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Evidence of Pathogenic Variation in *Sclerospora graminicola* Populations from Pearl Millet Regions of Nigeria

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ABSTRACT

The downy mildew pathogen is known to be pathogenically variable with many reported pathotypes from some millet producing countries. A downy mildew nursery screen was conducted between January and March 2003 to evaluate variation in the pathogenicity of fourteen *Sclerospora graminicola* populations collected across the pearl millet growing region in northern Nigeria. The oospore samples were collected from and designated as: Maiduguri, Benisheikh, Damaturu, Potiskum, Dadinkowa, Gashua, Nguru, Dutse, Azare, Kano East, Kano West, Zaria, Malunfashi and Gusau. The virulence of the pathogenic populations were evaluated on two pearl millet cultivars Ex-Borno, a susceptible cultivar and SOSAT, a newly developed downy mildew resistant cultivar. Sandy loam soil was mixed with the individual oospore populations in 25 cm plastic pots into, which seeds of the two pearl millet cultivars were separately sown and watered. As the seedlings emerged systemically infected by the pathogen, symptoms of damping off, seedling stunting and leaf streaking characteristic of the pathogen infection were monitored. The results demonstrated the presence of different pathotypes of *S. graminicola* in the Nigerian fields of pearl millet as shown by the differential virulence on the Ex-Borno and SOSAT cultivars. Three pathogen populations were highly virulent on Ex-Borno, while there were four populations highly virulent on SOSAT as evidenced by the measure of damping off and the severity of infection on surviving seedlings. Five pathogen populations namely, *Benesheikh, Nguru, Gashua, Potiskum and Dadinkowa* were found to be highly pathogenic to the cultivars under investigation. The inability to find a cultivar that is resistant to all pathotypes of the *S. graminicola* raises some concern about pearl millet production in Nigeria especially in the very dry areas, where it is the only cereal that can be successfully grown.

Key Words: Pearl millet; Downy mildew; S. *graminicola*; Oospores; Pathotypes; Virulence

INTRODUCTION

Pearl millet (*Pennisetum glaucum* (L.) R. Br.), the most important cereal crop of the semi-arid tropics, is the principal host of *Sclerospora graminicola* causing the downy mildew disease. Downy mildew is the most important disease of pearl millet in West Africa and India (Rachie & Majmudar, 1980; Gwary *et al*., 2002, 2006) and has been reported to cause crop losses from 10 to 60% (Nene & Singh, 1976). The pathogen is a diploid and biotrophic oomycete that has both sexual and asexual life cycles. The sexual life cycle produces oospores, which becomes soil borne and provide the primary source of inoculum to infect the successive crop. The asexual stage produces abundant sporangia, which germinate by releasing zoospores, secondary infective propagules causing systemic infections on the foliage. Pathogenic variations in populations of *S. graminicola* have been continually reported in different pearl millet growing countries (Ball, 1983; Ball *et al*., 1986; Thakur *et al*., 1998; 2001).

Different populations of the fungus are morphologically similar but differ in their infectivity. Variation in pathogenicity can be measured in terms of virulence and aggressiveness with which they infect. Introduction of new pearl millet cultivars sometimes leads to evolution of new pathotypes. For instance the large scale cultivation of hybrid millet in India has led to the evolution of new pathotypes of S. *graminicola* (Thakur & Rao, 1997; Thakur *et al*., 1999). Much is known about the pathogenic variability of this fungus elsewhere, but the Nigeria populations have not been investigated thoroughly, yet new millet cultivars and being introduced in Nigeria from different parts of the world. Objectives of this study was to determine the virulence and existence of pathotypes in *S. graminicola* fungus using old Ex-Borno (susceptible) and new SOSAT (resistant) pearl millet cultivars, which is widely grown in Nigeria.

MATERIALS AND METHODS

Pearl millet seeds and inocula. The trial was conducted at the Downy Mildew Screening Farm of University of Maiduguri between January and March 2003. Seeds of the two pearl millet cultivars Ex-Borno and SOSAT were obtained from previous season's crop. The purity of these seeds were tested and ascertained to be almost 100%. Ex-Borno is an old seed cultivar that is now known to be susceptible to *S. graminicola*, while SOSAT is a new

cultivar developed at the Lake Chad Research Institute Maiduguri. The pathogen populations were obtained from infected plants collected in the fields from farmer's fields. For this purpose plan was made to collect samples from locations across northern Nigeria, where pearl millet is widely grown (Fig. 1). Infected pearl millet inflorescence showing 'green ear' symptoms were harvested and sun dried.

Oospore populations of *S. graminicola* fungus were processed from infected pearl millet infected inflorescence collected in the field from fourteen different locations in Nigeria. Using clean mortar and pestle the sun dried infected plant materials were ground into a fine powder to help release the oospores. This ground plant materials containing oospores were sieved into 120 µm to eliminate large plant materials and resulting sieved powder containing oospores were stored in plastic containers in the refrigerator until used.

Soil inoculation. Twenty five centimeter plastic pots were filled with sandy loam soil leaving about 4 cm space for watering. To each pot 30 g of the ground plant material serving as treatment was in incorporated into the top 6 cm soil thoroughly. Each treatment was applied in the same way and replicated three times. The treatments were arranged in a randomized complete block design (RCBD). Pots were watered and left overnight and sowing twenty seeds were sown per pot in the evening.

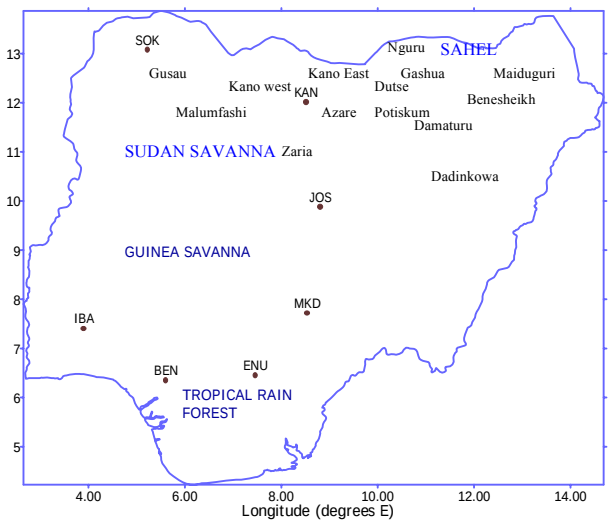
Monitoring and assessment of disease. For assessment of damping-off, seedlings were properly maintained by adequately watering. Damping-off due to downy mildew was monitored and assessed in terms of the percentage of seedlings that germinated and died soon; those that had damping-off showed symptoms and died within two weeks after emergence. Downy mildew incidence of established seedlings was measured by counting the seedlings showing symptom of infection and dividing this by the total number of seedlings and the result expressed as percentage. On the other hand, the disease severity was measured as the leaf area infected divided by the total leaf area and expressed as percentage.

Statistical analysis. Disease incidence and severity data were subjected to analysis of variance according to RCBD. The treatment means were compared using Duncan multiple range test.

RESULTS

Incidence of damping-off. Downy mildew virulence is normally exhibited in pearl millet seedlings with the appearance of damping off. The pathogen aggressively colonized and killed the seedlings in two weeks. Pathogen isolates from Nguru, Dadinkowa, Benesheikh, Kano west and Duste were very virulent on the susceptible cultivar, Ex-Borno. About 50% seedling mortality could be associated with each of these oospore inocula. Oospore inocula from Potiskum, Malumfashi and Damaturu caused the lowest

Fig. 1. Map of Nigeria showing the locations where Sclerospora graminicola populations were sampled in the northern region where pearl millet is grown



damping off on Ex-Borno (Table II). The remaining isolates were intermediate in their infectivity. The fungal isolates caused less damping-off on SOSAT cultivar except Zaria isolate, which caused more than 50% damping-off. Two of the isolates, Potiskum and Kano East did not cause any damping-off at all.

Disease incidence. The method used in this study ensured that the inoculum pressure was sufficiently high that there was no disease escape; the emerging seedlings were systemically infected leading to the development of "downy mildew" leaf lesions (Table III). The pathogen populations from Nguru, Dadinkowa, Benesheikh and Gashua, mostly infected the established seedlings of Ex-Borno. Dadinkowa, Benesheikh, Gashua and Potiskum pathogen populations also caused high disease incidence on SOSAT, while Nguru population had low incidence on this cultivar. Not all infected plants lead to crop losses as it has been known that some cultivars could outgrow low infections. However, severely infected plants had stunted growth and did not produce panicles. Instead, in some cases they lead to the total or partial transformation of the panicles to leafy structure called "green ear" symptoms of the disease.

Disease severity and classification of pathogen populations. The severity of infection by the pathogen populations on cultivars Ex-Borno and SOSAT were similar. They ranged from 2.4% on Ex-Borno to 43.5% and 47.2% on Ex-Borno and SOSAT, respectively (Table IV). Once again the pathogen populations from Benesheikh, Dadinkowa, Gashua and Nguru and in addition Potiskum are the most virulent compared to the others. Based on the severity rating, the isolated pathogen populations were categorized as highly virulent, moderately virulent and less virulent isolates. Populations of Benesheikh, Gashua and Nguru, Dadinkowa and Potiskum, were the most virulent among all fourteen (Table V).

Table I. *Sclerospora graminicola* oospores used for the trial from 14 locations in Nigeria

Entry No.	Isolate name	Location in Nigeria	Place of collection	Date of Collection
1.	Maiduguri	Borno State	Farmers field	September, 2002
2.	Benisheikh	Borno State	Farmers field	September, 2002
3.	Damaturu	Yobe State	Farmers field	September, 2002
4.	Potiskum	Yobe state	Farmers field	September, 2002
5.	Dadinkowa	Gombe State	Farmers field	September, 2002
6.	Gashua	Yobe State	Farmers field	September, 2002
7.	Nguru	Yobe State	Farmers field	September, 2002
8.	Duste	Jigawa State	Farmers field	September, 2002
9.	Azare	Bauchi State	Farmers field	September, 2002
10.	Kano West	Kano State	Farmers field	September, 2002
11.	Kano East	Kano State	Farmers field	September, 2002
12.	Zaria	Kaduna State	Farmers field	September, 2002
13.	Malamfashi	Katsina State	Farmers field	September, 2002
14.	Gusau	Zamfara State	Farmers field	September, 2002

Table II. Damping-off disease due to 14 Nigerian populations of *Sclerospora graminicola* on Ex-Borno and SOSAT pearl millet cultivars

		Damping-off disease (%) on:	
Entry No.	Isolate name	Ex-Borno cultivar	SOSAT cultivar
1.	Maiduguri	33.3bc	38.3bc
2.	Benisheikh	55.0ab	41.7ab
3.	Damaturu	18.3cd	43.3ab
4.	Potiskum	3.3d	0.0e
5.	Dadinkowa	58.3a	41.7ab
6.	Gashua	36.7bc	23.3d
7.	Nguru	61.7a	33.3bc
8.	Duste	50.0ab	38.3bc
9.	Azare	28.3bcd	10.0d
10.	Kano East	1.7d	0.0e
11.	Kano West	55.0ab	25.0cd
12.	Zaria	43.3bc	55.0a
13.	Malumfashi	11.7cd	38.3bc
14.	Gusau	36.7bc	38.3bc
CV (%)		15.7	20.8

Means in columns followed by the same letter (s) are not significantly (p = 0.05) different according to Duncan's Multiple Range Test.

Pathogen virulence is defined in this study as the capacity of an isolate to infect a host plant; it is measured quantitatively as disease incidence or disease severity. We define highly virulent isolates as those causing damping-off of seedlings, which is an extreme case of disease severity.

DISCUSSION

Our results show significant variation in the virulence of the isolates as measured by the symptom types and their intensities on the two differential hosts (Tables II & IV). The extent of this variability has enabled us to classify them into three pathogenic groups based on disease severity (Table V). Six of the 14 isolates are classified as highly virulent, 4 as moderately virulent, while 4 are rated less virulent. This demonstrates the existence of pathotypes of the fungus in the Nigeria. This result supports the earlier findings on pathogenic variation of Sclerospora graminicola using host differentials (Ball, 1983; Ball *et al.*, 1986; Singh, 1995; Thakur *et al.*, 1998). Apart from the work of Ball *et al.* (1986), which compared some African (including Nigeria) and India, this is the first elaborate study on the pathogenic variability of the fungus in Nigeria.

Table III. Disease incidence on susceptible (Ex-Borno) and resistant (SOSAT) pearl millet cultivars caused by 14 different Nigerian populations of *Sclerospora graminicola*

		Disease incidence (%)	
Entry No.	Isolate name	Ex-Borno	SOSAT
1.	Maiduguri	4.7c	2.8de
2.	Benisheikh	33.1ab	29.2a-c
3.	Damaturu	11.1bc	17.6b-e
4.	Potiskum	13.7bc	35.0ab
5.	Dadinkowa	18.5a-c	22.3b-d
6.	Gashua	21.8a-c	42.4a
7.	Nguru	36.7a	5.0de
8.	Duste	0.0c	5.5de
9.	Azare	8.6c	2.0c
10.	Kano East	7.0c	1.7e
11.	Kano West	6.1c	1.7e
12.	Zaria	0.0c	10.4c-e
13.	Malamfashi	2.4c	15.0c-e
14.	Gusau	3.0c	9.3de
CV (%)		19.5	31.1

Means in columns followed by the same letter (s) are not significantly (p = 0.05) different according to Duncan Multiple range test.

Table IV. Disease severity on susceptible (Ex-Borno) and resistant (SOSAT) pearl millet cultivars caused by 14 different Nigerian populations of *Sclerospora graminicola*

		Disease severity (%)	
Entry No.	Isolate name	Ex-Borno	SOSAT
1.	Maiduguri	4.7d	4.4d
2.	Benisheikh	43.5a	20.6bc
3.	Damaturu	10.6cd	14.4b-d
4.	Potiskum	15.7b-d	47.2a
5.	Dadinkowa	9.6cd	21.9b
6.	Gashua	28.7ab	38.9a
7.	Nguru	25.4bc	7.4cd
8.	Duste	0.0d	4.3d
9.	Azare	6.3d	2.3d
10.	Kano East	10.2cd	2.0d
11.	Kano West	4.7d	3.2d
12.	Zaria	0.0d	8.2b-d
13.	Malamfashi	3.0d	15.5b-d
14.	Gusau	2.4	9.1b-d
CV (%)		22.8	25.4

Means in columns followed by the same letter (s) are not significantly (p = 0.05) different according to Duncan Multiple range test.

The most virulent pathogen populations were from the northeastern part of Nigeria, where it is arid in places like Gashua and Nguru. However, Benesheik, Dadinkowa and Potiskum isolates, also highly virulent are from wetter part of the northeast. Most of the less virulent pathogen populations were from part of the region, where rainfall is relatively higher. Isolates having characteristic geographic relatedness, reflected similarity but those from un-related geographic locations showed heterogeneity of the pathogen. The number of pathogenic variation in the pathogen population influences its ability to adapt to fluctuating environmental conditions and the changing host cultivars. Variations in this pathogen influenced by changing host

Table V. Grouping of the *Sclerospora isolates* based on disease severity* expressed on Ex-Borno and SOSAT pearl millet cultivars

Highly virulent isolates		Moderately virulent isolates		Less virulent isolates	
Ex-Borno	**SOSAT**	**Ex-Borno**	**SOSAT**	**Ex-Borno**	**SOSAT**
Benisheikh	Benisheikh	Damaturu	Damaturu	Kano west	Kano west
Gashua	Gashua	Potiskum	Malumfashi	Duste	Duste
Nguru	Dadinkowa	Kano east	Gusau	Azare	Azare
	Potiskum	Dadinkowa	Zaria	Maiduguri	Maiduguri
			Nguru	Malumfashi	Kano east
				Gusau	
				Zaria	

*Highly virulent = more than 20.0% disease severity; Moderately virulent = 10-20% disease severity; Less virulent = less than 10% disease severity

have been observed in India. Following the introduction and mass cultivation of the genetically uniform single-cross F_1 hybrid pearl millet was reported to have led to the evolution of new pathotypes of the fungus (ICRISAT, 1996; Thakur *et al.*, 2004). There are as many as seven major pathotypes known to exist in hybrid-intensive states of India (Thakur *et al.*, 2004). This situation may not occur yet in Nigeria since most of the cultivars used are the open pollinated types. However, variation in *S. graminicolais* is known to arise largely through sexual processes (Pushpavathi, 2006). Un-regulated movement and exchange of grains and plant materials would mostly contribute to the evolution of new races in the country. The out-breeding capacity of *S. graminicola* shows the potentials of this fungus to produce new races that are capable of adapting to different environments (MacDonald & Linde, 2002).

The use of the method described here to detect pathogenic variation is still important in identification of resistant pearl millet and monitoring changes in *S. graminicola* in relation to changes in host for effective disease management (Thakur *et al*., 2001; Gwary *et al*., 2006). Pathogenicity or virulence has been used as the genetic marker in all studies, where variability has been assessed through virulence surveys, using host differentials containing different resistance genes. From this study although both pearl millet cultivars used here were equally challenged by the similar number of pathogen populations, it is clear that SOSAT still maintains its resistant characteristics, because there was less damping-off on this cultivar compared to Ex-Borno (Table II).

DNA finger printing techniques, like RAPD, SSR and AFLP are now being developed and used to detect genetic variations in the pathogen populations including the *S. graminicola* (Sastry *et al.*, 1995). The fingerprinting technique and the pathogenicity evaluation are complementary in the development of disease resistant cultivars (Jones *et al.*, 1995). This will help to a great extent in increasing and stabilizing the yields of pearl millet.

CONCLUSIONS

Sclerospora graminicola collected from pearl millet from different millet producing locations of northern Nigeria is here shown to have different virulence levels on the two pearl millet differentials, Ex-Borno (susceptible) and SOSAT (resistant). These differences in virulence are suggesting the presence of different pathotypes. This report is important for pearl millet improvement and other disease management strategies in Nigeria.

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