downy mildew incidence data among the 3–4 years of field screening tests.

within a week after inoculation in all the methods tested.

Evaluation of sorghum cultivars for resistance to downy mildew

Mean downy mildew incidence in the genotypes ranged from 0–54% in 61 genotypes sprayed with conidial suspension at the seedling stage. Twenty-one genotypes were free from downy mildew symptoms, 12 had 0.1 to 3% systemic disease, 13 genotypes had 3.1 to 10%, and the remaining genotypes had more than 10% downy mildew incidence in both greenhouse tests (Table 2). Although, the correlation coefficient between the greenhouse test and the field test was significant ($r = 0.68$, 60 df, $P < 0.01$), the disease incidence in greenhouse tests were higher than field tests in 40 genotypes. All the genotypes free from downy mildew in the greenhouse test were also disease-free in the field; but, the reverse was not true. Downy mildew incidence in the two greenhouse tests were statistically similar because the analysis of variance showed that the mean sum of squares (MS) for experiments was non significant (MS 5.70, 1 df). The correlation coefficient between the two green house tests was 0.98 (60 df, $P < 0.01$). This suggests that the seedling inoculation tests are repeatable.

Discussion

Among the six inoculation methods evaluated in the greenhouse, conidial spray inoculation of seedlings at plumule stage appeared most suitable for large-scale testing of sorghum genotypes for downy mildew resistance. This inoculation technique is simple repeatable, and effectively differentiated resistant genotypes among putatively resistant genotypes identified in the field. Of the 61 genotypes, 21 were free from downy mildew in the greenhouse and field tests. These were IS number 1331, 2473, 3546, 3547, 5743, 7144, 7179, 7528, 8185, 8276, 8283, 8607, 8864, 8906, 8954, 10710, 18757, 22227, 22228, 22229, and 22230.

The three methods of seedling inoculation closely correspond to the epidemiological processes occurring in nature, wherein wind dispersed conidia deposit on young seedlings and cause infection in near-saturated environments (Williams, 1984). The spray inoculation method was more advantageous because it was simple, rapid, and allowed uniform deposition of conidia on the entire seedling surface including the vicinity of growing points where infection normally occurs (Jones, 1970). Schmitt & Freytag (1974) also reported that conidial spray inoculation at seedling stage was most efficient in inducing severe downy mildew infection in corn and sorghum. In the conidial showering method, sporulating leaves placed above the plants acted as the source of conidia, that fell on the plants by gravitational force. However, this method was laborious, and required a large number of infected leaves, carefully selected to ensure uniform sporulation during incubation. Inspite of utmost precaution, some seedlings may not receive inoculum if the leaves immediately above them do not sporulate well. Craig (1976) avoided the latter disadvantage by devising an elaborate inoculation chamber to permit uniform air flow for consistent conidial deposition. Drop inoculation was also laborious and fraught with chances of inoculum loss by dripping of inoculum droplets from the erect surfaces of young seedlings that lacked unfolded leaves at the time of inoculation. The methods used to inoculate sprouted seeds do not represent what happens under natural conditions because sprouted seeds are not normally exposed to conidial inoculum.

Conidial spray inoculation of young seedlings of 61 putatively resistant genotypes resulted in varying levels of susceptibility (0–54%) in the greenhouse, whereas the same genotypes under field screening using the infector row method had shown < 5% susceptibility over a period of 3–4 years. Screening for resistance

to downy mildew in the field is essential to evaluate large numbers of genotypes, and to evaluate agronomic traits. It is often carried out using an infector row technique wherein conidia produced on the infected plants are dispersed by wind to infect test plants at susceptible stage. Production of inoculum is often determined by temperature and humidity which may not be always favorable for sporulation and infection, leading to disease escapes. The seedling spray inoculation method in the greenhouse achieved 100% disease in susceptible control and thus assured accuracy in detecting disease escapes in putatively resistant genotypes identified in the field. Alternatively, genotypes can be initially screened in the greenhouse, and selected plants can then be tested in the field for the adaptability of the resistance trait (Reddy et al., 1992). This technique would be more effective and economic in screening large numbers of breeding material in a resistance breeding programs.

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