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Assay of cross-infectivity between isolates of *Peronosclerospora sorghi* causing downy mildew on maize and sorghum

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Abstract
Downy mildew, caused by *Peronosclerospora sorghi* (Weston & Uppal) Shaw, is an economically important disease of maize (Zea *mays* L.) and sorghum [*Sorghum bicolor* (L.) Moench] in India. The symptoms of downy mildew on sorghum and maize are difficult to distinguish from each other using morphologic criteria. Hence cross infectivity assay was conducted to monitor the development of symptoms in maize and sorghum after reciprocal inoculation. When maize seedlings were inoculated with *P. sorghi* isolates from maize and sorghum, typical symptoms of downy mildew appeared 5 days after inoculation. Sorghum isolate induced more disease (82%) on maize compared to maize isolate (71%). When sorghum seedlings were inoculated with *P. sorghi* isolate from maize, typical symptoms of downy mildew were produced. In contrast, sorghum seedlings when inoculated with maize isolate did not produce any symptom of the disease indicating pathogenic variability among the isolates of *P. sorghi* from sorghum and maize.

Keywords: Cross inoculation, *P. sorghi*, downy mildew, maize, sorghum

Introduction
Diseases are serious constraints to maize production worldwide. Maize diseases can cause harvest losses, affect the quality of the harvested crop and cause storage losses. Among the various diseases, downy mildew, caused by *Peronosclerospora sorghi* (Weston & Uppal) Shaw, is a major limiting factor to maize cultivation worldwide [7]. *P. sorghi* can cause significant yield losses under favourable environmental conditions and yield losses can reach 50-100% in susceptible cultivars [10]. Although this disease can occur at any stage of maize development from seedling to harvest, the fungus primarily infects maize soon after the emergence of seedling, until one month after planting [10]. The leaves of affected plants show chlorotic streaks on the upper surfaces and white, cottony growth consisting of sporangiophores and sporangia on the corresponding lower surfaces. The leaves later become necrotic and finally dry. The tassels are malformed as small, green leaf-like structures, called "crazy top" that makes the plant unproductive. Epidemic development of the disease within a season is mainly due to airborne conidia and oospores are involved in the long distance movement through seed and soil [1]. The primary source of inoculum is infected seed and airborne spores play an important role in dispersal of the pathogen. Besides maize, *P. sorghi* has been reported to infect sorghum [*Sorghum bicolor* (L.) Moench], forage sorghum (*Sorghum vulgare* Pers.) and tanglehead (*Heteropogon contortus* L.) [6]. Although *P. sorghi* can infect both maize and sorghum, it typically does not complete sexual reproduction on maize; hence no oospores are formed [16]. Olanya and Fajemisin (1993) reported existence of two pathotypes of *P. sorghi*, one capable of infecting both maize and sorghum and the other specific to maize. The *P. sorghi* infecting sorghum and maize are difficult to distinguish from each other morphologically [2]. However, the evidence for molecular variability among *P. sorghi* isolates have been reported from many parts of the world, including India [9, 20], Africa [3], Indonesia [10] and the United States [16]. Such variabilities among pathogen populations are more likely to overcome genetic resistance [12]. Although *P. sorghi* can infect both maize and sorghum, it typically does not complete sexual reproduction on maize, and hence no oospores are formed [16]. Hence cross infectivity assay was conducted to monitor the disease development of symptoms in maize and sorghum after reciprocal inoculation and to know the primary infection in maize.

Materials and Methods

Plant material

Downy mildew susceptible maize seeds (CM 500) obtained from Indian Institute of Maize Research, New Delhi and downy mildew susceptible sorghum seeds (DMS 652) obtained from VC Farm, ARS, Mandya, Karnataka were used in this experiment. The seeds were sown in 30 cm diameter pots filled with sterilized field soil (clay loam with a pH of 7.5) at the rate of 5 seeds per pot and the plants were maintained separately in glass house chamber provided with 18-20^{o}C temperature and >95% RH at Department of Plant Pathology, TNAU during Jan, 2015.

Preparation of inoculum

In order to prepare pathogen inoculum, maize and sorghum leaves showing symptoms of downy mildew were collected from the experimental farm of Tamil Nadu Agricultural University, Coimbatore, India. The infected leaves were wiped out with wet absorbent cotton, cut into small pieces of 4-5 cm lengths and placed with their abaxial side facing up in 9 cm diameter Petri dishes lined with wet filter paper on both the sides. The plates were incubated in the dark for 6-7 h at 20°C for sporulation [13]. Conidia were harvested from the surface of leaves by gently washing them into cold distilled water using a camel hair brush. The concentration of conidia was adjusted to $1x10^{8}$conidia ml^{-1} and used as inoculum. At two leaf stage, the maize and sorghum seedlings were inoculated by spraying with conidial suspension of *P. sorghi* using a hand-held sprayer until run off (Fig. 1). The wetting agent, Tween 20 (0.02%) was mixed with the conidial suspension before inoculation. The inoculated seedlings were maintained at 20±2°C temperature with >95% relative humidity. At 25 days after sowing (DAS), percent disease incidence was measured. The inoculated plants were examined for leaf reaction 10 days after inoculation. Numerical values of 1, 2 and 3 were assigned to resistant (R), intermediate (I) and susceptible (S) leaf reaction classes respectively as described by [4]. Furthermore the presence of *P. sorghi* in infected maize and sorghum leaf tissues was monitored and confirmed by PCR using the developed SCAR primers [21].

Data analysis

Arc sine transformation of data on percentage of downy mildew incidence was done and Duncan's multiple range test (DMRT) was first applied to the transformed values and then transferred to the original means [8]. The data were analyzed using Ag Res statistical software, version 3.01 (Pascal Int1 Software solutions). The means of leaf reaction score and percent disease incidence were used to calculate the coefficient of correlation between the leaf reaction and downy mildew intensity utilizing SPSS statistical software, version 16.0 (IBM SPSS Statistics).

Results and Discussion

Cross inoculation tests were conducted to monitor the development of disease symptoms in maize and sorghum after inoculation with sorghum and maize isolates of *P. sorghi*. The leaf reaction scoring was initiated at 10 DAI with the development of first chlorotic symptom from the base of the leaf. The disease incidence assessment was measured at 25 DAS for both maize and sorghum when the symptom development was prominent with whitish downy conidial growth on underside of leaves and half leaf symptom with yellow interveinal chlorotic streaks. In severe cases, leaves were narrow with full yellow chlorotic symptom and mainly observed with stunted growth among the plants. When maize seedlings were inoculated with *P. sorghi* isolates from maize and sorghum typical symptoms of downy mildew appeared 10 days after inoculation (Fig. 1). The disease incidence was assessed at 25 DAS for both maize and sorghum when the symptom development was prominent with whitish downy growth of the fungus on underside of leaves and with yellow interveinal chlorotic streaks. Sorghum isolate induced more disease (82%) on maize compared to maize isolate (71%) (Table 1). When maize seedlings were inoculated with *P. sorghi* isolate from sorghum, typical symptoms of downy mildew were produced. In contrast, sorghum seedlings when inoculated with maize isolate did not produce any symptom of the disease indicating pathogenic variability among the isolates of *P. sorghi* from sorghum and maize. The presence of *P. sorghi* in infected maize and sorghum leaf tissues was further confirmed by PCR using the SCAR primers. No amplification product was observed in DNA extracted from sorghum leaves inoculated with maize isolate of *P. sorghi* (Fig. 2) indicating pathogenic variability among the isolates of *P. sorghi* from sorghum and maize. The occurrence of different pathotypes of *P. sorghi* has been documented [15, 6, 7, 5, 19]. Dange (1976) [6] reported that isolate of *P. sorghi* present in Rajasthan (India) was pathogenic to maize and tanglehead [*Heteropogon contortus* (L.) Beauv.] but not to sorghum. Frederiksen and Renfro (1977) reported that *P. sorghi* from Karnataka (India) infects maize and sorghum but not *H. contortus*. Schmitt and Freytag (1977) [18] demonstrated that the strain of *P. sorghi* from Thailand differed from that of Texas strain in its pathogenicity on sorghum and has greater virulence to maize differentials. In Texas (USA), two pathotypes (Pathotype 1 and 2) of *P. sorghi* have been identified by differential pathogenicity on sorghum [5]. Among them, Pathotype 2 caused higher percentage of mildew in differential sorghum cultivars than Pathotype 1. The differences in the pathogenicity among isolates of *P. sorghi* from maize and sorghum in the present study may be due to differences in genetic diversity of pathogens [11, 9, 10]. Prabhu *et al*. (1984) [17] conducted cross inoculation studies to determine the host range of *P. heteropogani* on maize and sorghum. The conidial inoculum of *P. heteropogani* was collected from grass and whorl inoculated on maize (Ganga 5 and CM 500) and sorghum (DMS 652 and IS 643). Typical downy mildew symptoms were observed on maize not on sorghum posing a critical threat to maize indicating a wide variability in pathogenic and molecular characters among the isolates.

Table 1: Cross infectivity of *P. sorghi* on maize and sorghum under greenhouse conditions

Treatment	Number of leaves in each reaction class			% of leaves in reaction class			Mean leaf reaction score	% disease incidence
	R	I	S	R	I	S		
Maize inoculated with *P. sorghi* sorghum isolate	8	21	36	12	32	56	2.4	82 (64.89)[a]
Maize inoculated with *P. sorghi* maize isolate	20	22	26	21	31	48	2.1	71 (57.42)[c]
Sorghum inoculated with *P. sorghi* maize isolate	60	0	0	100	0	0	1.0	0.0 (0.29)[d]
Sorghum inoculated with *P. sorghi* sorghum isolate	16	18	26	27	30	43	2.2	73 (58.69)[b]

Plants were inoculated with *P. sorghi* at two leaf stage and the incidence of downy mildew was recorded 30 days after sowing
Numerical values of 1, 2 and 3 were assigned to resistant (R), intermediate (I) and susceptible (S) reactions respectively. A mean score was calculated for the leaf reactions of each treatment
Data are mean of five replications
Data followed by the same letter in a column are not significantly different (*P*=0.05) from each other according to DMRT

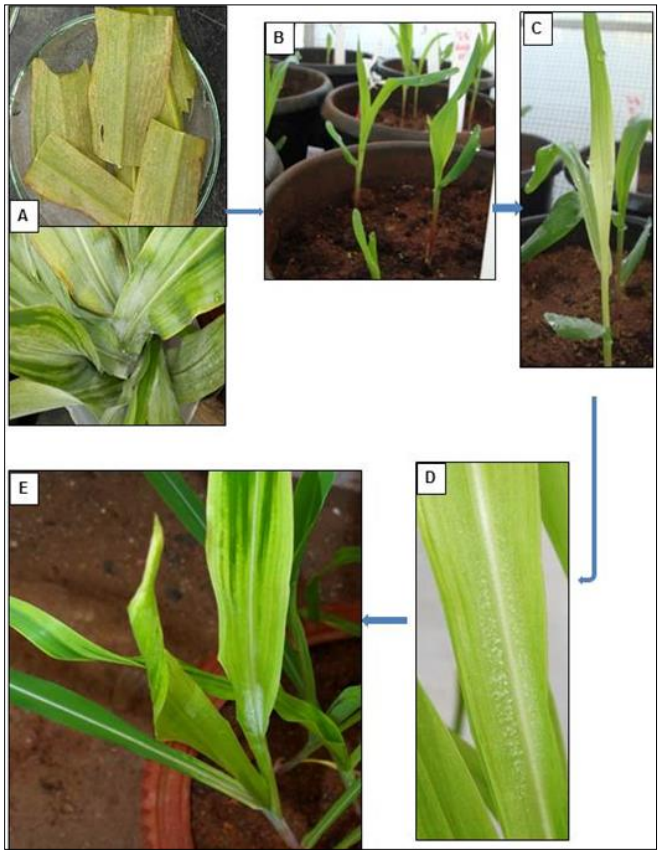

Fig 1: Artificial inoculation and development of downy mildew symptoms in maize
A). Multiplication of conidial inoculum B). Stage of Inoculation C). Symptom expression of basal chlorosis at 7 DAI D). Appearance of whitish downy growth at 15 DAI E). Maize plant with chlorotic streaks and sporangiophore growth.

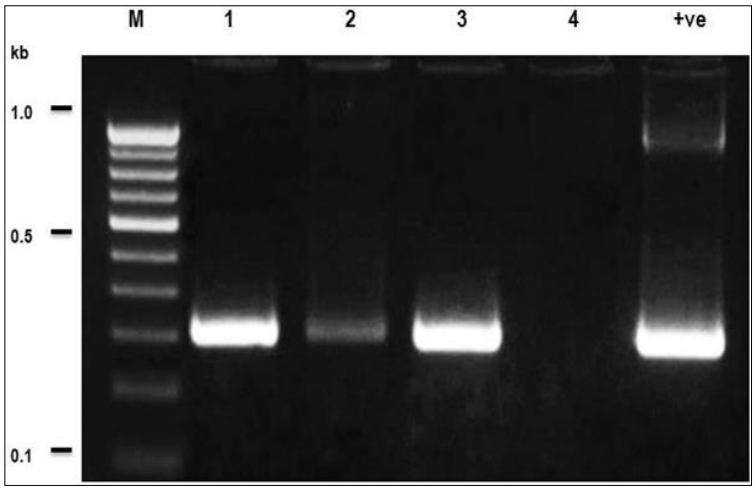

Fig 2: Agarose gel electrophoresis of PCR products from maize and sorghum inoculated with *P. sorghi* Lane M, 100 bp DNA ladder; Lane 1, Maize inoculated with *P. sorghi* sorghum isolate; Lane 2, Maize inoculated with *P. sorghi* maize isolate; Lane 3, Sorghum inoculated with *P. sorghi* sorghum isolate; Lane 4, Sorghum inoculated with *P. sorghi* maize isolate; Lane +ve, pGEM-T vector containing cloned PCR fragment.

Conclusions

The development of downy mildew symptoms in maize after cross inoculation with *P. sorghi* from sorghum implied a threat to maize which could be a source of infection in maize. This in turn suggest that sorghum or weeds can be avoided grown in and around maize can be a promising alternate strategy to be followed to manage maize downy mildew. Even though the research has been carried out elsewhere, clear cut evidence hasn't been elucidated. This research explained the cross inoculation assay between the isolates of *P. sorghi* from maize and sorghum clearly for the first time.

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