

Full Length Research Paper

Inventory of fungi associated with fonio (*Digitaria exilis*, Stapf) diseases and evaluation of the phenotype of 15 fonio mutant lineages against two isolates of *Curvularia lunata* in Burkina Faso

Niaone Mandinatou*, Issa Wonni, Mahamadou Sawadogo, Soumana Kone, Abdourasmane Konate and Abalo Kassankogno

Institute of the Environment and Agricultural Research, Joseph Ki-ZERBO University, Burkina Faso.

Received 8 August, 2023; Accepted 7 June, 2024

Curvulariosis is one of the main fungal diseases of fonio, appearing at the vegetative growth stage and causing yield losses of up to 45%. The aim of this study was to invent the fungal species that infest fonio and to evaluate the phenotype of 18 lines developed or generated by mutagenesis against curvulariosis. A collection survey was carried out in the main fonio production zones. The lines were screened under semi-controlled conditions using a split plot design with two factors, the first of which included the 18 fonio lines and the second isolates of *Curvularia lunata*. The fungal inventory identified 15 fungal genera from the seeds and leaves collected, of which *Curvularia* sp was the most frequent. Of the two isolates used for line screening, the Cu Sindou isolate was the more aggressive and virulent. However, V1 lines proved resistant to both isolates. To better assess the phenotyping of these lines, testing in real-life conditions in production areas, depending on their ecology, is necessary.

Key words: fonio, genus Fungi, *Curvularia lunata*, pathogenic, resistance, Burkina Faso.

INTRODUCTION

Fonio (*Digitaria exilis* Stapf) is a monoecious herbaceous plant of the Poaceae family, belonging to the genus *Digitaria*. It is the oldest African cereal native to Mali (Cruz, 2007). Nutritionists rediscovered this food at the beginning of the 21st century because of its high digestibility, recommending it for people with allergies,

especially gluten intolerance. Fonio is considered a minor cereal of economic importance in only a few regions of West Africa. It is a hardy crop that requires little cultivation care and is often relegated to marginal, depleted, and leached soils (Cruz et al., 2011). In these ecologies, fonio achieves an average yield of 650 kg/ha.

*Corresponding author. E-mail: niaonemandinatou@gmail.com.

Depending on the country, fonio yields vary between 200 and 900 kg/ha (Vodouhe and Achigan-Dako, 2006). In Burkina Faso, despite the fact that the majority of the population is farmers, the problem of food insecurity persists. To alleviate this insecurity, research should focus on the promotion of neglected species such as cereals. The different fonio accessions could help to address seasonal food insecurity (Blench, 2012). Fonio is only grown in semi-arid zones (Boucle du Mouhoun and Nord) and humid zones (Hauts Bassins and Cascades) in Burkina Faso. From 2020 to 2021, national fonio production was estimated at 10,758 tonnes (DGESS/MAAH, 2020).

To address this insecurity, research should focus on promoting neglected crops such as cereals, aiming to compensate for the cereal deficit by increasing fonio production. With increasing food insecurity in the interior of the country, there is a need for the promotion of so-called secondary crops (Blench, 2012). Despite the willingness of some producers to grow the crop, fonio cultivation has declined in recent years due to several technical constraints, including the use of low-yielding cultivars, inadequate weeding, high crop losses, challenges in post-harvest management, and poor socio-economic evaluation (FiBL et al., 2013). Additionally, parasitic problems significantly reduce yields. Several fungal species such as *Phyllachora sphaerosperma*, *Helminthosporium* spp., *Puccinia cahuensis*, *Fusarium* spp., and *Curvularia* spp. are responsible for fungal diseases of the fonio plant (Dansi et al., 2010; Akanmu et al., 2013). Limited research has been conducted to confirm the causal agent of these fungal diseases (Zinsou et al., 2020). In particular, curvulariosis has been reported as the most important fungal disease of fonio, causing yield losses of around 45% (Zinsou et al., 2020).

The fungus has become a recurrent disease in cultivated cereals (Dioulasso, 2021). Very few studies have been carried out to analyze the behavior of fonio varieties with respect to curvulariosis.

The search for resistant varieties to reduce constraints is proving necessary. For this reason, this study was initiated to inventory the different fungal species that attack fonio and to evaluate the behavior of 18 fonio mutant lines against two isolates of *Curvularia* sp.

MATERIALS AND METHODS

Study site

The study was carried out at the phytopathology laboratory at INERA/Farako-Bâ, located 10 km south-west of the town of Bobo-Dioulasso on the Bobo-Banfora axis. Its geographical coordinates are 4°20' west longitude, 11°06' north latitude and 450 m altitude. Figure 1 shows the map of geographical location of the INERA/Farako-Bâ research institute housing the experimental site. Figure 2 shows the map of the study survey site.

The plant material consisted of 18 lines, comprising 15 mutant lines and three varieties (CVF109, CVF234, and Solosso). The mutant lines were obtained with the support of the International

Atomic Energy Agency. The mutation was induced by gamma irradiation of the seeds at three doses (150 Gy, 300 Gy, and 450 Gy). After an initial multiplication, the 150 Gy dose was utilized to obtain a larger population. Characterization of the second generation led to the selection of 15 lines, including five per variety showing the best performance compared to the control (1).

Fungal material

The fungal material consisted of two isolates of *Curvularia lunata*, designated CuBF01 and CuBF02. These isolates came from the results of this study and were isolated from symptomatic samples collected in 2021 in Sindou and Farako-Bâ respectively. They were selected on the basis of their pathogenicity and geographical origin.

Sampling collected

Samples were collected during the 2021 rainy season in the Hauts-Bassins, Cascades, and Boucle du Mouhoun regions from fonio growers' plots. A radius of 10 km was observed between the plots surveyed and symptomatic leaves were sampled along the diagonals. The number per province varied according to the number of sites and fields visited. The surveys were mainly conducted in five provinces divided into agro-ecological zones, enabling 24 fields to be visited, including eight in Kossi. Seeds of three fonio varieties (CVF109, CVF234, and Solosso) obtained from INERA and produced during the 2020 rainy season, along with ten symptomatic leaves of varieties, were collected during the 2021 dry season in on-station trials.

Inventory of the different fungal species on fonio

The blotting paper method was used for this activity. Four hundred seeds per variety and the collected leaf fragments (20) were first disinfected with 70% ethanol for 30 s and then with 1% bleach for one minute. The organs were then placed in Petri dishes on blotting paper sprayed with sterile distilled water. The dishes were incubated in a chamber at a constant temperature of 25°C under near ultraviolet light, alternating with darkness every 12 h. Identification was made with a binocular loupe 72 h after incubation. Monospores of recurrent fungi were subcultured on PDA medium and incubated for 10 days. The spores were observed under the electron microscope, and the species were identified using the identification key of Mathur and Kongsdal (2003).

Evaluation of the phenotype of lines against two isolates of *Curvularia lunata*

Experimental design

The experimental design used was a two-factor split plot from July to October 2021. The primary factor consisted of three varieties for the pathogenicity test and 18 lines for the varietal screening activity, and the secondary factor included the two isolates of *Curvularia* sp. Each line was replicated in three pots with a control. The seeds of the lines were cleaned with 70% ethanol for 30 s, then in 1% bleach for one minute, rinsed with distilled water, and then sown in small pots.

Inoculation method

Two weeks later, the seedlings were transplanted at a rate of four

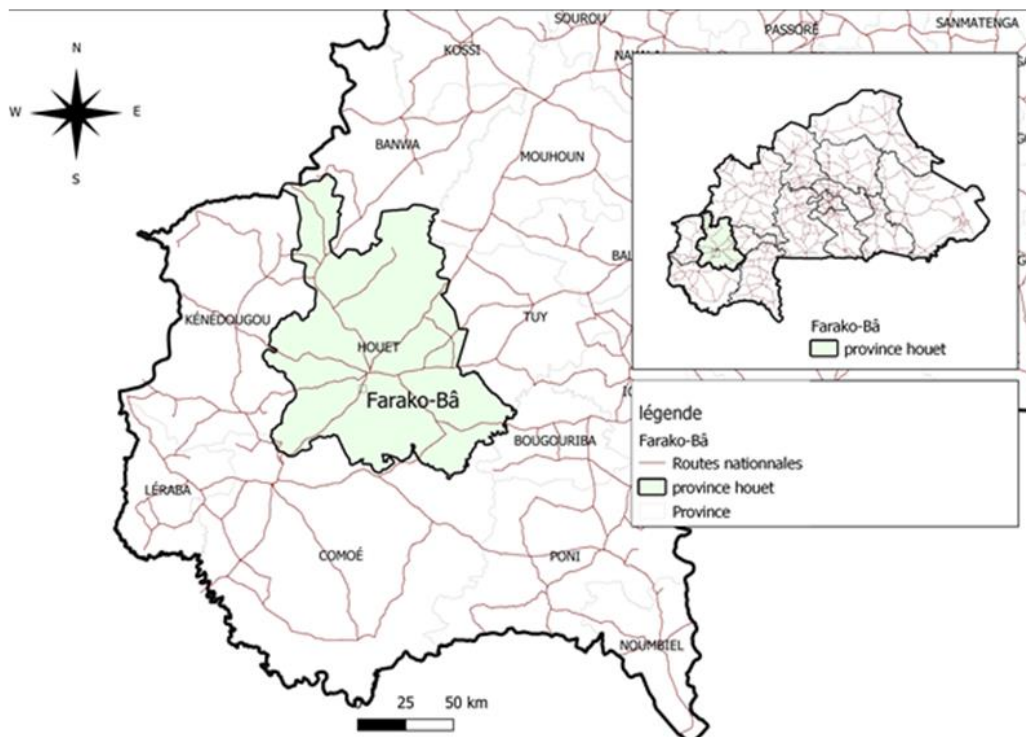


Figure 1. Map showing the geographical location of the INERA/Farako-Bâ research institute housing the experimental site.
Source: QGIS 3.28.2 (2022), Plant material used for screening.

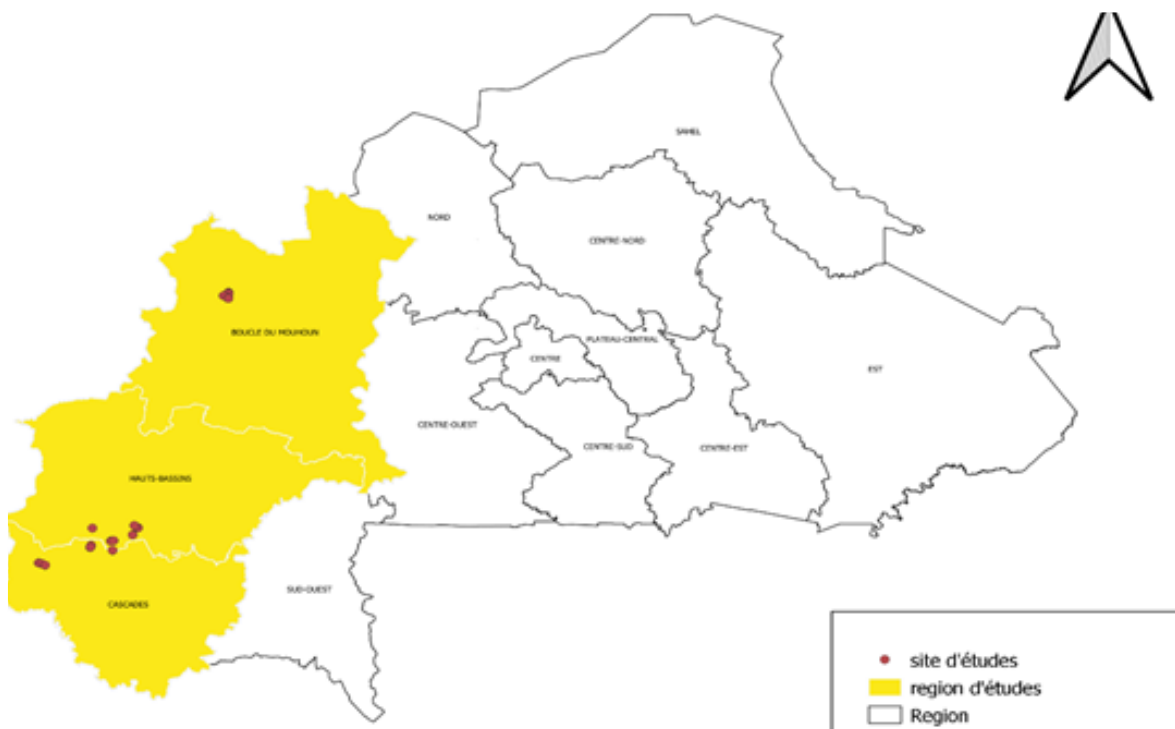


Figure 2. Map of the study.
Source QGIS; 2023.

Table 1. Characteristics of the lines tested in this study.

Line	Mutants	irradiation waves	NT (tillers)	DFM(Day)	HP (cm)	NRP	PGr (g)/stand
CVF109	V1	0Gy	85	90	79.7	3	27.3
CVF109	V1D1-11-2	150Gy	42	102	87.2	3	19.7
CVF109	V1D1-2-3	150Gy	45	116	95.3	4	20.5
CVF109	V1D1-2-5	150Gy	40	113	84.9	3	13.7
CVF109	V1D1-3-5	150Gy	55	124	83.4	3	18.2
CVF109	V1D1-6-4	150Gy	80	105	83.9	3	40.3
CVF234	V2	0Gy	33	101	90.9	3	17.3
CVF234	V2D1-2-5	150Gy	33	115	93.4	3	25.4
CVF234	V2D1-3-4	150Gy	64	95	85.7	3	30.3
CVF234	V2D1-6-1	150Gy	48	106	84.8	4	23.3
CVF234	V2D1-8-2	150Gy	63	92	97.0	3	34.7
CVF234	V2D1-8-4	150Gy	66	104	86.6	3	17.3
Solosso	V3	0Gy	63	88	85.2	3	67.3
Solosso	V3D1-11-2	150Gy	30	103	87.6	3	33.7
Solosso	V3D1-2-3	150Gy	71	87	79.9	2	33.4
Solosso	V3D1-3-1	150Gy	61	90	85.9	3	44.3
Solosso	V3D1-5-3	150Gy	52	88	92.6	2	49.7
Solosso	V3D1-6-2	150Gy	62	90	97.8	3	47.0

NTP: number of tillers per plant; DFM: Maturity count days after sowing, HP: Plant Height, NRP: Number of flower racemes per panicle; PGr: Grain weight per plant.

Table 2. Disease severity scale of vales (1992).

Typical symptoms	Number of lesions	Severity note (1 à 9)
no symptom	0	1
b rarely	< 20	2
b many	≥ 20	3
bg rarely	< 10	4
Bg many	≥ 10	5
bG rarely	< 10	6
bG many	≥ 10	7
pG rarely	< 5	8
pG many	≥ 5	9

b: small brown spot (absence of sporulation); bg: lesion with brown border and grey centre; bG: large bg; pG: lesion with or without border and grey centre.

sprigs per pot and then thinned down to three sprigs per pot. The apical part of the 21-day-old seedlings (4-leaf stage) was sprayed with 3.104 spores/ml using the method of Kauffman et al. (1973). After inoculation, the seedlings were placed in a humidified incubation chamber for 24 h to promote infection and then transferred to the greenhouse.

Evaluation of the level of resistance of fonio lines

The evaluation was carried out on the 5th, 10th, and 15th day after inoculation. The Vales (1992) scale (Table 2) was used to rate severity. Plants were considered resistant with a severity score of 1 to 3; moderately resistant with a severity score of 4; moderately susceptible with a severity score of 5 to 6; susceptible with a

severity score of 7; and very susceptible with a severity score of 8 to 9.

Evaluation of the extent of disease progression on varieties depending on the isolates

The Area Under the Disease Progress Curve (AUDPC) was calculated according to the formula of Shaner and Finney (1997): $AUDPC = \sum [(D_i + D_{i+1}) / 2] \times (t_{i+1} - t_i)$; where D_i and D_{i+1} represent the percentage measures of disease severity observed at times; t_i and t_{i+1} represent the time interval between two observations. AUDPC was calculated taking into account the severity of leaf symptoms at 5; 10 and 15 days after inoculation. According to Shaner and Finney (1997), any cultivar with an AUDPC below 20 is considered

Table 3. Distribution of survey areas by number of samples taken.

Province	Locations	Number of fields	Number of samples	Types of symptoms	Coordinates of fields	
					Latitude	Longitude
Houet	Farako-Bâ	2	20	Brown stain dry edges	11.06	4.20
Houet	Péni	4	12	Brown stain dry edges	10.9701851	4.497435
Houet	Toussiana	4	12	Brown stain dry edges	10.96765833	4.83425333
Kéné Dougou	Toussiamasso	1	3	Brown stain dry edges	10.913489	4.5380323
Comoé	Kolokolo	2	6	Brown stain dry edges	12.8314628	3.8688527
Léraba	Sindou	3	9	Brown stain dry edges	10.6884151	5.2291152
Kossi	Simbadougou	8	24	Brown stain dry edges	12.8574517	3.8323892
Total: 5	7	24	86			

resistant.

Collection of agro-morphological data

The study focused on five quantitative characteristics because of their importance in the evaluation of fonio varieties. These are:

- i) Plant height. This was measured on the main stem (or the longest stem) using a ruler graduated from the surface of the soil to the top of the tallest panicle;
- ii) The length of the flag leaf. This is the last leaf under the panicle; it was measured using a double decimeter and expressed in cm;
- iii) The number of racemes per panicle. This was counted by hand and represents all the racemes that make up the panicle;
- iv) The length of the racemes per panicle. This was measured (cm) from the point where the racemes were attached to the end of the longest raceme;
- v) Grain yield per plant. The panicles of each plant were harvested and threshed in plastic bags, and the paddy grains obtained at a moisture content of 14% were weighed.

Data analysis

Microsoft Excel 2016 was used for data entry. The

evaluation of the infection rate was based on the frequency F (%). XLSTAT 2016.02.27444 software was used for the statistical analysis of the data, which included analysis of variance followed by comparison of the means of the agro-morphological characteristics using the Newman-Keuls test with a threshold of 5%. An analysis of variance was then conducted to compare the pathogenicity of the *Curvularia* sp isolates with respect to the lines.

Correlation tests between agro-morphological characteristics and the curvulariosis phenotype were carried out using Pearson's method with a threshold of 5%, and hierarchical ascending classification (CAH) for the characteristics of plant height, above-ground biomass, and severity score on the 15th day after inoculation, again using XLSTAT 2016.02.2744.

RESULTS

Samples collected at different sites

The surveyed areas have a northern and southern Sudanese climate and are divided into three regions (Table 3). A total of five provinces were surveyed, including six villages, namely Farako-Bâ, Péni, and Toussiana in the province of Houet; Toussiamasso in the province of Kéné Dougou. These two provinces are part of the Hauts-

Bassins region. The villages of Kolokolo and Sindou, in the provinces of Comoé and Léraba respectively, are in the Cascades region. The village of Simbadougou is in the province of Kossi in the Boucle du Mouhoun region. Twenty-four fields were explored, with eight in the province of Kossi alone, reflecting the high production in the area.

Inventory of fungi associated with fonio fungal diseases

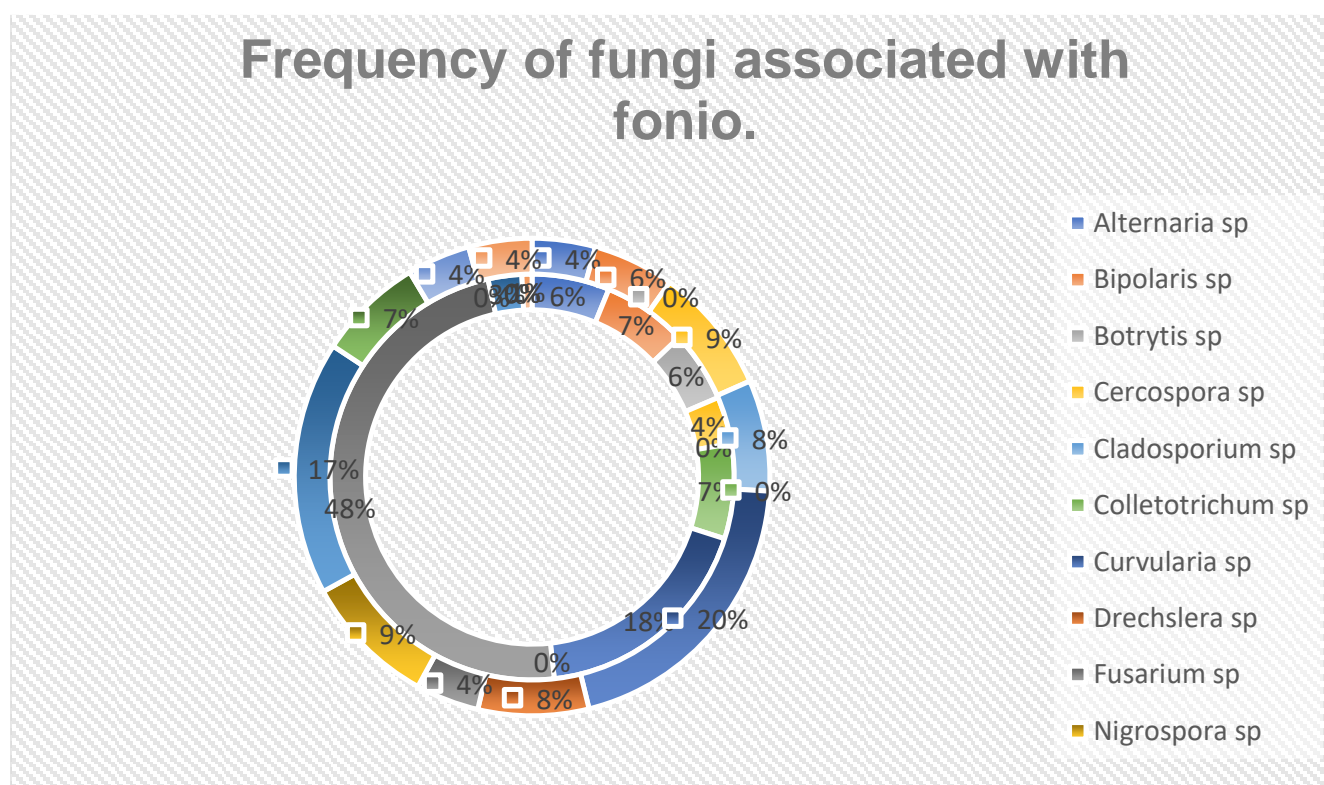
Fungal pathogens and frequency of fungal genera associated with fonio organs

Based on the samples analyzed, 14 fungal genera were identified, but at varying rates of frequency. These are *Alternaria*, *Bipolaris*, *Botrytis*, *Cercospora*, *Cladosporium*, *Colletotrichum*, *Curvularia*, *Drechslera*, *Fusarium*, *Nigrospora*, *Penicillium*, *Phoma*, *Rhizopus*, and *Stenocarpella*. According to the frequency rates of the fungi identified, the fungal genera with a strong presence on the seeds (Figure 3) are the genus *Fusarium* with 44.75%, followed by *Curvularia*

Table 4. Distribution of fungi genera by surveyed site.

Sites	Comoé		Houet		Léraba		Kossi	
Fungi	%F	I	%F	I	%F	I	%F	I
<i>Alternaria</i> sp	0	0	0	0	0	0	20	0.2
<i>Bipolaris</i> sp	20	1	20	0.4	20	0.5	0	0
<i>Curvularia</i> sp	70	1	74	0.9	20	0.5	68	1
<i>Drechslera</i> sp	0	0	40	0.3	30	1	0	0
<i>Penicillium</i> sp	80	1	87	0.4	0	0	78	0.9
<i>Phoma</i> sp	0	0	33	0.4	0	0	0	0
<i>Stenocarpella</i> sp	0	0	0	0	20	0,5	0	0

F: Frequency of fungi, I: Incidence of fungi.

**Figure 3.** Frequency of fungi associated with fonio.

sp (17%). On the leaves, *Penicillium* was the most frequent fungal genus at 80%, followed by *Curvularia* at 59.13%. The genera *Cladosporium* and *Nigrospora* were observed specifically on the leaves. *Botrytis* sp is present on seeds and absent on leaves. However, *Curvularia* sp was present on all organs at a high rate. Moreover, the *Curvularia* genus is the most frequently identified on both seeds and leaves.

The province of Houet has the greatest diversity of fungal genera (05), followed by the province of Léraba (04). Of all the genera, *Curvularia* is the one listed in all

the localities. Table 4 shows the distribution of fungal genera according to surveyed sites and organs.

Lineage response to inoculation with two *Curvularia* isolates

The analysis of variance (Table 5) showed a significant difference between the lines for incidence at the three inoculation dates. For isolate S1 on the first date, the highest severity score was obtained from variety V3T (4.33), and the lowest from variety V2D1-3-4 (1.22). For

Table 5. Severity of curvulariosis as a function of time and resistance status of lineages.

Lineages	Genotype type	5DAI		10DAI		15DAI	
		S1	S2	S1	S2	S1	S2
V3D1-5-3	Mutant	3.77 ^{bc}	5.667 ^f	5.889 ^b	6.556 ^e	7.444 ^c	7.667 ^d
V3T	Unmutant	4.33 ^c	5.222 ^{ef}	5.333 ^{ab}	5.778 ^{de}	5.889 ^{abc}	6.778 ^{cd}
V2D1-6-1	Mutant	3.778 ^{bc}	4.778 ^{def}	5.222 ^{ab}	5.667 ^{de}	6.556 ^{bc}	6.222 ^{bcd}
V1T	Unmutant	3.222 ^{abc}	4.2 ^{bcef}	4.000 ^{ab}	5.111 ^{cd}	5.778 ^{abc}	6.778 ^{cd}
V2T	Unmutant	2.778 ^{abc}	4.556 ^{cdef}	4.222 ^{ab}	4.889 ^{cd}	6.111 ^{abc}	6.111 ^{bcd}
V2D1-2-5	Mutant	3.222 ^{abc}	3.7 ^{abcd}	4.333 ^{ab}	4.556 ^{bcd}	5.333 ^{abc}	5.333 ^{abc}
V2D1-8-2	Mutant	2.889 ^{abc}	3.333 ^{abcd}	4.333 ^{ab}	4.000 ^{abc}	5.444 ^{abc}	4.889 ^{abc}
V2D1-8-4	Mutant	3.222 ^{abc}	3.333 ^{abcd}	3.889 ^{ab}	3.889 ^{abc}	5.333 ^{abc}	4.444 ^{abc}
V3D1-6-2	Mutant	2.333 ^{abc}	2.889 ^{abc}	3.889 ^{ab}	3.333 ^{ab}	4.667 ^{abc}	4.667 ^{abc}
V1D1-6-4	Mutant	2.667 ^{abc}	2.667 ^{ab}	3.333 ^{ab}	3.667 ^{abc}	4.222 ^{abc}	4.444 ^{abc}
V3D1-2-3	Mutant	2.000 ^{ab}	2.111 ^a	3.556 ^{ab}	3.333 ^{ab}	4.333 ^{abc}	5.778 ^{bcd}
V1D1-2-3	Mutant	2.222 ^{ab}	2.333 ^{ab}	2.556 ^{ab}	3.333 ^{ab}	4.222 ^{abc}	4.444 ^{abc}
V3D1-3-1	Mutant	2.222 ^{ab}	2.556 ^{ab}	3.111 ^{ab}	3.222 ^{ab}	3.889 ^{ab}	4.000 ^{ab}
V1D1-11-2	Mutant	2.111 ^{ab}	2.444 ^{ab}	3.333 ^{ab}	3.111 ^{ab}	3.444 ^{ab}	4.222 ^{abc}
V1D1-2-5	Mutant	1.890 ^{ab}	2.333 ^{ab}	2.943 ^{ab}	3.000 ^{ab}	3.553 ^{ab}	2.999 ^a
V3D1-11-2	Mutant	1.444 ^a	2.111 ^a	2.111 ^a	2.889 ^{ab}	2.889 ^a	3.000 ^a
V1D1-3-5	Mutant	1.889 ^{ab}	2.333 ^{ab}	2.000 ^a	2.667 ^a	2.667 ^a	2.889 ^a
V2D1-3-4	Mutant	1.222 ^a	2.000 ^a	2.000 ^a	2.444 ^a	2.667 ^a	2.778 ^a
Pr > F		0	<0.0001	0.006	<0.0001	0	<0.0001
Significatif		Oui	Oui	Oui	Oui	Oui	Oui

Pr : Probability ; Sign : Meaning , DAI : Days After Inoculation, S1 : isolate 1; S2 : isolate 2

the second observation date, the highest score was obtained by the variety V3D1-5-3 (5.88), and the lowest score was observed on the varieties V2D1-3-4 and V1D1-3-5 (2). For the last date, the same variety V3D1-5-3 (7.44) scored highest, and varieties V2D1-3-4 and V1D1-3-5 scored lowest (2.66). For the S2 isolate on the first date, the highest severity score was obtained by variety V3D1-5-3 (5.66), and the lowest by variety V1D1-3-5 (2). On the second date, variety V3D1-5-3 had the highest score (6.55), and variety V1D1-3-5 the lowest (2.44). On the last date, the same variety V3D1-5-3 obtained the highest score (7.66), and the variety V1D1-3-5 the lowest score (2.77). The results of the evaluation of the level of sensitivity or resistance of the varieties are presented in Table 5. The varieties V1D1-11-2, V1D1-3-5, V2D1-3-4, and V3D1-11-2 were resistant to isolate S1. However, varieties V1D1-11-2, V1D1-3-5, V2D1-3-4, and V2D1-8-2 were resistant to isolate S2. Variety V3D1-5-3 was highly susceptible to isolate S2 and susceptible to isolate S1.

Area under the disease progress curve (AUDPC)

The area under disease progress curve (AUDPC) on the cultivars on days 5, 10, and 15 after inoculation is shown in Figure 4. Disease development on each cultivar varied

depending on the isolate. On all cultivars, AUDPC was greater than 20% for all isolates. The variety V3D1-5-3 had the highest area of progression for the two isolates S1 (66.94) and S2 (80.28). The lowest area was recorded for line V2D1-3-4 (22.78) for isolate S1 and V1D1-3-5 (29.17) for isolate S2.

Hierarchical ascending classification of lines according to their performance and behavior with *Curvularia* sp

This allowed the lines to be classified into three groups (Figure 5). The first group consisted of two lines, V1D1-11-2 and V1D1-6-4, characterized by a high mean height of 81.61 cm, a high mean biomass of 20.83 g, and a low severity score of 3.83 (Table 6). The second group contained the largest number of lines with nine: V1D1-2-3, V1D1-2-5, V1D1-3-5, V1T, V2D1-3-4, V2D1-6-1, V2D1-8-2, V3D1-11-2, and V3D1-3-1. This group had an average plant height of 66.07 cm, an average kernel weight of 18.97 g, and an average severity score of 4.19. The third group included seven lines V2D1-2-5, V2D1-8-4, V2T, V3D1-2-3, V3D1-5-3, V3D1-6-2, V3T; the lower-performing and most severely affected lines. It had an average plant height of 54.84 cm, an average kernel weight of 15.33 g and an average severity score of 5.59

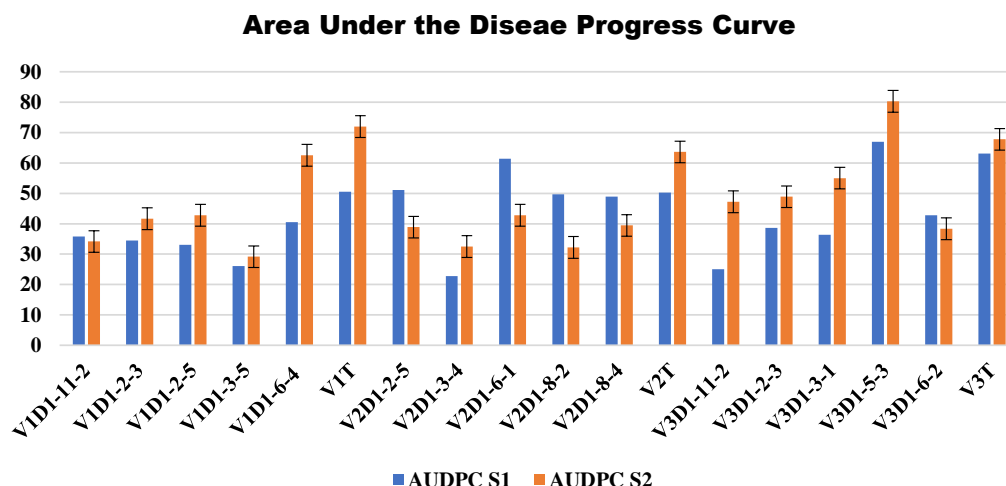


Figure 4. AUDPC on the lines tested by *Curvilaria* sp. Isolates.

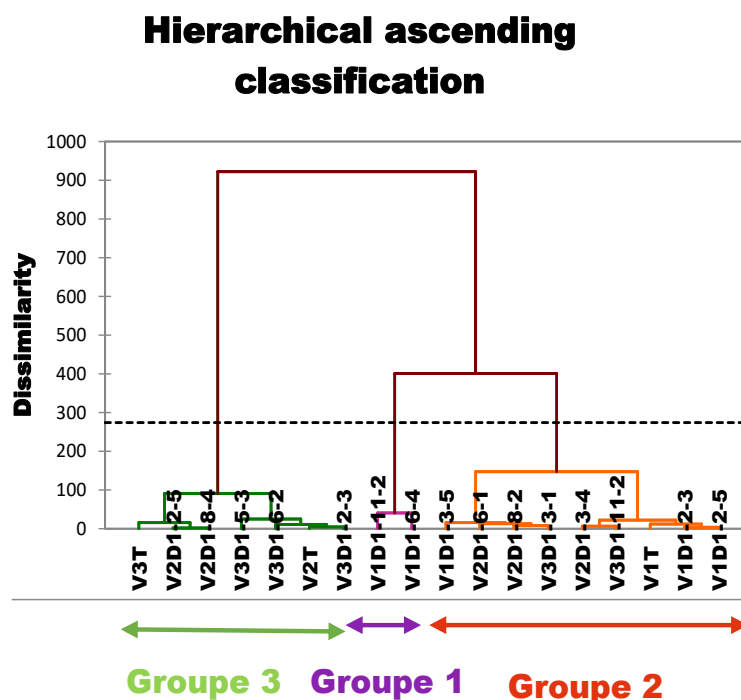


Figure 5. Hierarchical classification of the lines in ascending order according to their performance.

Table 6. Agro-morphological performance and sensitivity of AHC groups.

Group	HP	BM	15 DAI S1
1	81.61	20.83	3.83
2	66.07	18.97	4.19
3	54.84	15.33	5.59

HP: Plant height; BM: Back biomass DAI: Days after inoculation, S1: isolate 1.

DISCUSSION

Fonio is generally grown in areas with high rainfall. In some cases, it is part of dietary habits and customary principles. Previous studies (Cruz et al., 2011) have shown that the Boucle du Mouhoun region is the leading producer in terms of both area and production. This explains the high number of samples compared to the other regions studied. There was a high frequency of

three mushroom genera on this site. The results of the analysis of leaf samples and seeds using a magnifying glass, microscope, and fungal identification keys identified 14 fungal genera, including 10 genera observed on seeds, eight genera observed on leaves collected at the station, and seven genera on leaves collected during the survey. These 14 genera are *Alternaria* sp, *Bipolaris* sp, *Botrytis* sp, *Cercospora* sp, *Cladosporium* sp, *Colletotrichum* sp, *Curvularia* sp, *Drechslera* sp, *Fusarium* sp, *Nigrospora* sp, *Penicillium* sp, *Phoma* sp, *Rhizopus* sp, *Stenocarpella* sp. Work on other Poaceae, particularly sorghum, has shown the presence of most of these fungi on sorghum samples in Burkina Faso (Zida et al., 2008). Some of these fungi, such as *Fusarium moniliforme*, *Penicillium* sp, and *Phoma* sp, are toxigenic (Magan and Aldred, 2005; Murphy et al., 2006). The genus *Alternaria* was found on both leaves and seeds in our study. Similar results have been reported by some authors. According to Meddah et al. (2010), *Alternaria* damage can be observed on leaves, stems, and fruits of host plants. The genus *Curvularia* is one of the most common species found on both sorghum seeds and seeds of wild Poaceae species (Bonzi et al., 2012). The same genus is present both on fonio seeds and on the collected leaves, with relatively high frequencies. These results show the extent to which this fungus can affect Poaceae species. The two isolates of *Curvularia* Sp were virulent against fonio lines because they induced leaf changes. These changes were also observed on fonio plants in Benin by Valerien et al. (2020). Similar results were observed on inoculated plants by André et al. (2004). They pointed out that *Curvularia* is an important species responsible for macular leaf diseases. According to El Abdellaoui et al. (2005), the symptoms characteristic of curvulariosis can be explained by a compatible interaction between host and fungus.

The results of the analysis of variance of the mean severity scores showed a difference in the pathogenicity of the two isolates. Isolate S2 was virulent against all three cultivars and had a higher severity score than isolate S1. Indeed, Johnson and Taylor (1976) found that the pathogenicity of the parasite can be reflected by the number of spores produced by the pathogen on the host. The symptoms of *Curvularia* on *Digitaria* leaves are characterized by numerous brown to black, elongated, or circular spots with darker outlines. Infection begins at the periphery of the leaf and progresses towards the center, with severely affected leaves eventually wilting and dropping. In the course of the experiment, the symptoms of curvulariosis on fonio leaves were confused at the beginning of the infection, but eventually became distinct from those caused by the other fungi, helminthosporiosis, and blast. The same observation was made on rice plants by Dioulasso (2021) and Hassikou et al. (2002). Analysis of variance comparing the two isolates with the control showed no difference. In addition, the lines showed different responses to the two isolates of

Curvularia Sp. The variability observed in the degree of pathogenicity may correspond to host-parasite genotypic interactions (Bousslim et al., 1997). Similar results were found by Hassikou et al. (2002) on Moroccan isolates of *C. lunata*, which damaged the leaves of rice cultivars grown in Morocco with different pathogenicities.

The analysis of variance of the scores assigned to the lines showed that lines V1D1-11-2 and V1D1-2-3 all had low levels of infection with the two isolates, whereas V3D1-5-3 had high levels of infection with isolate S1 and was very susceptible to isolate S2. The areas of disease progression of the lines were scored differently with different amplitudes depending on the line, confirming the variable susceptibility of the lines. The CAH was constructed using three characteristics, namely height, dry biomass, and severity score, which allowed the population to be divided into three groups. The 1st group was considered to be the best group with the best agronomic performance and the lowest severity score.

The 2nd group contains the majority of the population with average performance in both agronomic and severity scores, and the 3rd group contains lines with high severity scores and low agronomic scores. This structuring can be explained by the fact that the behavior of a population of host plants towards a pathogen is determined by the genotype of these plants (Lepoivre, 1989).

Conclusion

The aim of this study was to make an inventory of the different species found on fonio, and we were able to identify the different fungal genera associated with fonio cultivation. We were able to count 15 genera of fungi distributed on the different organs of the plant. Given the high frequency of the genus *Curvularia* sp on both seeds and leaves, our study focused on the isolation and pathogenicity of this genus. Observation of the symptoms on the leaves of the fonio lines showed that *Curvularia* sp was pathogenic. Regarding the aggressiveness of the two isolates used, the results showed that isolate S2 was more aggressive than isolate S1. The determination of varietal resistance showed that lines V1D1-11-2 and V1D1-3-5 were resistant to both isolates, whereas V3D1-5-3 was susceptible to both isolates, with a very high difference in susceptibility for isolate S2. The smallest area of progression was found in V1D1-3-5. The latter could be proposed as a resistant cultivar for heavily infested areas. In the future, the study should be repeated to confirm the response of resistant lines in the field, and multilocation testing should be considered to confirm the phenotype of the lines

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors thank the International Atomic Energy Agency (IAEA) for providing the mutant material and the entire team of the Phytopathology Laboratory of the INERA Farako-Bâ Research Station for their support.

RÉFÉRENCES

- Akanmu AO, Abiala MA, Odebode AC (2013). Pathogenic Effect of Soilborne Fusarium Species on the Growth of Millet Seedlings. *World Journal of Agricultural Sciences* 9(1):6068. DOI: 10.5829/idosi.wjas.2013.9.1.1721
- Blench RM (2012). Vernacular Names for African Millets and Other Minor Cereals and Their Significance for Agricultural History. *Archaeological and Anthropological Sciences* 8(1):1-8.
- Bonzi S, Somda I, Zida E, Sérémé P (2012). Efficacy of plant extracts and effect of seed soaking duration on treatment of sorghum seed naturally infected by *Colletotrichum graminicola* and *Phoma sorghina*. *Archives of Phytopathology and Plant Protection* 45:1404-1410.
- Cruz JF (2007). Upgrading quality and competitiveness of fonio for improved livelihoods in West Africa. First year activity report. INCO FONIO, Specific International Scientific Cooperation Activities. Montpellier, France: CIRAD.
- Cruz JF, Beavogui F, Drame D (2011). Le fonio, une céréale africaine. *Collection Agricultures tropicales en poche*. Editions. Quae / Cta / Presses agronomiques de Gembloux: Versailles, France 175 p.
- Dansi A, Adokounou SH, Vodouhè R (2010). Diversity, conservation and related wild species of fonio millet (*Digitaria* spp.) in the northwest of Benin. *Genetic Resources and Crop Evolution* 57:827-839. DOI: 10.1007/s10722-0099522-3.
- DGESS/MAAH (2020). *Annuaire des statistiques agricoles 2018*. Burkina Faso. http://cns.bf/IMG/pdf/annuaire_agriculture_2020_def.pdf
- Dioulasso B. Caractérisation des symptômes foliaires et évaluation du pouvoir pathogène de quelques isolats de *Curvularia lunata* sur trois variétés de riz (*Oryza sativa* L) produites au Burkina Faso. *Afrique Science* 19(4):106-17.
- FIBL (2013). *African Organic Agriculture Training Manual*. Version 2.0 July 2013. Edited by Gilles Weidman and Lukas Kilcher. Research Institute of Organic Agriculture FIBL, Frick
- Hassikou R, Hassikou K, Touhami AO, Douira A (2002). Effet in vitro et in vivo de quelques fongicides sur *Curvularia lunata*. *Institut Agronomie et Vétérinaire* 22(4):205-213. <https://core.ac.uk/download/pdf/230579279>.
- Johnson R, Taylor AJ (1976). In *Investigations of the Race - Specificity of Host Resistance*. Plant Breeding Institute pp. 97-119.
- Lepoivre M, Boudbid H, Petit JF (1989). Antiproliferative activity of γ -interferon combined with lipopolysaccharide on murine adenocarcinoma: dependence on an L-arginine metabolism with production of nitrite and citrulline. *Cancer Research* 49(8):1970-6.
- Magan N, Aldred D (2005). Condition of formation of ochratoxin A. in drying transport and in different commodities. *Food Additives and Contaminants Supplement* pp. 10-16.
- Mathur SB, Kongsdal O (2003). Common laboratory seed health testing methods for detecting fungi. <https://www.semanticscholar.org/paper/Common-laboratory-seed-health-testing-methods-for-Mathur-Kongsdal/e572a6c5d9207f6aadaf5c97956c1930da2ad75b>
- Meddah N, Ouazzani Touhami A, Douira A (2010). Mycoflore associée au bananier (*Musa accuminata* L.), variété Grande naine, cultivé sous serre dans la région du Gharb (Maroc). *Bulletin de l'Institut Scientifique, Rabat, Section Sciences de La Vie* 32(1):1-11.
- Murphy PA, Hendrich S, Landgren C, Bryant CM (2006). Food mycotoxins: an update. *Journal of Food Science* 71(5):R51.
- Valerien ZA, Emmanuel S, Mahfouz D, Gildas T, Ayouba KT, Néhal DM, Cyrille K (2020). *Curvularia* leaf spot on white fonio accessions in the commune of Boukoumbe in north Benin. pp. 7695-7706
- Vodouhè SR, Achigan-Dako EG (2006). *Digitaria exilis* (Kippist) Stapf. In: Brink M, Belay G, (eds). *Plant Re-sources of Tropical Africa*. Oegstgeest, The Netherlands: Backhuys Publishers pp. 59-63
- Zida PE, Sérémé P, Leth V, Sankara P, Somda I, Néya A (2008). Importance of seed-borne fungi of sorghum and pearl millet in Burkina Faso and their control using plant extracts. *Pakistan Journal of Biological Science* 11(3):321-331.
- Zinsou AV, Sekloka E, Dramane M, Tchamadon G, Kora TA, Djaouga MN, Kanlindogbe C (2020). *Curvularia* leaf spot on white fonio accessions in the commune of Boukoumbe in north Benin. *Journal of Animal and Plant Sciences* 44(3):7695-7707. <https://doi.org/https://doi.org/10.35759/JANmPISci.v44-3.2>