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Evaluation of Differential Pearl Millet Varieties for Pathological Characterization of *Sclerospora graminicola* (Sacc.) Schroet Isolates in Niger

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A B S T R A C T

Pearl millet downy mildew, caused by the fungus *Sclerospora graminicola*, is the most destructive disease of pearl millet present in all production areas. Eight varieties of pearl millet (*Pennisetum glaucum* (L.) R. Br.) were tested at six locations in Niger, including five INRAN stations (Bengou in the Sudanian zone, Kalapaté, Konni, Tarna, and Magaria in the Sahelian-Sudanian zone) and one farmland location (Toumnia in the Sahelian zone). The main objective of the study was to evaluate the resistance level of these varieties and the virulence of *S. graminicola* isolates in these locations. The experiment was conducted using a randomized complete block design with three replications. The tested varieties showed different responses to *S. graminicola* isolates at different locations. The varieties SOSAT-C88 and HKP showed stable resistance in all six locations. Analysis of the resistance-to-sperm ratio (R:S) allowed for the distinction of four groups of *Sclerospora graminicola* isolates according to their virulence level: Group I – Highly virulent (HV), consisting of the *S. graminicola* population from Bengou; Group II – Intermediate virulence (MV), comprising the isolates from Kalapaté and Konni; Group III – Intermediate virulence, but less aggressive than the previous low virulence group (V), formed by the isolates from Magaria and Tomnia; and Group IV – Low virulence (LV), composed solely of the Tarna isolate, representing the lowest virulence level. The variety SOSAT-C88 demonstrated stable resistance in all six locations. The Bengou isolate proved to be the most virulent and can be used for screening millet breeding lines for the development of hybrids best adapted to Niger.

Introduction

Originating in West Africa, pearl millet [*Pennisetum glaucum* (L.) R. Br.] was domesticated in this region approximately 8,000 years ago (IRD/UM, 2015). Pearl millet is primarily cultivated in the arid and semi-arid tropical regions of Africa and India, where agro-climatic conditions are very challenging and no other species can

thrive (Botorou and Boureima, 2004; Ati *et al.,* 2015). In West Africa in general, and particularly in the Sahel, pearl millet remains the most widely cultivated cereal due to its tolerance to drought, poor soils, and low phosphorus content. In Niger, despite ranking second in the world for pearl millet production (FAOSTAT, 2022), several abiotic and biotic factors make it very difficult to improve the yield of this cereal. Regarding biotic factors, diseases play a significant role in reducing pearl millet yield and productivity (Lakshmana, 2008). Indeed, pearl millet production is hampered by several diseases, among which fungal diseases cause the most serious problems. Pearl millet downy mildew, caused by *Sclerospora graminicola* (Sacc.) Schroet, is one such fungal disease that poses a significant challenge to pearl millet production (Thakur *et al.,* 2011). *S. graminicola* is an obligate parasitic fungus found in more than 50 countries worldwide, particularly in temperate and tropical regions of Africa, Asia, the Americas, Europe, and Oceania (Grahame, 2014). It is one of the main limiting factors for pearl millet production in pearl millet-growing areas (Gwary *et al.,* 2007; Rao *et al.,* 2007). Pearl millet downy mildew is responsible for significant crop losses, ranging from 10% to 80% (Sudisha *et al.,* 2008; Thakur *et al.,* 2011; Grahame, 2014). Therefore, it is necessary to develop pearl millet genotypes that are both resistant to downy mildew and high-yielding. Much work has been done on various aspects of this disease (Mahmood *et al.,* 2001; Thakur *et al.,* 2011; Manga and Arun, 2012; Arun *et al.,* 2012). However, the gradual decline in varietal resistance in pearl millet (Thakur *et al.,* 2004), the variability within the species *S. graminicola*, and the widespread use of a pearl millet genotype in an environment conducive to disease development are all major challenges in managing pearl millet downy mildew. With its mixed reproductive system (asexual and sexual), *S. graminicola* is one of the pathogens that easily overcomes varietal resistance (McDonald and Linde, 2002). According to Christet *et al.,* (1987), the lifespan of a downy mildew resistance gene in pearl millet for most commercial hybrids in India varies from 3 to 5 years due to pathogen variability. This variability of the pathogen *S. graminicola* makes selecting resistant varieties difficult. To study the variability of pathogens such as *S. graminicola*, differential host varieties, resistant or susceptible, cultivated in various locations are necessary (Vanderplank, 1984). In Niger, little work has been carried out on *S. graminicola* isolates. The present study aims, firstly, to test pearl millet varieties in several locations for their resistance to downy mildew, and secondly, to evaluate the virulence of *S. graminicola* isolates from these locations.

Materials and Methods

Experimental Sites

The study was conducted at INRAN research stations in Bengou (11°58′ N, 3°33′ E, 182 m) in the Sudanian zone, and in Kalapaté (13°12′ N, 2°56′ E, 197 m), Konni (13°47′ N, 5°7′ E, 264 m), Tarna (13°27′ N, 7°6′ E, 380 m), Magaria (13°44′ N, 9°36′ E, 401 m), and Toumnia, in a rural setting in the Sahelian-Sudanian zone.

Plant Material

Eight (8) varieties of pearl millet were used in this experiment (Table 1). Of these varieties, six are listed in the National Catalogue of Plant Species and Varieties of Niger, one is a new cross from the national breeding program, and the other is a local variety. Four of these six varieties are resistant to downy mildew and one is susceptible to this disease.

Experimental Design

The experimental design was a randomized complete block design with three replications. Each plot consisted of 2 rows of 6 m long spaced by 1 m. Plant hills were spaced by 1 m within the rows. Plots were separated by 1 m, and replications by 1.6 m. Each replication covered an area of 91 m², for a total trial area of 295.4 m². Sowing was carried out with a micro-dose of DAP (diammonium phosphate) fertilizer at a rate of 6 g per hill. Three weedings were performed: the first, two weeks after sowing, the second three weeks after the first, and the third at the pearl millet flowering stage. Two applications of urea at a rate of 5 g per hill were also carried out at the first and second weedings. Thinning was performed to 1 plant per hill, for a total of 12 plants in each experimental plot.

Observations

In each plot, the total number of plants was recorded at emergence. At the physiological maturity stage of pearl millet, the number of plants affected by pearl millet downy mildew was recorded to calculate the incidence of pearl millet downy mildew according to the formula of William (1984):

$$\text{Incidence (\%)} = \frac{n}{N} \times 100$$

Where n is the number of diseased plants and N is the total number of plants in the plot.

The severity of pearl millet downy mildew was also noted at physiological maturity on a scale of 1 to 5, where 1 = no infection, 2 = 20% of productive tillers infected, 3 = 50% of productive tillers infected, 4 = 80% of productive tillers infected, and 5 = all tillers infected or the entire plant dead. Severity is calculated using the following formula (Demba, 1994; Thakur *et al.,* 2011):

Sévérité (%) = [{(1-1).n1+ (2-1).n2 + (3-1).n3 + (4-1).n4 + (5-1).n5}/(5-1). N] × 100, with n1, n2, n3, n4 et n5 the total number of plants in each class from 1 to 5.

Statistical Analysis

Data collected on the incidence and severity of pearl millet downy mildew was subjected to analysis of variance using Genstat software, 14th edition. To assess the variability of the pathogen responsible for pearl millet downy mildew (*Sclerospora graminicola* isolate) at each location, quantitative data (disease incidence) were transformed into qualitative data, R and S, where "R" designates "resistant variety with a disease incidence ≤ 20%" and "S" designates "susceptible variety with a disease incidence > 20%" (Rao *et al.,* 2007). Using these qualitative data, a dendrogram of the ratio (R:S), with R = number of resistant varieties and S = number of susceptible varieties, was generated using the R software.

Results and Discussion

The incidence of the disease varied significantly between varieties ($P = 0.001$), between locations ($P < 0.001$), between varieties and locations, and between varieties and years (Table 2). Analysis of variance also showed a significant difference between varieties and locations ($P < 0.001$) in disease severity (Table 2).

Table 3 illustrates the mean incidence of pearl millet downy mildew at the different experimental location. The Bengou location in the Sudanian zone and the Kalapaté location in the Sahelian-Sudanian zone recorded the highest incidence of the disease, at 29.02% and 27.08%, respectively. The lowest mean incidence was recorded at Toumnia (12.32%), located in the central-eastern Sahelian-Sudanian zone, compared to the mean of 20.44% for all six locations.

Across all locations, the varieties GB 8735 and GAMOJI recorded the highest average incidence rates, at 33% and 24.49% respectively, while the varieties SOSAT C88 (7.22%) and HKP (16.58%) had the lowest average incidence rates (Table 3). Therefore, the latter varieties proved to be the most resistant, while the varieties GB8735 and GAMOJI were the most susceptible. Within a single location, varieties reacted differently. For example, in Bengou, the varieties BG 8735 and ICRITABI*HKB had the highest average incidence rates, at 46.62% and 38.05% respectively, while the varieties SOSAT C88 (4.29%) and HKP (20%) recorded the lowest incidence rates. In contrast, in Magaria, the varieties GAMOJI (30.36%) and GB 8735 (30.6%) had the highest average incidence rates, while the varieties SOSAT C88 (4.96%) and ICMV IS 89305 (11.2%) had the lowest incidence rates (Table 3). These variations observed among the millet varieties tested suggest that they react differently to an attack by the pathogen *S. graminicola*.

The mean severity of pearl millet downy mildew ranged from 5.75% in Toumnia to 14.74% in Bengou. Across all locations, the highest mean severity rates were observed with the varieties GB 8735 (14.54%) and GAMOJI (11.14%), while the varieties SOSAT C88 (2.99%) and HKP (7.06%) had the lowest mean severity rates. Within locations, severity varied from one variety to another (Table 4). In Bengou, the highest severity rates were observed with the varieties GB 8735 (24.32%) and ICRITABI * HKB (23.91%), and the lowest were recorded with the varieties SOSAT C88 (2.15%) and HKP (9.88%). Whereas in Magaria, the varieties GAMOJI (13.99%) and GB 8735 (15.26%) have the highest mean severity and the varieties SOSAT C88 (1.24%) and ICMV IS 89305 (4.59%) have the lowest disease severity (Table 4).

Quantitative data on the incidence of pearl millet downy mildew (Table 3) are translated into qualitative data (Table 5). Thus, the varieties SOSATC88 and HKP showed stable resistance in all six locations, while the varieties ICRITABI*HKB and ICMVIS 89305 showed stable resistance in four of the six locations (Table 5). The varieties ZATIB and ICMVIS 99001 showed stable resistance in three locations, while GAMOJI and GB 8735 were resistant in two and one location, respectively (Table 5). This qualitative data clearly reveals the

variability in virulence between locations. In Bengou, two varieties were found to be resistant out of the eight tested, compared to four resistant varieties in Kalapaté and Konni, seven resistant varieties in Tarna, and six resistant varieties in Magaria and Toumnia (Table 5).

Using the (R:S) ratio, a dendrogram classifying the pathogenesis of *S. graminicola* from the six locations was performed (Figure 1). This classification revealed that the pathogen from Bengou was highly virulent, with only two resistant varieties, while those from Magaria and Toumnia were moderately virulent, with six resistant varieties. The least virulent *S. graminicola* pathogen was found at the Tarna location, where seven out of eight varieties were resistant (Figure 1). The significant difference observed in the "variety x location" interaction indicates that the virulence of the *S. graminicola* population varies from one location to another.

In the pathosystem of pearl millet downy mildew, disease incidence levels indicate quantitative differences in pathogen virulence and host resistance (Sharma *et al.,* 2011). Eight pearl millet varieties were observed at six locations, including five INRAN agronomic research stations and one farm location in different agroecological zones of Niger (Table 1). This study revealed that the varieties behaved differently at the locations. The SOSAT C88 variety, with incidences below 20% at all locations, maintained its resistance status. The HKP variety, although presumed susceptible to downy mildew, proved resistant during this study, with an average incidence of 7.06% (Table 3). These results are similar to those of Halilou *et al.,* (2017) and Hayyo *et al.,* (2020) who showed that the SOSAT C88 and HKP varieties are resistant against *S. graminicola* in the Tarna experimental station. The varieties GB 8735 and GAMOJI, with average incidences of 33% and 24.49% respectively, are the most susceptible. The study also showed that the average incidence of the disease varies from one location to another. Thus, the average incidence, which was 12.32% in Toumnia, rises to 29.02% in Bengou, compared to an overall average of 20.44% for all sites (Table 3). This suggests the existence of variations in meteorological conditions and/or pathogen populations in these locations. Studies on the incidence of millet downy mildew in rural areas and the morphological characterization of *S. graminicola* isolates in different locations in Niger, conducted by Issa *et al.,* (2021), note that the incidence of millet downy mildew varies from 0.6% to 31.8%, with an overall average of 11.12%. The highly significant difference observed between the different localities in this experiment (Table 2) confirms the idea of Thakur *et al.,* (2004) who believe that the significant effect observed with the interaction “varieties x localities” suggests that the virulence of the *S. graminicola* pathogen population is different from one locality to another. To understand the virulence profile of the *S. graminicola* population in the six locations, quantitative data on pearl millet downy mildew incidence were transformed into qualitative data using R and S (Table 4). The different varieties tested showed varying reactions depending on the location. The varieties SOSATC88 and HKP showed stable resistance in all six locations (Table 5). The variety ICMV IS 89305 and the new cross “ICRITABI*HKB” were resistant in five locations. This suggests that they have more or less stable resistance. In contrast, the varieties GAMOJI and GB8735 were resistant in only one or two locations, thus having limited resistance. These results are similar to those of Flor (1971), who suggested that the genetic resistance of a genotype in one location may not be effective in another due to pathogen variability.

Similarly, Thakur *et al.,* (2004) showed that the pathogen population can differ from one locality to another and causes variations in the susceptibility of the varieties tested in different locations. Based on the resistance-to-sufficiency ratio (R:S) and the reactions of the varieties tested in the six locations, the S. graminicola pathogen population can be classified into four virulence groups. The first group, corresponding to Bengou pathogen population, exhibits high virulence (HV), with only two resistant varieties out of eight. The second group, consisting of the Kalapaté and Konni populations, is virulent, with four resistant varieties out of eight.

The third group, comprising Magaria and Toumnia, exhibits moderate virulence (MV), with six resistant varieties out of eight. Finally, the fourth group, represented by Tarna, corresponds to low virulence (LV), with seven resistant varieties out of eight (Figure 1). This classification highlights a clear gradation in the virulence of the populations according to the locations studied. The studies by Sharma *et al.,* (2011) on virulence diversity in *Sclerospora graminicola* isolates from northern India in the state of Uttar Pradesh reveal the existence of several groups of isolates of this pathogen. According to these authors, *S. graminicola* isolates have varying levels of virulence, ranging from less virulent to highly virulent.

Table.1 Pearl millet genotypes used in the experiment

Varieties	Source
GAMOJI[a]	INRAN
GB 8735 [a]	ICRISAT
HKP [c]	INRAN
ICMV IS 89305[a]	ICRISAT
ICMV IS 99001[c]	ICRISAT
ICRITABI * HKB[b]	INRAN
SOSAT C88[a]	ICRISAT
ZATIB[c]	INRAN

Note: [a] = resistant to downy mildew; [b] = new cross; [c] = susceptible to downy mildew

Table.2 Analysis of variance of the incidence and severity of millet downy mildew in tested varieties from six locations in Niger

Sources of variation	Incidence		Sévérité	
	d.f	F pr	d.f	F pr
Varieties	7	0,001 **	7	< 0,001 **
Location	5	0,001 **	5	< 0,001 **
Years	1	0,064 ns	1	0,017 *
Varieties x Location	35	0,015 *	35	0,205 ns
Varieties x Years	7	0,671 ns	7	0,455 ns
Location x Years	5	0,001 **	5	< 0,001 **
Varieties x Location x Years	35	0,694 ns	35	0,876 ns

Note: d.f = degrees of freedom; Fpr = probability; ** = significant at 1%; * = significant at 5%; ns = not significant; localities = 'isolates' of *Sclerospora graminicola*.

Table.3 Mean of incidence of pearl millet downy mildew on tested varieties in six locations in Niger

Varieties	Bengou	Kalapaté	Konni	Tarna	Magaria	Toumnia	Mean
GAMOJI	29,92	19,44	24,81	15,51	30,36	26,92	24,49
GB 8735	46,62	38,64	43,43	17,85	30,6	20,83	33
HKP	20,00	19,44	18,06	18,43	16,54	6,94	16,58
ICMV IS 89305	31,94	22,22	12,63	17,5	11,2	8,33	17,3
ICMV IS 99001	28,56	40,91	21	18,56	15,87	11,24	22,69
ICRITABI * HKB	38,05	17,68	15,4	22,32	14,85	11,83	20,02
SOSAT C88	4,29	16,67	4,72	9,97	4,96	2,78	7,23
ZATIB	32,78	41,67	22,5	13,28	13,49	9,72	22,24
Mean	29,02	27,08	20,32	16,68	17,23	12,32	20,44

Table.4 Mean of severity of pearl millet late blight on tested varieties in six locations in Niger

Variétés	Bengou	Kalapaté	Konni	Tarna	Magaria	Toumnia	Mean
GAMOJI	12,91	6,94	12,75	6,89	13,99	13,37	11,14
GB 8735	24,32	14,77	16,41	7,59	15,26	8,88	14,54
HKP	9,88	6,25	10,07	8,87	5,38	1,91	7,06
ICMV IS 89305	17,65	7,64	9,92	7,64	4,59	4,43	8,65
ICMV IS 99001	14,28	17,42	15,19	6,76	8,58	3,67	10,98
ICRITABI * HKB	23,91	7,45	9,41	10,88	8,31	6	10,99
SOSAT C88	2,15	6,25	4,72	2,87	1,24	0,73	2,99
ZATIB	12,85	18,06	15,9	7,96	5,61	6,98	11,23
Mean	14,74	10,6	11,8	7,43	7,87	5,75	9,70

Table.5 Reaction of the different varieties tested in the six locations

Variétés	Bengou	Kalapaté	Konni	Tarna	Magaria	Toumnia
SOSAT C88	R	R	R	R	R	R
HKP	R	R	R	R	R	R
ICRITABI * HKB	S	R	R	S	R	R
ICMV IS 89305	S	S	R	R	R	R
ZATIB	S	S	S	R	R	R
ICMV IS 99001	S	S	S	R	R	R
GAMOJI	S	R	S	R	S	S
GB 8735	S	S	S	R	S	S
Ratio (R:S)	2:6	4:4	4:4	7:1	6:2	6:2
Note: R = incidence ≤ 20%; S = incidence >20%,						

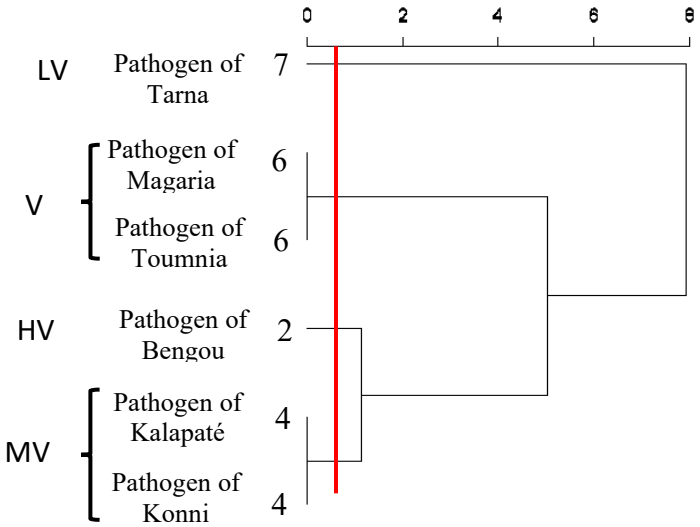

Figure.1 Dendrogram classifying *S. graminicola* pathogens according to their (R:S) ratio, where R = number of resistant genotypes with a downy mildew incidence ≤ 20%; S = number of susceptible genotypes with a downy mildew incidence > 20%. HV = highly virulent; V = virulent; MV = moderately virulent; LV = weakly virulent

In conclusion, the main objective of this study was to test pearl millet varieties for their resistance to pearl millet downy mildew and to evaluate the virulence of *S. graminicola* isolates in six locations in Niger.

The variety SOSAT C-88 demonstrated stable resistance in all six locations. It therefore represents an important source of resistance to pearl millet downy mildew for the continued characterization of other *S. graminicola* isolates from Niger. Given its high agronomic performance, listed in the National Catalogue of Plant Species and Varieties of Niger, this variety can be recommended to millet producers in the country.

The study identified four groups of *S. graminicola* isolates in the six locations in Niger where the experiment was conducted. The Bengou isolate proved to be the most virulent and can be used for screening millet breeding lines for the development of hybrids best adapted to Niger.

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Author Contributions

Halilou Hayyo: Conceived the original idea and designed the model and wrote the manuscript. Karimou Issa: Designed the model and the computational framework and analysed the data.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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