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Downy mildew inoculum in maize seeds: Techniques to detect seed-borne inoculum of *Peronosclerospora sorghi* in maize

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Summary

Peronosclerospora sorghi, the causal organism of maize downy mildew, occurs either in the form of vegetative mycelium or in the form of oospores in the pericarp, endosperm and embryo of maize seeds. Mycelium and oospores were detected after macerating the seeds in 5% NaOH with 0.015% trypan blue for 24 hours at normal room temperature and separating the seed components by agitating the seeds in a water stream and by hand-picking. Quick maceration of the material was achieved by heating the seeds in 2.5% NaOH at 80 °C for four hours. Of the 17 seed samples tested, mycelium was recorded in all samples while oospores were detected only in eight. The percentage of seeds with mycelium infection in the pericarp and the embryo ranged from 16 to 100 and from one to 15 respectively. The percentage of seeds with oospores in the pericarp and in the embryo varied from one to 35 and one to 12 respectively.

Résumé

Techniques pour détecter les semences porteuses d'inoculum de Peronosclerospora sorghi *chez le maïs*

Peronosclerospora sorghi, organisme responsable du mildiou du maïs, se rencontre soit sous forme de mycelium végétatif, soit sous forme d'oospores dans le péricarpe, l'endosperme et l'embryon des semences de maïs. Mycelium et oospores furent détectés après macération des semences dans 5% de NaOH avec 0,015% de bleu trypan pendant 24 heures à température normale de la pièce et séparation des composants des semences en agitant celles-ci dans un courant d'eau et en les recueillant manuellement. Une macération rapide du matériel fut exécutée en chauffant les semences dans 2.5% de NaOH à 80 °C pendant quatre heures. Sur les 17 échantillons de semences analysés, du mycelium fut relevé sur tous les échantillons, tandis que des oospores ne furent détectées que dans 8 échantillons. Le pourcentage de semences infectées par du mycelium dans le péricarpe et l'embryon variait de 16 à 100 et de 1 à 15 respectivement. Le pourcentage de semences avec des oospores dans le péricarpe et l'embryon variait de 1 à 35 et de 1 à 12 respectivement.

Zusammenfassung

Verfahren zum Nachweis samenbürtigen infektiösen Materials von Peronosclerospora sorghi *bei Mais*

Peronosclerospora sorghi, der Erreger des falschen Mehltaus von Mais, tritt entweder in der Form vegetativen Mycels oder in der Form von Oosporen im Perikarp, Endosperm und Embryo von Maiskörnern auf.

Mycelium und Oosporen wurden nachgewiesen, nachdem die Körner 24 Stunden lang bei normaler Zimmertemperatur in 5% NaOH mit 0,015% Trypanblau mazeriert und die Samenteile durch Rühren der Samen in fließenden Wasser und durch Handauslese getrennt worden waren. Eine rasche Mazeration des Materials wurde durch ein Erhitzen der Samen über vier Stunden bei 80 °C in 25% NaOH erreicht. Bei den 17 untersuchten Saatgutproben wurde Mycel in allen, Oosporen dagegen nur in 8 Proben festgestellt. Der Prozentsatz der Körner mit Mycelinfektionen im Perikarp und im Embryo lag zwischen 16 und 100 bzw. 1 und 15. Der Prozentsatz der Körner mit Oosporen im Perikarp und im Embryo schwankte zwischen 1 und 35 bzw. zwischen 1 und 12.

Introduction

Sorghum downy mildew of maize (*Zea mays* L.) and sorghum (*Sorghum bicolor* (L) Moench) incited by *Peronosclerospora sorghi* (Weston & Uppal) C. G. Shaw, is an important disease in tropical and sub-tropical areas. Systemically infected sorghum plants can produce oospores within the glumes (Safeeulla, 1976). Oospores have also been detected in seed pericarps and mycelial fragments have been observed in the endosperm of seeds collected from systemically infected plants (Safeeulla, 1976). In maize seed-borne inoculum in the form of mycelium in the pericarp, endosperm and embryonic tissues has been detected (Jones, Leeper and Frederiksen, 1972; Safeeulla and Shetty, 1977) and recently oospores have also been detected in the pericarp, endosperm and embryo (Muralidhara Rao, 1982). Detection of oospores and mycelium in maize seed tissues indicates seed infection and suggests the need for greater care in the movement of seed material. The role of seed-borne inoculum in downy mildew disease transmission has been discussed by Safeeulla and Shetty (1977) and Pupipat (1976).

At present there is no well described routine procedure for detecting seed-borne inoculum of *P. sorghi* in maize seed that can be practised in seed testing stations and in plant quarantine laboratories. In this paper a simple, convenient, quick and reliable standardised procedure is described.

Materials and methods

Collection and storage of seed samples

In December 1981 seed samples were collected from different maize growing areas of South India, where sorghum downy mildew had been reported. Seeds thus collected were stored in paper packets at normal room temperature (28 °C).

Washing test

A washing test was used to determine whether the oospores were surface-borne in the seed samples. One hundred seeds from each sample were immersed in 25 ml of distilled water with a drop of Tween 20 and shaken for 10 minutes. The water was centrifuged at 2500 rpm for 10 minutes. The supernatant solution was discarded and the sediment was resuspended in 2 ml of distilled water. Eight drops of the suspension from each sample were examined under the compound microscope.

Procedure for detecting oospores and mycelium in seed tissues

Method I

Seed components were separated by modifying the procedures used for extracting pearl millet embryos, to detect downy mildew mycelium (Shetty, Khanzada, Mathur and Neergaard, 1978) and wheat embryos, to detect loose smut mycelium (Khanzada, Rennie, Mathur and Neergaard, 1980).

1. *Soaking and staining*: 400 seeds from each sample were soaked in a litre of 5% NaOH with 0.015% trypan blue for 24 hours at room temperature (28 °C).

2. *Extraction*: Soaked seeds were agitated in running tap water. Seed components were separated using three sieves of 5 mm, 4 mm and 3 mm pore size in descending order. The embryos were collected on the lowest sieve which had 3 mm pores. Great care was taken to avoid breaking the components. In another test the seed components viz, embryo, pericarp and disrupted endosperm were separated by hand instead of using the sieves. The endosperm portion of the seed dissolved in the alkali solution and could not be collected as a separate component.

3. *Dehydration*: The embryos and pericarps were placed in a 9 cm diameter strainer and dehydrated separately in rectified spirit for two minutes.

4. *Separation*: The dehydrated embryos were transferred to a funnel which had its stem connected to a rubber tube with a pinch cock. Approximately 200 ml of lactic acid + glycerol + water mixture (1:2:1, v/v/v) was added. The embryos floated and the chaff sank and was run off using the pinch cock. The chaff was washed in the mixture five or six times until a clear suspension of the embryos was obtained. The pericarps were separated by hand using a brush and forceps.

5. *Clearing*: Embryos and pericarps were separately transferred to a 250 ml beaker each with lactic acid + glycerol mixture (1:2, v/v). The beakers were heated until the liquid boiled and it was then allowed to cool before examination.

6. *Examination*: The lactic acid + glycerol mixture together with the embryos was poured in a grooved plate fitted into the upper lid of a 9 cm petri dish. The embryos were submerged in the lactic acid + glycerol mixture and examined at 16x and 40x magnification using a stereobinocular microscope. The pericarps were examined using the same procedure.

7. *Recording*: Infected embryos and pericarps with stained mycelium and oospores of *P. sorghi* were counted and the percentage infection was calculated for each sample.

Method II

In this method, the seed sample with the maximum oospore and mycelial infection was used. Four different concentrations of NaOH viz, 2.5%, 5%, 7.5% and 10% were prepared. One litre of each concentration of NaOH was placed in a 2 litre flask and 0.015% trypan blue was added and stirred with a glass rod to ensure complete dissolution of the stain. Four hundred seeds were randomly selected from the sample and soaked in each concentration. The flasks were kept in an oven maintained at 80 °C. The time taken for complete maceration was noted for each concentration. Macerated seeds were removed from the solution and agitated in running tap water. The compo-

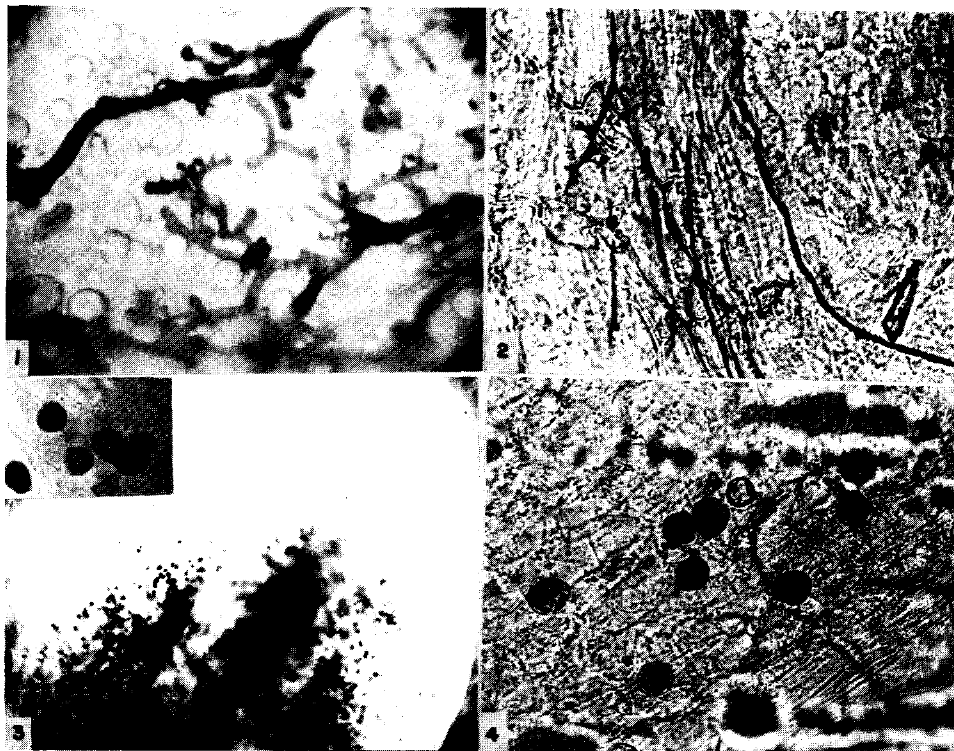

Figure 1. *Peronosclerospora sorghi* mycelium in maize embryo 450 ×.
Figure 2. *P. sorghi* mycelium in the pericarp of maize 150 ×.
Figure 3. *P. sorghi* oospores in the embryo 40 × (Inset: enlarged portion of the embryo showing the oospores 150 ×).
Figure 4. Oospores of *P. sorghi* in the pericarp 150 ×.

nents were dehydrated, separated, cleared and examined as described in method I. The percentage of embryos and pericarps with oospores and mycelium of *P. sorghi* was calculated.

Results

Before processing, the seed samples were placed on white paper and examined to detect the occurrence of shrivelled seeds, but none was seen. This observation indicated that seed infection of maize by *P. sorghi,* may not cause shrivelled seed. No oospores were detected in the seed washings of any of the samples.

Mycelium of *P. sorghi* was observed in the embryo (figure 1), endosperm and pericarp (figure 2) of all seed samples (table 1). Oospores were observed in the embryo (figure 3), endosperm and pericarp (figure 4). Of the 17 seed samples tested, oospores

Table 1. Percentage incidence of *Peronosclerospora sorghi* inoculum in maize seed samples.*

Sample number	Percentage** seeds infected			
	Oospores		Mycelium	
	pericarp	embryo	pericarp	embryo
1	0	0	100	0
2	0	0	98	0
3	0	0	96	0
4	0	0	100	0
5	0	0	16	1
6	0	0	18	4
7	1	0	32	4
8	0	3	100	3
9	2	4	100	4
10	0	1	100	1
11	0	0	48	0
12	0	0	100	0
13	0	0	92	0
14	12	0	75	12
15	14	2	78	13
16	12	2	75	15
17	35	12	70	13

* Seeds were macerated in 5% NaOH at room temperature for 24 hours.
** Data are based on 400 seeds analysed per sample.

Table 2. Efficiency of different concentrations of NaOH in determining the seed-borne inoculum of *Peronosclerospora sorghi* in maize seeds.

Concentration of NaOH	Time required for complete maceration	Percentage infection	
		Oospores*	Mycelium**
2.5%	4 hours	35.50^{A}	70.4^{C}
5.0%	3 hours	35.75^{A}	70.0^{C}
7.5%	2 hours	25.00^{B}	70.2^{D}
10.0%	$1\frac{1}{2}$ hours	24.25^{B}	68.2^{D}

A-B and C-D; Newman-Keuls multiple range test (Zar, 1974); Values followed by the same letters are not significantly different (P = 0.05).
The data were processed after 'Arcsin' transformation.
* Percentage oospore infection in method 1 = 35.00
** Percentage mycelial infection in method 1 = 70.00

were detected in eight (table 1). The percentage of oospore infection was highest in samples 14 to 17. Some of the seeds showed mycelium and oospore infection.

Oospores were stained blue and had three thick wall layers. The diameter of the oospores ranged from 32.5 to 40 μm. Normally the mycelium was long, branched, robust, coenocytic and often broken. In some seeds mycelial fragments looked plasmodial. In the first method, the time required for complete maceration of the seed was 24 hours. The oospores and mycelium were stained satisfactorily and detection was straightforward. In the second method, less time was required for maceration and it varied with the NaOH concentration used. Maceration was complete within one and half hours when 10% NaOH was used. In the case of 2.5%, 5% and 7.5% NaOH the time taken was four hours, three hours and two hours respectively. However, as the concentration of NaOH increased, staining became fainter. From the multiple range test (table 2), it is clear that the treatment effects of 2.5% and 5% NaOH do not differ significantly.

Discussion

The presence of mycelium of *P. sorghi* in maize seeds has been shown using microtomy of mature seeds (Jones *et al.*, 1972; Safeeulla and Shetty, 1977). Muralidhara Rao (1982) reported oospores of *P. sorghi* in maize seed components. A detailed procedure to detect seed-borne inoculum in a seed lot is needed.

Khanzada *et al.* (1980) used the embryo extraction procedure to assess loose smut infection in wheat seed lots. Shetty *et al.* (1978) modified the embryo extraction procedure to detect downy mildew mycelium in pearl millet embryos.

In the present investigation a successful attempt has been made to evolve and standardise a routine and efficient technique for the detection of downy mildew (*P. sorghi*) inoculum in maize seeds. The mycelium was net-like and in some cases mycelial fragments were distributed in all parts of the seed in addition to the occurrence of oospores. Oospores were stained blue and were easily detected. Oospores of *P. sorghi* were not detected after washing seeds as they are not surface-borne. In contrast, in sorghum and pearl millet the oospores are formed on the seed surface or embedded in the glumes and hence are easily detected in washing tests (Safeeulla, 1976). In soybean seed samples downy mildew infected seeds can be detected by visual examination or using a washing method since the oospore contamination forms a white crust on the seed surface (Pathak, Mathur and Neergaard, 1978).

Of the two maceration procedures described here, the first is time consuming but the staining is satisfactory. The rapid technique (method II) achieves a quick maceration. The two lower concentrations viz., 2.5% and 5% NaOH were quite satisfactory as far as maceration and staining were concerned but as the concentration of NaOH was increased to 7.5% and 10% staining became fainter though maceration was quicker.

Maize is known to be attacked by *Sclerophthora macrospora* (Sacc.) Thirum., Shaw & Naras., *S. rayssiae* var. *zeae* Payak and Renfro; *Sclerospora graminicola* (Sacc.)

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