



# Evidence of pathogenic variation in *Sclerospora graminicola* populations from pearl millet growing regions in Senegal

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## Abstract

Downy mildew of pearl millet induced by *Sclerospora graminicola* is a major disease that causes significant yield losses in most pearl millet production areas worldwide. The pathogenic variability of *S. graminicola* populations from four agro-ecological zones in Senegal was assessed under field conditions. A set of 19 pearl millet lines, including 12 downy mildew-resistant differential lines with five improved cultivars and two local checks was evaluated during two consecutive rainy seasons (2015 and 2016) at Bambey, Nioro, Kolda and Sinthiou, representing the main pearl millet-growing zones of Senegal. Significant differences were observed among the tested lines, pathogen origin, year and their interactions for resistance to *S. graminicola*. Except for ICMR 356 and 7042 S, the downy mildew-resistant differential lines differed in their resistance to the pathogen from the four agro-ecological zones over the years, providing evidence that the *S. graminicola* isolates represented different races of the pathogen. Among the locations where the experiments were established, the highest average of downy mildew incidence over the two years was recorded at Nioro ( $18.7 \pm 2.6\%$ ), Kolda ( $17.6 \pm 1.8\%$ ) and Bambey ( $12.1 \pm 2.3\%$ ), while the mean downy mildew incidence was lowest at Sinthiou ( $6.9 \pm 2.9\%$ ). This finding indicated that populations of pathogen from Kolda, Nioro and Bambey are more virulent compared to the one from Sinthiou.

**Keywords** Pearl millet · Downy mildew · Pathogenic variability · Senegal

## Introduction

More than 500 million people depend for food and income on pearl millet, a crop grown for grain and fodder on approximately 28 million ha worldwide (Siddaiah et al. 2017). The grain is mainly used for human consumption, but is also used as animal feed or forage. In West and Central Africa, pearl millet production has considerably increased since 1980 (ca. 130%) (Siddaiah et al. 2017). In Senegal, it is the most important food

crop together with sorghum, rice, maize and fonio (FAOSTAT 2016). During the rainy season 2014, pearl millet was grown on approximately 720,000 ha with a production of 410,000 t and a yield of  $571 \text{ kg ha}^{-1}$  (FAOSTAT 2016). The main regions contributing to grain production in Senegal are Diourbel, Fatick, Kaolack, Kaffrine (Groundnut Basin), Kolda (Casamance) and Tambacounda (Eastern Senegal).

Despite the importance of this crop, the national average grain yield is low compared to India or other West and Central Africa countries. In addition, pearl millet yield has not been consistent during the past 20 years, varying between 464 and  $768 \text{ kg ha}^{-1}$  (FAOSTAT 2016). Hence, to sustain pearl millet production, breeding for hybrid cultivars, which normally have higher grain yield compared to the open pollinated ones, has been suggested as a possible alternative. However, hybrid cultivars are more susceptible to downy mildew disease due to their narrow genetic base and low uniformity. Therefore, resistance to downy mildew should be one of the major selection criteria when implementing a hybrid breeding program and promising hybrid parents should be tested for resistance to downy mildew at known disease hotspots in Senegal.

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Downy mildew (DM) caused by the obligate oomycete *Sclerospora graminicola* is the most economically important disease of pearl millet worldwide and has been a persistent and major problem in the pearl millet-growing areas of Africa and Asia (Sharma et al. 2015). It causes yield losses from 10 to 60%, but this loss in grain production can be higher under favorable conditions and when a single cross hybrid F1 is grown in successive years (Sharma et al. 2015). In 1971, an epidemic caused by this fungus in India was observed on the popular hybrid HHB 67. The resulting loss in grain production was estimated at around 3.3 million metric tons (Singh et al. 1993). In Senegal, DM of pearl millet constitutes the major disease that affects grain production, with grain yield losses often ranging between 0.2 to 21% (Mbaye 1986).

*S. graminicola* is known to be largely heterothallic although homothallicism has also been noted (Michelmore et al. 1982). These characteristics make this fungus highly variable (Sudisha et al. 2008). Its pathogenic and genetic variations have largely been studied in the past years, disclosing a considerable variability in different pearl millet-growing countries according to the growing area, cultivars and seasons (Ball 1983; Ball et al. 1986; Sivaramakrishnan et al. 2003; Thakur et al. 2004; Pushpavathi et al. 2006; Sharma et al. 2015). The first evidence of this pathogenic variation was reported by Bhat (1973) in a F1 hybrid cultivar which was found to be resistant at Mysore but susceptible on other Indian locations. Likewise, a field screening conducted in India has shown that

out of 123 pearl millet germplasm accessions from 15 countries across Africa and Asia that were identified as resistant, only 21 remained resistant when the screening was conducted again 14 years later (Thakur et al. 2009). Therefore, a well-known resistant cultivar in a given geographic region or time may not be resistant in a different zone or in another period owing to the pathogenic variability of the fungus. Ball (1983) demonstrated that the West African isolates of *S. graminicola* were generally more virulent compared to those from India. In Senegal, however, the pathogenic variability of this fungus in the different agro-ecological zones where pearl millet is grown has not yet been investigated.

For effective resistance breeding programmes, it would be necessary to study the pathogenic variability of *S. graminicola* populations from Senegal in order to identify suitable hotspot where field screening for resistance should be conducted. Consequently, a project was initiated to compare fungal populations from the main pearl millet-growing areas of Senegal.

## Materials and methods

**Experimental material** The experimental material consisted of a set of 19 entries, including 12 downy mildew resistant (DMR) lines, five improved cultivars and two local cultivars (Table 1). Seeds of the DMR differential lines, the DM susceptible check and the five improved cultivars were obtained

**Table 1** List of plant material used in the study

Entry Number	Designation	Description	Seed source
1	700,651	DMRDL	ICRISAT, Niger
2	852B	DMRDL	ICRISAT, Niger
3	ICMB 841B-P3	DMRDL	ICRISAT, Niger
4	843B	DMRDL	ICRISAT, Niger
5	ICMB 88004	DMRDL	ICRISAT, Niger
6	ICMB 90111-P2	DMRDL	ICRISAT, Niger
7	81B-P6	DMRDL	ICRISAT, Niger
8	7042 DMR	DMRDL	ICRISAT, Niger
9	ICMP 451-P6	DMRDL	ICRISAT, Niger
10	ICMP 85410-P7	DMRDL	ICRISAT, Niger
11	ICMR 356	DMRDL	ICRISAT, Niger
12	7042 S	DMRDL	ICRISAT, Niger
13	ICMV IS 90309	Improved variety	ICRISAT, Niger
14	ICMV IS 90311	Improved variety	ICRISAT, Niger
15	ICMV IS 92326	Improved variety	ICRISAT, Niger
16	SOSAT-C88 (Pat)	Improved variety	ICRISAT, India
17	SOSAT-C88 (ISC)	Improved variety	ICRISAT, Niger
18	Souna 3	Popular Senegalese variety (control)	Bambey
19	IBMV 8402	Popular Senegalese variety (control)	Bambey

DMRDL = Downy mildew resistant differential line

from the Genetic Resources Division of ICRISAT Niger, while the seeds of the two checks were obtained from Senegalese national research. Souna 3 is the most popular cultivar, while IBMV 8402 is known to have resistance to downy mildew. The lines were evaluated for resistance under field conditions in four different agro-ecological zones in Senegal.

**Experimental design** Field evaluation were conducted for two consecutive rainy seasons (2015 and 2016) in four locations where pearl millet is mainly cultivated: Bambey (13° 49' 12" North, 13° 55' 11.99" East), Niore (13° 45' 0" North, 15° 48' 0" West), Kolda (12° 52' 48" North, 14° 39' 0" West) and Sinthiou Maléme (14° 43' 12" North, 16° 0' 36" East) (Fig. 1). For each of the individual experiment, the land was ploughed and harrowed and fertilizer (15 N-15P-15 K) was applied at a rate of 150 kg ha<sup>-1</sup>. The 19 genotypes were evaluated in a randomized complete block design with three replications. Sowing was done with spacing of 0.80 m between rows and 0.40 m between plants within a row in plots of 4.8 m for each evaluated entry. Urea was applied as top dressing at a rate of 100 kg ha<sup>-1</sup>, 50% at 15 days after sowing and 50% at 30 days after sowing. All the recommended cultural practices were applied. Sprinkler irrigation was provided when needed to maintain high relative humidity for favourable disease development.

**Data collection** Disease incidence was recorded 30 days after sowing (das) by counting the total number of plants and the number of infected plants. Entries were classified as described according to the following scale by Sharma et al. (2015): (i) resistant ( $\leq 10\%$  incidence); (ii) moderately resistant (10.1 to 20% incidence); (iii) susceptible (20.1 to 50% incidence) and (iv) highly susceptible ( $>50\%$  incidence). Relative variation,

an indicator for the stability of resistance in the cultivars, was calculated as described by Thakur et al. (2004) by dividing standard deviation over years across locations with the square root of [mean incidence  $\times$  (1 - mean incidence)]. The air temperature and relative humidity from sowing to 30 das were recorded.

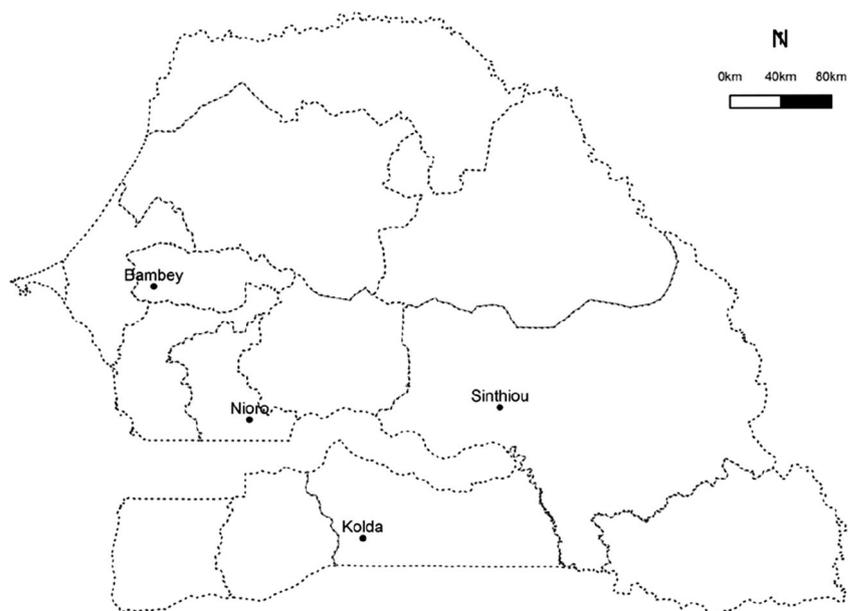
**Statistical analysis** Analysis of variance (ANOVA) for DM incidence (%) was computed using SAS 9.4 to determine significant differences among years, locations, host genotypes and their interactions. Test of homogeneity of variances was confirmed with the Bartlett test for homogeneity of group variances before combined analysis. Significant effects of treatments were determined by magnitude of F values ( $P \leq 0.05$ ). Relative downy mildew variation was compared among the pearl millet lines, locations and years.

## Results

**Seasonal conditions** The weather conditions from sowing to 30 das are presented in Table 2. In general, the weather conditions were the same over years and locations and were favourable for DM disease development. The minimum temperature ranged between 22.9 °C and 24.8 °C and the maximum between 32.1 °C and 33.1 °C. Relative humidity was also observed during the same period. Minimum and maximum values ranged from 66.3 to 68.0% and 95.1 to 100%, respectively.

**Variation in resistance for downy mildew** Analysis of variance showed a significant difference ( $P < 0.01$ ) between pathogen origin, host genotypes, years and their interactions for DMI (Table 3).

**Fig. 1** Map of Senegal showing downy mildew field screening locations



**Table 2** Temperature and relative humidity of the four test sites from sowing to 30 days after sowing in 2015 and 2016

Site	Year	Relative Humidity (%)			Temperature (°C)		
		Minimum	Maximum	Mean	Minimum	Maximum	Mean
Bambey	2015	65.1	99.7	82.4	24.8	33.1	28.9
Nioro		68.0	96.1	82.1	22.9	32.1	27.5
Sinthiou		65.4	100.0	82.7	23.5	32.6	28.0
Kolda		66.3	95.1	80.7	23.6	32.7	28.1
Bambey	2016	67.0	99.4	83.2	24.8	33.0	28.9
Nioro		67.5	97.1	82.3	24.0	33.0	28.5
Sinthiou		66.6	97.3	82.0	23.8	32.4	27.2
Kolda		67.0	97.5	82.3	24.1	32.9	28.5

The tested lines showed differential responses to downy mildew between populations of the pathogen. All the 12 differential downy mildew resistant lines varied in their relative position for DM and confirmed their status as differential lines, except for ICMR 356 and 7042 S (Table 4). ICMR 356 was resistant across locations and years whereas 7042 S was highly susceptible during the two years in all the four locations. The improved cultivars were consistently resistant across location during the two years.

Among the tested lines, the improved cultivars from ICRISAT along with lines 700,651, 843B, ICMB 88004, ICMB 90111-P2, ICMP 85410-P7 and ICMR 356 were the most resistant with downy mildew incidence ranging from 2.8 to 9.4% across locations. The line 7042 S was highly susceptible showing an average disease incidence of 93.9%. Relative variation in incidence of downy mildew of the tested lines varied between 0.28 and 0.89. The two downy mildew resistant differential lines ICMP 451-P6 and 852 B exhibited higher relative variation. In contrast, lines ICMV IS 90311 and ICMR 356 showed low relative downy mildew variation.

The highest DMI over the two years was recorded at Nioro (18.7%), Kolda (17.6%) and Bambey (12.1%) while the mean DMI was lowest at Sinthiou (6.9%) (Table 5). The differences in DMI over the two years were significantly different. A

higher average DMI was obtained during the rainy season 2016 (19.0%) compared to that of 2015 (8.6%).

## Discussion

Weather conditions, particularly temperature and relative humidity, are known to play an essential role and can have an impact on the development and spread of DM of pearl millet. Analysis of these two parameters at the respective trial sites during the rainy seasons of 2015 and 2016 showed that conditions for DM development were fully met according to Thakur et al. (2011) who stated that DM development is observed in high relative humidity (85–90% RH) and moderate temperature (20–30 °C) conditions. The average temperature and relative humidity recorded for the four environments and over years were 28.2 °C and 82.2%, respectively.

Characterisation of virulence over time at different locations is essential to guide pathologists and breeders on which populations of the pathogen to use in the screening process. Pearl millet lines differed in their resistance level, suggesting that different populations of *S. graminicola* were present in the various locations in Senegal where the tests were conducted. The evidence of pathogenic variation was also reported in pearl millet lines evaluated in different environments across Africa and Asia and other crops, such as cucumber, evaluated in Asia, Europe and North America (Shetty et al. 2002; Gwary et al. 2007; Thakur et al. 2007; Sharma et al. 2013).

Disease incidence in the sensitive DM line 7042 S was high with an average of 94% across the four locations over the two years and served as an indicator of good disease pressure. This high DM incidence on line 7042 S has also been reported in Senegal during the past years by Gupta (1986), who conducted an international pearl millet DM nursery at Bambey and Nioro during three consecutive years and recorded a disease incidence varying between 74 and 95% on this susceptible line.

**Table 3** Analysis of variance on effects of location, year, line, and their interaction for downy mildew incidence

Source of variation	df	Type III SS	Mean Square	Pr > F
Genotype (G)	18	174,584	9699	<.001
Location (L)	3	12,257	4086	<.001
G x L	54	23,597	437	<.001
G x Y	18	16,669	926	<.001
G x Y x L	54	23,156	429	<.001
Year (Y)	1	14,341	14,341	<.001
Y x L	3	6079	2026	0.003
Error	302	40,049	135	

**Table 4** Average downy mildew incidence and relative variation on tested pearl millet lines across locations during 2015 and 2016 rainy seasons

Variety	Bambey	Kolda	Nioro	Sinthiou	Mean across location	SE	Relative variation
700,651	1.4	18.3	11.0	0.0	7.7	3.1	0.57
852B	1.7	19.3	20.0	4.0	10.9	5.9	0.87
ICMB 841B-P3	1.8	23.7	20.0	0.0	11.0	4.3	0.65
843B	5.1	23.5	6.0	1.7	9.1	2.5	0.43
ICMB 88004	4.7	24.9	21.6	1.3	13.1	4.4	0.64
ICMB 90111-P2	13.6	6.9	14.1	0.9	8.9	3.1	0.53
81B-P6	14.4	12.6	8.3	2.3	9.4	3.6	0.60
7042 DMR	12.6	21.7	55.2	9.7	24.8	5.0	0.57
ICMP 451-P6	39.4	3.3	40.0	0.0	21.6	8.4	0.89
ICMP 85410-P7	0.8	7.1	22.9	1.2	8.0	3.8	0.68
ICMR 356	1.0	1.5	7.9	1.0	2.8	1.1	0.33
7042 S	94.8	100.0	97.2	83.6	93.9	1.9	0.38
ICMV IS 90309	10.8	17.9	6.9	1.5	9.2	3.0	0.48
ICMV IS 90311	5.4	0.8	0.0	3.6	2.7	0.9	0.28
ICMV IS 92326	1.4	11.1	8.1	2.9	5.9	2.3	0.48
SOSAT-C88-Pat	2.5	6.6	16.8	4.7	7.1	2.6	0.33
SOSAT-C88-ISC	6.0	13.3	7.3	1.9	7.6	1.7	0.47
Souna 3	10.5	18.2	2.7	7.3	9.7	2.8	0.46
IBMV 8402	2.0	4.8	3.6	2.6	3.3	1.3	0.34

The findings from this study revealed that the pathogen populations from Nioro, Kolda and Bambey are more virulent than the one from Sinthiou. Thus, the field screening of the pearl millet breeding materials should be conducted in Nioro, Kolda and Bambey. Girard and Delassus (1978) and Mbaye (1986) reported that a high DM incidence, up to 60%, was observed in the groundnut basin where Bambey and Nioro are located, while incidence as low as 3% was observed in Sinthiou. Therefore, there is apparently pathogenic variation across locations, indicating the existence of populations of the pathogen differing in aggressiveness. Also, Nioro, Bambey and Kolda are among the locations where pearl millet is mainly produced compared to Sinthiou (ANSD 2014). This should orient pearl millet pathologists and breeders on the choice of pathogen populations to be used in the

screening process for identifying breeding materials for an effective resistance to DM in Senegal.

Stability of the resistance of some of these lines has been tested by ICRISAT and their partners, under the International Pearl Millet Downy Mildew Nursery collaborative research. This collaborative research used 11 pearl millet lines as host differentials in trials carried out in different locations of India and West Africa. Results from this work provided evidence of the presence of variable pathogen populations in different locations, with higher downy mildew incidence average observed at Bagauda (Nigeria) and Durgapura (India) and lower downy mildew incidence average in India at Coimbatore and Aurangabad (Singh et al. 1993; Singh 1995; Thakur et al. 2004). In the present study, most of the lines differed in their reaction to downy mildew in the different locations and years indicating differences in the pathogen virulence in Senegal.

ICMR 356 was identified as the most resistant line as it exhibited the lowest relative variation among the tested lines. Therefore, this line may be used as a donor parent for stable DM resistance by the Senegalese pearl millet improvement programme. In contrast, ICMP 451-P6 and 852 B lines showed the highest relative variation across the tested locations. This indicates that the resistance observed on these lines is restricted to certain populations of the pathogen, so they should not be used as parents for developing pearl millet lines with stable resistance. However, these two lines are used as downy mildew and rust resistance donors in the pearl millet hybrid programme at ICRISAT Patancheru (Kumar et al. 1995). The line ICMP

**Table 5** Comparison of the pearl millet downy mildew incidence among different locations and years

		DMI mean (%)	SE	Relative variation
Location	Bambey	12.1	2.3	0.76
	Nioro	18.7	2.6	0.78
	Sinthiou	6.9	2.9	0.75
	Kolda	17.6	1.8	0.72
Year	2015	8.6	1.5	0.80
	2016	19.0	1.9	0.72

451-P6 has also shown resistance to downy mildew in Kolda and Sinthiou. Thus, it can be used by the pearl millet programme to develop lines with downy mildew and rust resistance effective for Kolda and Sinthiou locations.

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