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A MODIFIED METHOD FOR DETECTING OOSPORES OF *PERONOSCLEROSPORA SORGHI*, THE CAUSAL ORGANISM OF SORGHUM DOWNY MILDEW IN SUDAN GRASS SEEDS (*SORGHUM SUDANENSE*)

SHAARAWY M.A., A.M. ABDEL-MONEM AND A.A. EL-WAKIL

Plant Pathology Res. Inst., Agric. Res. Center, Giza, Egypt

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Abstract

The washing technique was developed for detecting oospores of the sorghum downy mildew (*Peronosclerospora sorghi*) on seed surface or in fine plant debris (seed transmitted infection) in sudan grass (*Sorghum sudanense).* Modification was based on grinding the seed bulk for 2 min before washing followed by filteration,centrifugation and adding drops of iodine tincture to the washing sediments. Grinding increased the number of oospores in washing sediment from the different seed parts , *i.e.,* glumes , pericarp or perianth as well as from plant debris. On the other hand , iodine tincture stained the starch particles, prolonged the time provided for microscopic examination and made the examination easier than in the common technique. Although mycelium was observed also in some different seed parts, especially the endosperm tissues, the internal infection (endosperm and scutellum) could not be confirmed yet.

Systemic infection of plants produced from infested seeds was proved in the greenhouse using sterilized soil (pathogen-free). Meanwhile, no systemic infection was observed in plants resulting from non-infested seeds. Moreover, soaking sudan grass seeds in a suspension of the fungicide Apron (metalaxyl) at concentration of 750 ppm for 2 hours before sowing ,increased the emergence and reduced seed transmission of *Peronosclerospora sorghi.*

INTRODUCTION

Sorghum downy mildew (*Peronosclerospora sorghi*) Weston&Uppal C.G.Shaw is one of the most serious diseases of corn and sorghum in the temperate zone (Frederiksen and Renfro, 1977). The economic importance of the disease was studied by Frederiksen *et al.* (1969) in Texas. Sorghum downy mildew was first recorded in 1928 on sorghum and maize (corn) in the experimental station at Giza, Ministry of Agriculture , Egypt. The disease was thought to be introduced by the packing material from India (Melcher, 1931).

Reactions of some varieties of sudan grass (*Sorghum sudanense*) to the disease showed that they were all highly susceptible (El-Shaarawi *et al.*, 1990). Symptoms of the disease may occur either as systemic or local infections.Systemic form of the disease was often caused by seedling infection with oospore in the soil or by conidia soon after seedling emergence (Frederiksen *et al.*, 1973).

Seed transmission of sorghum downy mildew was studied by some investigators (Bain & Alfred, 1969; Jones *et al.*, 1972; Singh & Milliano , 1989 and Yao *et al.*, 1990). Moreover, *P.sorghi* was recovered from many seed samples collected from different countries such India, Venezuela and Argentina. The pathogen was present as oospores embedded in glumes , sticking to the seed surface and as mycelium in the pericarp and endosperm. The disease was reduced if glumes were removed and seeds were surface sterilized (Ahmed and Majumder, 1986) . Oospore on seed surface and in plant debris and mycelium in embryo of pearl millet seed has been observed by detection in washing sediment and embryo examination for *Sclerospora graminicola* (Shetty and Mathur, 1981). Moreover, staining methods have been used to detect sorghum downy mildew (oospores and mycelium) in different seed parts by some aforementioned investigators.

Lakshmanan (1992) found that sorghum downy mildew (*P.sorghi*) was not controlled by some fungicides applied as a dry seed treatment, but soaking seeds in 0.2 % metalaxyl , 0.2 % metalaxyl-ziram and 0.1 % carboxin for 2 hrs was effective to reduce disease incidence.

A quick and easy technique for detecting effective inoculum of downy mildew in sorghum grass seeds is urgently required for plant quarantine purposes. In this paper, an evaluation of modified washing test with different categories of sudan grass seeds, seed transmission of sorghum downy mildew and fungicidal seed treatment were described .

MATERIAL AND METHODS

(A) Detection of sorghum downy mildew inocula in sudan grass grain :
(1) Examination of washing sediment :

Ten sorghum sudan grass samples were collected from Kafr El-Sheikh and El-Gharbia Governorates. All samples were collected from fields showing sorghum downy mildew infection. Seeds were classified into two categories (1) with glumes and plant debris and (2) without glumes and plant debris. Two working seed samples, 400 seeds each ; were drawn from each category for washing test (usual test and modified test), as well as a third working sample (400 seeds each) for detection of mycelium inside seeds.

a. A washing test : Detection of oospores loosely adhering to the seed surface or in fine plant debris was carried out by examination of seed washing sediment. 400 seeds shaked separtely in portions , 100 each , for 10 min in 10 ml distilled water to which some drops of detergent were added as a wetting agent. The suspension of each working sample were centrifuged for 10 min at 2300-2500 rpm and the sediment was resuspended in 2 ml of distilled water and suspension drops were examined under compound microcope X200 using a haemocytometer (Shetty and Mathur, 1981) .

b. A modified washing test : Detection of oospores was made in different parts of seeds (glumes; pericarp) and different plant debris as well. The grains were either ground for 2 min in a coffee grinder or crushed in small plastic bags (5x15 cm) with a wooden hammer and distilled water was used to wash the residual tissues from bags . Each sample of 400 seeds was shaked as mentioned before and filtered through 2 layers of cheesecloth to eliminate ground tissues.The suspension of each working sample was centrifuged for 10 min at 2300-2500 rpm and the sediment was resuspended in 2 ml of distilled water with some drops of iodine tincture to stain starch particles and to make examination under compound microscope easier.

(2) Examination of downy mildew mycelium in different parts of grains of sudan grass:

a. In embryo and scutellum :Embryos were extracted from each sample, as reported in ISTA Handbook on seed health testing (Shetty and Mathur, 1981) .400 seeds of sudan grass were soaked for 24 hrs at 22 °C -25°C, in 5 % aqueous solution of sodium hydroxide containing 0.005 % trypan blue . The extracted embryos were collected and cleared by boiling for 15 min in lactophenol . Moreover, some chaff were collected and clarified in boiling lactophenol and examined at X 12-25 under a stereoscopic microscope with substage illumination . Compound microscope X 200 was used for confirmation.

b. In different seed parts : It is a rapid method for detecting oospores embedded in glumes and mycelium in the pericarp and endosperm according to Ahmed and Majumder (1986) and Yao *et al.* (1990) . Seeds (400 seeds)were soaked in 5% aqueous solution of sodium hydroxide at 80°C for one hr,washed three times with sterile distilled water, then stained in 0.05% lactophenol blue at 80 °C for30 min and examined microscopically.

(B) Effect of fungicidal seed treatment on different levels of sorghum downy mildew infection of sudan grass:

This experiment was carried out (in pots No.30) in the greenhouse, in August 1996, using seed samples with different levels of oospores. Six seed samples of sudan grass were selected having different levels of spore load / seed i.e. 00, control sample; 20 , sample No.1 ; 39 , sample No.2 ; 86 , sample No. 5 ; 102 , sample No.8 and 109 oospores in sample No. 9 to observe disease transmission (three hundred seeds for each level) .

One hundred seeds of each level were soaked in 750 ppm of metalaxyl (Apron 35 %) for 2 hrs then sown immediately in pots . Another one hundred seeds of each level were soaked in distilled water for 2 hrs then sown immediately and the third set was sown without any pretreatment . The last 2 groups of each level served as control and data were collected as an average of untreated seed . The result of emergence % and No. of diseased plants were recorded at 20 days and 45 days , respectively, after planting.

RESULTS AND DISCUSSION

(A) Detection of sorghum downy mildew inocula :

(1) Examination of washing sediment :

The oospore (embedded in fine plant tissue or free in washing sediment) are spherical hyaline with light yellow walls measuring nearly 35 µm in diameter . Rusults of ten sudan grass seed samples are tabulated in Table (1). Results indicated that spore load /seed differs from sample to sample . Mean values of spore load / seed of ten different samples were arranged descendantly as follows : 109 oospores , sample No.9 ; 102 oospores , sample No.8 ; 96 oospores , sample No. 5 ; 63 oospores , sample No. 4 & No. 10 ; 47 oospores , sample No. 7 ; 39 oospores , sample No. 2 ; 31 oospores , sample No. 6 ; 24 oospores , sample No.3 and 20 oospores , sample No. 1 . All these seed samples were collected from fields showing or exhibiting symptoms of downy mildew disease. Values of spore load were affected by crushing samples and plant debris accompanied 8 out of the ten samples .

In addition , 4 out of 5 samples were affected by grinding has a low value of spore load (≤ 39 oospore / seed) . Moreover , adding iodine tincture to washing sediment stained starch particles and made microscopical examination easier (negative staining in field) and preserved washing sediment longer for examination . Generally, grains with plant debris and glumes have rather more oospore than grains without plant debris .However , sometimes plant debris are attached as pedicles .

(2) Detection of mycelium of downy mildew in sudan grass :

Two methods were used to detect the mycelium in seeds (Table 1) . Examination of embryos and endosperm proved that all seed samples were infected. The bluish stained mycelium is thick and net like , present either as few hyphal threads or massively invading the whole tissues. However, The method of Shetty and Mathur , (1981) requires more time to observe the mycelium and collect some chaff to examine the mycelium of sorghum downy mildew. The second method (Ahmed and Majumder , 1986) and (Yao *et al* ., 1990) is quicker to detect inocula of sorghum downy mildew. However, sometimes oospore could not be detected by this method alone.

Oospore inocula is very important for the transmission of the disease during long storage (Yao *et al* . , 1990) . Mycelium of downy mildew was not present in some seed samples infested with oospores and some seed samples which had endospermic tissue were infected by mycelium and no oospores, especially when samples were in a small quantity. Therefore, detecting oospore inocula is very important for plant quarantine purposes. Grinding seed samples helped to release more oospores which may be embedded in plant tissue in low quantity . However , Shetty and Mathur (1981) reported that quantitative determination of oospores may not be essential .

Thereby, direct examination of effective inocula (oospores) of seed borne downy mildew disease was easier and less expensive and did not require a sophisticated equipment .However , there was an attempt made by Yao *et al* ., (1990) to detect sorghum downy mildew in seeds of some sorghum varieties by DNA hybridization . Although, the result of DNA hybridization might be confused with mycelium infection (non-effective inocula) , especially in international exchange of germplasm , but it could be an appropriate procedure to prevent the introduction of a suspicious material to disease-free areas .

(B) Effect of fungicidal seed treatment on different levels of sorghum downy mildew of sudan grass :

Data of fungicidal seed treatment on some seed samples infested with different spore loads / seed are presented in Table (2) . Systemic infection occurred as chlorotic streaks along the leaves which turned dark brown . Fungicidal seed treatment by soaking seeds in 750 ppm metalaxyl with different seed loads reduced the number of systemically-diseased plants . Emergence percentage of sudan grass increased by using fungicidal seed treatments . Besides , seed samples were found infected by some other pathogenic fungi such as *Fusarium* spp . which might affect emergence during germination .

It had been also found that number of systemically infected plants was 4-5 plants/100 seeds when spore load/seed ranged from 20-30 oospores and was 5-8 plants/100 seeds when spore load/seed ranged from 86-109 oospores. However, it might depend on oospore phase; temperature and distribution of oospores inside bulked samples. No systemically infected plants were observed from non-infested grain.

Table 1. Spore load / seed and mycelium infection of sorghum downy mildew estimated by washing test and staining method of different seed samples of sudan grass

Sample No.	Grain without plant debris		Grain with plant debris		Mean	Mycelium present inside seed* (endosperm and scutellum)
	non-crushed seed	crushed seed	non-crushed seed	crushed seed		
1	16	16	16	31	20	+
2	31	47	31	47	39	+ +
3	16	31	16	31	24	+
4	47	47	78	78	63	+ +
5	78	94	78	94	86	+ + +
6	16	31	31	47	31	+
7	47	47	47	47	47	+ +
8	94	94	109	109	102	+ +
9	109	109	109	109	109	+
10	47	47	78	78	63	+ + +
Mean (1)	50	56	59	67	- -	- -
Mean (2)	53		63		- -	- -

* + infection less than 2 %
+ + infection less than 5 %
+ + + infection less than 10 %

The present results of seed transmission of sorghum downy mildew were in accordance with findings recorded by a number of authors (Bain and Alfred, 1969; Tones, *et al.*, 1972; Singh and Milliano, 1989; Yao *et al.*, 1990) . Moreover , the importance of mycelial inocula to transmit the disease may require further studies . The observed symptoms were similar to the typical symptoms of sorghum downy mildew disease caused by *P. sorghi* described by some investigators . (Richard *et al* . , 1969 and Anonymous , 1973) .

Result of fungicidal treatment is nearly in conformity with those reported by Lakshmanan (1992) and Sadoma (1995) . The first author soaked grains in a high dose (0.57 gm / 100 ml water) of metalaxyl to have a complete control of the disease; however, emergence % was decreased at this dose . In our experiment , grains were soaked in lower dose (0.2 gm / 100 ml water) of metalaxyl to reduce downy mildew disease and avoid a harmful effect of the fungicide on seed germination.

Table 2. Effect of chemical seed treatment on different levels of sorghum downy mildew of sudan grass in pots .

Sample No.	Spore load per seed (oospores)	Emergence %		No. of diseased plants / 100 seeds	
		Untreated	Treated	Untreated	Treated
Control	0	75	80	0	0
1	20	79	80	4	2
2	39	72	82	5	3
5	86	74	82	8	3
8	102	80	84	5	2
9	109	87	88	8	3
Mean	59	77.8	82.7	5.00	2.00

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طريقة محورة للتعرف على وجود الجراثيم البيضية لفطر البياض الزغبي في حبوب السورجام والسودان جراس وانتقال المسبب المرض بواسطة الحبة

محمد شعراوى - عبد الله عبد المنعم - عبد الفتاح الوكيل

معهد بحوث أمراض النباتات - مركز البحوث الزراعية - الجيزة - مصر

إستخدم تكنيك معدل لزيادة كمية الجراثيم البيضيه في راشح الغسيل . وقد وجد أنها طريقه سهلة وتسمح بخروج وفصل الجراثيم البيضيه من الأجزاء المختلفة للحبوب (الأجزاء الزهرية و القنابع) وبقايا النباتات الملازمة أحيانا للحبوب وذلك بطحن أو جرش عينات الحبوب لفترة قصيرة . وفي نفس الوقت فإن إضافة بضع نقط من صبغة اليود يؤدى إلى صبغ حبيبات النشا باللون الأزرق وإظهار الجراثيم البيضيه بوضوح خلال الفحص الميكروسكوبي علاوة على أنه يحافظ على معلق الجراثيم لأطول فترة ممكنة.

كما لوحظت إصابات داخليه بميسليوم فطر البياض الزغبي خصوصا في الإندوسبرم - ومع ذلك فإن يلزم إجراء مزيد من الدراسات لإثبات أهمية الميسليوم في نقل المرض تحت الظروف المصرية.

ولوحظت الإصابة الجهازية بالبياض الزغبي عندما زرعت الحبوب المصابة في تربة نظيفة - وقد وجد أن نقع حبوب السودان جراس في مبيد الأبرون (٧٥٠ جزء في المليون) لمدة ٢ ساعة رفع نسبة الإنبات وقلل من فرص انتقال المرض . ولم يلاحظ إصابات من الحبوب السليمة عند زراعتها في تربة نظيفة .