

Full Length Research Paper

Morphological, molecular and pathogenic characterization of *Fusarium* species infecting quinoa (*Chenopodium quinoa* Willd) in Burkina Faso

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Quinoa (*Chenopodium quinoa* Willd.), recently introduced in Burkina Faso, is facing fungal diseases, particularly those caused by *Fusarium oxysporum*. This study aimed to characterize 44 fungal isolates collected from infected quinoa organs. Morphological characterization on Potato Dextrose Agar (PDA) medium revealed high variability: 57.9% of isolates exhibited pink pigmentation and 62.4% produced dense mycelia. Colony diameters ranged from 2.26 to 9.00 cm, and spore concentrations reached up to 2.41×10^7 conidia/ml. Molecular analysis confirmed that 35 isolates belonged to the *Fusarium* genus, including 32 identified as *F. oxysporum*. Hierarchical cluster analysis revealed three distinct groups based on growth and sporulation characteristics. Pathogenicity tests indicated significant variability, with severity indices up to 91.67% and a seedling damping-off rate (SDR) of 25%. A weak but positive correlation was observed between radial growth and virulence depending on the inoculation method. These findings provide a basis for quinoa varietal selection and the development of sustainable fungal disease management strategies in Burkina Faso.

Key words: *Fusarium* species, quinoa, diversity, polymerase chain reaction (PCR), pathogenicity, Burkina Faso.

INTRODUCTION

Quinoa, like all crops, is vulnerable to pathogens, responsible for diseases whose severity depends on the

pathogen and environmental conditions. Known diseases include downy mildew caused by *Peronospora* species,

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fusariosis (*Fusarium* species), leaf spot (*Ascochyta hyalospora*), brown spot (*Phoma exigua*), damping-off (*Rhizoctonia solani*) and Sclerotinia (*Sclerotium rolfsii*) (El-Moity et al., 2015; Danielsen and Munk, 2004).

In Burkina Faso, our previous studies revealed various fungal diseases on quinoa, including damping-off, crown rot, stem rot, plant wilting, panicle browning followed by complete plant desiccation, and chlorosis with necrotic brown leaf spots. Browning and desiccation were the most severe symptoms observed. *Fusarium oxysporum* was identified in most pathologies. Pathogenic studies have shown that this fungus can reduce yield by 58% compared with uninfected controls (Nebié, 2021).

The genus *Fusarium*, a cosmopolitan group of fungi, comprises around 300 phylogenetically distinct species, grouped into 23 species complexes (Dongzhen et al., 2020). This fungus is attracting particular attention due to its impact on many crops worldwide, resulting in considerable economic losses (Chandra et al., 2011). In addition, over 70 species of *Fusarium* are considered opportunistic pathogens, capable of causing serious infections in immunocompromised patients (Bansal et al., 2019). The mycotoxins produced by some *Fusarium* species can also cause serious health problems in animals and humans (Bertero et al., 2018).

F. oxysporum is a cosmopolitan pathogen capable of producing mycotoxins hazardous to humans. Its ability to infect various plant tissues and organs at all stages of development results in reduced yield and lower quality of agricultural products (Ryabova et al., 2021). Diseases caused by *Fusarium* spp., particularly *F. oxysporum*, represent a major challenge for crop management (Arie, 2019).

Consequently, thorough characterization of this plant pathogen is essential to develop effective management strategies. Molecular diagnostics, using markers such as translation elongation factor (EF1- α), β -tubulin, calmodulin and internal transcribed spacing (ITS) (Palacios et al., 2021), is a crucial tool for identifying, classifying and differentiating *Fusarium* spp. In addition, the variability of *F. oxysporum* can vary considerably around the world. It is therefore necessary to assess this variability in *F. oxysporum* isolates from different agro-ecological zones in Burkina Faso.

This is the background to the present study, the main aim of which is to characterize *F. oxysporum* isolates obtained from diseased quinoa organs in Burkina Faso. This characterization will be based on morphological, molecular and pathogenicity analyses. The results of this study will enable us to gain a better understanding of the diversity of this pathogen, and to develop appropriate management strategies to protect the quinoa crop in Burkina Faso.

MATERIALS AND METHODS

Study sites

The fungal material for this study was collected from three distinct agro-ecological zones of Burkina Faso, as detailed in Table 1. The selection of these zones was based on their varied climatic and agricultural conditions, which allowed for the collection of a diverse range of fungal isolates. All subsequent laboratory work, including morphological characterization, molecular analyses, and pathogenicity tests, was conducted at specialized research centers located in Bobo-Dioulasso and Farako-Bâ.

Fungi material

A total of 44 *Fusarium* spp. isolates were obtained from symptomatic quinoa plants showing signs of wilt and leaf infection. The samples were collected between 2020 and 2021 from various sites within the three designated agro-ecological zones. This collection strategy, targeting symptomatic plant parts, ensured the isolation of potentially pathogenic strains. Morphological characterization of these isolates was performed on PDA medium, and microscopic observation of conidia and other fungal structures was carried out.

Plant

The quinoa variety used for pathogenicity testing was Fenï, selected for its good adaptation to local agro-climatic conditions and its desirable agro-morphological characteristics (Dao et al., 2019). This variety is known to be susceptible to *Fusarium* infections, making it a suitable host for evaluating the pathogenicity of the isolated strains.

Morphological characterization of isolates

Fusarium spp. isolates were vivified on Potato Dextrose Agar (PDA) medium and incubated at 22°C, under a 12 h UV light alternating with 12 h darkness for 5 days. For each isolate, four mycelial explants (5 mm in diameter) were taken from the margin of 5-day-old colonies and individually transferred to the center of Petri dishes containing PDA. The experimental set-up was a completely randomized block with 44 treatments (isolates), each repeated four times. Traits evaluated included mycelial growth rate (colony diameter measured at 4, 7 and 10th days after incubation), sporulation and cultural traits. Sporulation was assessed on day 10^(e). Each colony was covered with 10 ml sterile distilled water, scraped and filtered to recover conidia. The number of conidia was estimated using a hemacytometer (Fuchs-Rosenthal cell). Suspensions that were too concentrated were diluted if necessary.

Cultural traits observed included colony texture (cottony, velvety, rough), mycelium density, color (pinkish, gray, whitish, etc.) and margin shape. These observations were carried out according to standardized methods for *Fusarium* identification (Leslie and Summerell, 2006).

Assessing the pathogenicity of isolates

Substrate preparation and sowing

A substrate consisting of two-thirds mature compost and one-third

Table 1. Geographic location and collection sites of the *Fusarium* spp. isolates.

No.	Code	Localities	Latitude	Longitude	No.	Code	Localities	Latitude	Longitude
1	BAA1	Banfora	10° 38' 15" N	4° 45' 55" O	23	KMA2	Kombissiri	11° 51' 21.44" N	1° 55' 58.38" O
2	BAA2	Banfora	10° 38' 15" N	4° 45' 55" O	24	KOA1	Koloko	10° 40' 59" N	4° 34' 19" O
3	BTA1	Gbomblora	10° 23' 48" N	2° 59' 38" O	25	KOA2	Koloko	10° 40' 59" N	4° 34' 19" O
4	BTA2	Gbomblora	10° 23' 48" N	2° 59' 38" O	26	LA1	Lanfièra	12° 47' 11" N	3° 28' 17" O
5	DA1	Di	13° 01' 06" N	3° 34' 33" O	27	LA2	Lanfièra	12° 47' 11" N	3° 28' 17" O
6	DA2	Di	13° 01' 06" N	3° 34' 33" O	28	OA1	Ouagadougou	12° 10' 53" N	1° 38' 04" O
7	DIA2	Diabo	11° 31' 23" N	0° 00' 27" E	29	OA2	Ouagadougou	12° 10' 53" N	1° 38' 04" O
8	DJA1	Djibo	13° 56' 6.73" N	1° 47' 7.37" O	30	SA1	Solenzo	12° 11' 42" N	4° 05' 12" O
9	DJA2	Djibo	13° 56' 6.73" N	1° 47' 7.37" O	31	SA2	Solenzo	12° 11' 42" N	4° 05' 12" O
10	DPA1	Diapaga	11° 46' 27" N	2° 03' 29" E	32	SAA1	Saria	11° 50' 52" N	1° 27' 55" O
11	DRA1	Dori	13° 37' 58.51" N	0° 15' 51.58" O	33	SAA2	Saria	11° 50' 52" N	1° 27' 55" O
12	DRA2	Dori	13° 37' 58.51" N	0° 15' 51.58" O	34	SMSS1	Soumouso	10° 46' 09" N	4° 20' 38" O
13	FA1	Fara	11° 18' 23" N	2° 47' 52" O	35	SMSS4	Soumouso	10° 46' 09" N	4° 20' 38" O
14	FBSS1	Farako-Bâ	10° 42' 7.50" N	1° 21' 9.92" E	36	TCA1	Tchériba	12° 15' 44" N	3° 05' 09" O
15	FBA2	Farako-Bâ	10° 42' 7.50" N	1° 21' 9.92" E	37	TCA2	Tchériba	12° 15' 44" N	3° 05' 09" O
16	IA1	Iolonioro	10° 46' 26" N	3° 26' 39" O	38	TEA1	Tenkodogo	11° 46' 25" N	0° 22' 08" O
17	IA2	Iolonioro	10° 46' 26" N	3° 26' 39" O	39	TEA2	Tenkodogo	11° 46' 25" N	0° 22' 08" O
18	KA1	Kouaré	11° 29' 34" N	0° 08' 04" E	40	TIA2	Tiéfora	10° 21' 53" N	4° 36' 36" O
19	KA2	Kouaré	11° 29' 34" N	0° 08' 04" E	41	TTA1	Titao	13° 48' 15" N	2° 04' 40" O
20	KBA1	Kelbo	13° 49' 32" N	1° 13' 54" O	42	TTA2	Titao	13° 48' 15" N	2° 04' 40" O
21	KBA2	Kelbo	13° 49' 32" N	1° 13' 54" O	43	WA1	Wéléni	10° 50' 37" N	5° 20' 35" O
22	KMA1	Kombissiri	11° 51' 21.44" N	1° 55' 58.38" W	44	YA1	Yendéré	10° 00' 34" N	5° 05' 14" O

sand was sterilized at 120°C for 4 h. After cooling, it was distributed in 9-liter pots. The quinoa seeds (var. Feni) were disinfected in a 2% NaClO solution for 5 min, rinsed with sterile water and then sown. The crop was maintained in a greenhouse for 4 weeks under semi-controlled conditions.

Substrate inoculation

Conidial suspensions (10⁶ conidia/ml) were prepared from 10-day-old sporulating colonies. One kilogram of sterile substrate was homogenized with 10 ml of this suspension for each isolate. After incubation at 22°C, under a cycle of 12 h UV light alternating with 12 h darkness for 10 days, the substrate was distributed in pots (4 replicates per isolate). A negative control (sterile water) was included.

Leaf inoculation

For leaf inoculation, two methods were used for foliar assessment.

Method 1: Inoculation by wounding

Micro-injuries were made on the surface of quinoa leaves using

carborundum. A volume of 6 µl of conidial suspension (10⁶ conidia/ml) was deposited on each leaf (Nalam et al., 2016; Fonseca-Guerra et al., 2022).

Method 2: inoculation with mycelial explants

Four mycelial explants (5 mm) of each isolate were deposited on spreading leaves in moistened Petri dishes, mycelial side in contact with the main vein (Pal and Testen, 2020). Each isolate was evaluated on 4 leaves, with 15 replicates, in a completely randomized block design comprising 44 isolates and one control.

Pathogenicity assessment

Leaf symptoms (chlorosis, necrosis) were recorded 5 days after inoculation. A severity scale of 1 to 4 was used: 1 = no symptoms, 2 = < 25% of surface affected, 3 = 25 to 50%, 4 = > 50%. The severity index (IS) was calculated according to the following formula (Nalam et al., 2016):

$$I(s) (\%) = 100 \times \sum (I \times n_i) / (N \times K)$$

where I: severity grade, n_i: number of leaves with this grade, N: total number of leaves, K: maximum grade.

Three parameters were measured for soil infestation:

- 1) Seeding damping-off at 5th day after sowing (SDR)
- 2) Mean plant height (MPH)
- 3) Mean root length at day 10th (MRL)
- 4) Mean plant weight (MPW)

Molecular characterization of isolates

Deoxyribonucleic acid (DNA) extraction

Fungal isolates were subcultured on PDA medium and incubated for seven days at room temperature. Mycelium was scraped from the surface and immediately frozen at -20°C for 24 h, before being frozen for 48 to 72 h. Genomic DNA was extracted from the freeze-dried mycelium using the Wizard® Genomic DNA Purification kit (Promega), following a slightly modified protocol. Approximately 200 to 500 mg of the mycelium was transferred to a tube containing 600 µl of nuclear lysis solution. The mixture was incubated at 65°C for 15 min before proceeding with the standard steps of the manufacturer's protocol. The extracted DNA was then quantified using a NanoDrop™ 1000 spectrophotometer (Thermo Scientific) to assess its purity and concentration. To ensure optimal conditions for the PCR, the DNA was diluted to a working concentration of 20 ng/µl.

PCR amplification and analysis

To characterize the isolates, two genetic regions were targeted using Polymerase Chain Reaction (PCR): the Internal Transcribed Spacer (ITS) region, widely used as a universal barcode for fungi, and the Elongation Factor 1-alpha (EF-1α) gene, which offers higher resolution for discriminating between *Fusarium* spp. The primers used for the ITS region were ITS-Fu-f (5'-CAACTCCCAAACCCCTGTGA-3') and ITS-Fu-r (5'-GCGACGATTACCAGTAACGA-3'), while those for EF-1α were EF1-983F (5'-GCTCCYGGHCAATCGTGAYTTYAT-3') and EF1-2218R (5'-ATGACACCRACRGCRACRGTYTG-3').

The PCR for the ITS region followed these conditions: initial denaturation at 94°C for 3 min, followed by 35 cycles of denaturation at 95°C for 1 min, hybridization at 56°C for 30 s, and extension at 72°C for 1 min, with a final extension of 7 min at 72°C. For the EF-1α gene, a protocol adapted from O'Donnell et al. (2022) was applied, consisting of an initial denaturation at 95°C for 5 min, followed by 30 cycles of 94°C for 1 min, 60°C for 50 s, and 72°C for 1 min, with a final extension of 3 min at 72°C.

The amplification products were analyzed by electrophoresis on a 1.2% agarose gel, run at 120 volts for one h. After migration, the gel was stained with ethidium bromide and visualized under UV light. The electrophoretic profiles for 35 of the 44 isolates showed the presence of a single, distinct band of approximately 400 bp. This size is consistent with the expected amplicon for the targeted ITS and EF-1α regions, suggesting that these isolates belong to the genus *Fusarium*. Based on the specificity of the primers used and their known targets, the results further indicate a strong likelihood that the species is *F. oxysporum*.

Data analysis

Quantitative data collected on mycelial growth, sporulation,

seedling traits, and severity indices were used to evaluate differences among *Fusarium oxysporum* isolates. Prior to statistical analyses, assumptions of normality and homogeneity of variances were verified. Normality was assessed using the Shapiro-Wilk test, while Levene's test was applied to confirm variance homogeneity. When assumptions were met, one-way analysis of variance (ANOVA) was conducted to detect significant differences among isolates for each variable. Mean separation was performed using Student's t-test at a 5% significance level ($p \leq 0.05$). A hierarchical ascending classification (HAC) was performed to group isolates based on their morphological and pathogenic characteristics. The analysis was conducted in R software (R Core Team, 2022), using Euclidean distance and Ward's method of aggregation. All statistical analyses were carried out using R (version 4.2.2).

RESULTS

Morphotypes of *Fusarium* spp.

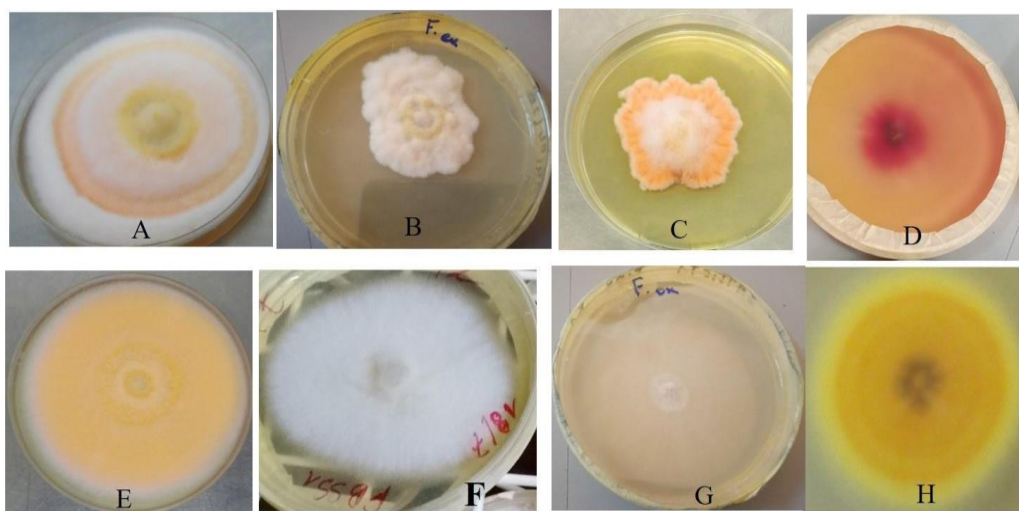
Analysis of the qualitative morphological characteristics of *Fusarium* spp. isolates revealed high phenotypic variability on PDA medium, as presented in Table 2 and illustrated in Figure 1. A diversity of colony colors was observed. Pink coloration was the most frequent (57.90%), followed by whitish pink (35.40%). Less common shades included whitish (2.20%), orange (2.20%), and purple, pink (2.20%). These variations are visible in the micrographs (Figure 1A to F). Regarding mycelial density, three development types were identified: dense mycelium (62.40%), low-density mycelium (20.20%; Figure 1E and F), and absence of mycelial growth (17.40%; Figure 1G). The elevation and texture of the colonies also varied: 37.10% of isolates exhibited aerial mycelium, while 38.20% showed slightly elevated growth with a velvety appearance (Figure 1E and F), and 24.70% had flat colonies (Figure 1G). Margin morphology revealed two main types: regular (46.60%; Figure 1A and E) and irregular (53.40%; Figure 1B and C), with no dominant pattern observed. Pigmentation was not consistently observed across isolates. A total absence of pigmentation was recorded in 82% of the samples. However, some isolations exhibited purple (11.20%; Figure 1D), pink (2.20%), or white, purple (2.20%; Figure 1H) pigmentation.

Analysis of morphological variability in *Fusarium* spp. isolates

The study of mycelial growth and certain biological

Table 2. Averages of qualitative characteristics of *F. oxysporum* isolates expressed as a percentage

Feature	Terms and conditions	Percentage of isolates
Colony color	Pink	57.90
	Whitish	02.20
	Orange	02.20
	Whitish pink	35.40
	Purple/Pink	02.20
Mycelium density	Dense	62.40
	Low density	20.20
	Absent	17.40
Elevation of mycelium	Aerial	37.10
	Slightly aerial	38.20
	Flat	24.70
Margin shape	Regular	46.60
	Irregular	53.40
Pigmentation	Absent	82.00
	Purple	11.20
	Pink	02.20
	White purple	02.20

**Figure 1.** Different morphotypes of *Fusarium* spp.

characteristics of *Fusarium* spp. isolates revealed significant variability between the isolates tested (Table 3). Analysis of radial growth on days 4^e, 7^e and 10^e after incubation (JAI) showed very highly significant differences

between isolates (observed $F = 12.96$ to 19.52 ; $p < 0.001$), confirming the heterogeneity of mycelial development. The mean diameters recorded increased progressively over time: 3.96 mm at 4 JAI, 6.20 mm at 7

Table 3. Morphological and growth variability of *Fusarium* spp. isolates.

Characters	F observed	Minimum (mm)	Maximum (mm)	Average (mm)	CV (%)
D1 (mm)	12.96***	2.26	6.8	3.96	20.13
D2(mm)	19.52***	3.27	9	6.2	19.1
D3(mm)	15.17***	3.78	9	7.47	15
NC	24.02***	16.10 ³	24 × 10 ⁷	16.4 × 10 ⁶	26.98
NB	16.49***	0	4	01	20.11

CV: coefficient of variation; JAI: days after incubation; NC: number of conidia per mL; NB: number of bands; D1, D2 and D3 respectively colony diameter measured at 4th, 7th and 10th days after incubation.

JAI, and 7.47 mm at 10 JAI. The coefficient of variation (CV), between 15% and 20%, indicates a moderate dispersion of values within isolate populations. Significant variability was also noted for physiological traits: the number of conidia produced per ml of inoculum (NC) showed a wide range (16×10^3 to 24.10^7), with an average of 16.4×10^6 and a high CV of 26.98%, reflecting a high inter-isolate dispersion. The number of bands formed (NB) was also highly variable (from 0 to 4), with an average of around 1 band and a CV of 20.11%.

Classification of isolates according to quantitative characteristics

By analyzing quantitative characteristics related to mycelial growth (colony diameter at 4, 7 and 10 days after incubation) and conidial production (NC), we were able to classify *Fusarium* spp. isolates using hierarchical ascending classification (HAC). This classification was based on Euclidean distance and Ward's aggregation method, renowned for its robustness in multivariate data analysis.

The resulting dendrogram (Figure 2) was truncated at a threshold to identify three distinct classes of isolates. Class I comprises 21 isolates. This class is characterized by weak to moderate mycelial growth at the different evaluation dates, and average conidial production. It could correspond to isolates with slow but stable development. Class II contains 11 isolates. These show rapid mycelial growth combined with high sporulation capacity. This group could contain particularly vigorous and potentially more competitive isolates. Class III contains 13 isolates, distinguished by high radial growth, but relatively low conidial production. This dissociation between vegetative growth and asexual reproduction may reflect different biological strategies within the population.

This structuring reveals significant phenotypic diversity

within *F. oxysporum* isolates, in relation to their growth and sporulated power. These results are useful in guiding the selection of representative isolates for pathogenicity or genetic studies.

Relationship between radial growth and sporulation of *Fusarium* spp. isolates

To examine the existence of a relationship between mycelial growth and sporulation of *Fusarium* spp. isolates, colony size measured at 10 days after incubation (10 JAI) was compared with the number of conidia produced per ml of inoculum. The results showed a very weak positive correlation between these two parameters ($R^2 = 0.052$), indicating that the increase in colony size was accompanied by only a slight increase in sporulation (Figure 3). This weak correlation suggests that other biological or physiological factors strongly influence spore production.

Molecular diagnosis of *Fusarium* spp. isolates

PCR analysis using primers ITS1-Fu-f / ITS4-Fu-r, targeting ribosomal DNA, confirmed that most isolates belonged to the *Fusarium* genus. Of the 44 isolates tested, 35 were positive, showing an expected band size of around 400 bp after agarose gel migration. These results indicate that these 35 isolates most probably belong to the *Fusarium* genus (Table 4). For specific identification, primers EF1-983F and EF1-2218R, targeting the elongation factor 1-alpha (EF1 α) gene, were used (Table 4). The expected size of the amplified product is around 70 bp, characteristic of the *F. oxysporum* species. Of the 35 isolates previously identified as *Fusarium*, 32 isolates showed a positive amplification profile with these primers, confirming their membership of the *F. oxysporum* species.

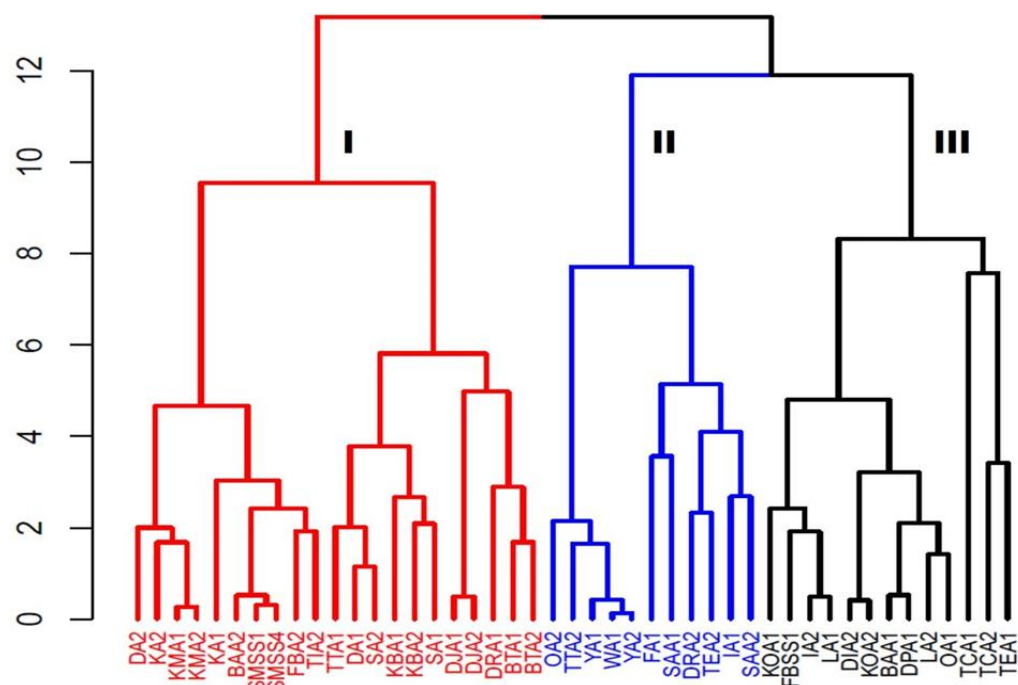


Figure 2. Different classes of *Fusarium* spp. Isolates.

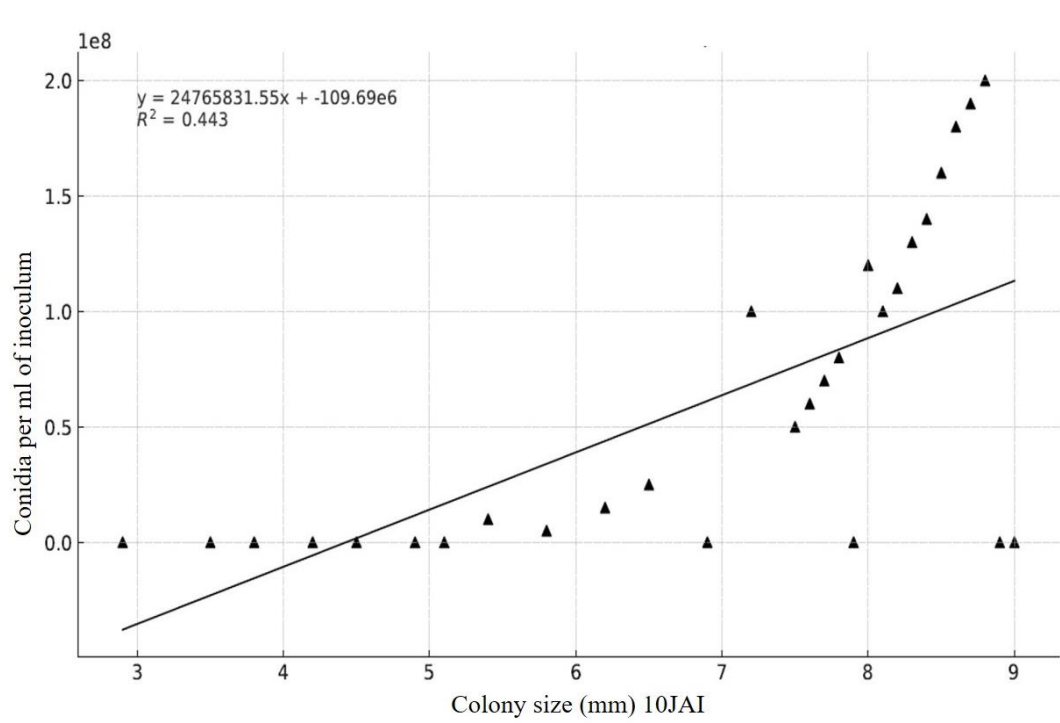


Figure 3. Correlation between radial growth and spore production of *Fusarium* spp. isolates.

Table 4. List of isolates amplified by the different primers.

No.	Code	Pair of primers		No.	Code	Pair of primers	
		ITS1 and 4-Fu	EF1 and 2			ITS1 and 4-Fu	EF1 and 2
1	BAA1	-	-	23	KMA2	-	-
2	BAA2	+	-	24	KOA1	+	+
3	BTA1	+	+	25	KOA2	-	-
4	BTA2	+	+	26	LA1	+	+
5	DA1	+	+	27	LA2	+	+
6	DA2	+	+	28	OA1	+	+
7	DIA2	+	+	29	OA2	+	+
8	DJA1	+	+	30	SA1	+	+
9	DJA2	+	-	31	SA2	+	+
10	DPA1	+	+	32	SAA1	-	-
11	DRA1	+	+	33	SAA2	+	+
12	DRA2	+	+	34	SMSS1	+	+
13	FA1	+	+	35	SMSS4	+	+
14	FBSS1	-	-	36	TCA1	-	-
15	FBA2	+	+	37	TCA2	+	+
16	IA1	+	+	38	TEA1	+	+
17	IA2	+	+	39	TEA2	+	+
18	KA1	+	+	40	TIA2	+	+
19	KA2	+	+	41	TTA1	+	+
20	KBA1	+	+	42	TTA2	-	-
21	KBA2	+	+	43	WA1	-	-
22	KMA1	-	-	44	YA1	+	+

+: Indicates an amplified isolate; -: indicates a non-amplified isolate

Table 5. Pathogenicity of isolates and results of analysis of variance.

Characters	F	Minimum	Maximum	Mean	Sd	CV (%)
SII (%)	72.89***	0	66.67	23.31	± 17.04	18.73
SIE (%)	23.40***	0	91.67	34.04	± 21.18	20.62
MRL (cm)	7.04***	1.71	5.65	3.04	± 00.81	23.27
TMH (cm)	6.60***	8.63	13.71	10.7	± 01.11	19.1
MPW (g)	1.22	0.01	0.83	0.11	± 00.07	3.06
SDR (%)	20.59***	0	25	10.41	± 06.43	27.62

Sd: Standard deviation; CV: coefficient of variation; SII: severity index by inoculum deposition; SIE: severity index by explant a deposition; MRL: mean root length; TMH: mean plant height; MPW: mean plant weight; SDR: seedling damping-off rate; ***, highly significant; F: Fisher's value.

Pathogenicity analysis of isolates of *Fusarium* spp.

For this activity, isolates found to be PCR-negative were simply discarded for pathogenicity characterization. The results of the analysis of variance applied to the pathogenic characteristics of the isolates are presented in

Table 5. The severity index by inoculum deposition (SII) ranged from 0.00 to 66.67%, with a mean of 23.31 ± 17.04% and a coefficient of variation (CV) of 18.73%. The explanta deposition severity index (SIE) ranged from 0.00 to 91.67%, with a mean of 34.04 ± 21.18% and a CV of 20.62%. Average root length (MRL) fluctuated between

Table 6. Comparison of pathogenicity of isolates versus control.

Character	df	P-value	CV (%)	Test-T
SII (%)	35	0.0001	31.59	23.83***
SIE (%)	35	0.0001	20.47	34.78***
MRL (cm)	35	0.0001	25.42	-2.29***
MPH (cm)	35	0.0001	10.14	-2.248***
MPW (g)	35	0.0001	27.27	0.02***
SDR (%)	35	0.0001	29.91	10.65***

df: degree of freedom; CV: coefficient of variation; SII: severity index by inoculum deposition; SIE: severity index by explanta deposition; MRL: mean root length; MPH: mean plant height; MPW: mean plant weight; SD: seedling damping-off rate; ***: highly significant.

1.71 and 5.65 cm, with a mean of 3.04 ± 0.81 cm. Average seedling size (MPH) ranged from 8.63 to 13.71 cm, with an average of 10.70 ± 1.11 cm. MPW ranged from 0.01 to 0.83 g, with a mean of 0.11 ± 0.07 g. Finally, the rate of damping-off (SDR) ranged from 0.00 to 25.00%, with an average of $10.41 \pm 6.43\%$ and a CV of 27.62%. Fisher tests revealed highly significant differences ($p < 0.001$) between isolates for all traits assessed, except for MPW, which showed no significant difference.

Pathogenicity of isolates of *Fusarium* spp.

Statistical comparison between *F. oxysporum* isolates and the uninfected control, using Student's t-test, revealed highly significant differences ($p < 0.0001$) for all pathogenic traits assessed (Table 6). The inoculum deposition severity index (SII) and explanta deposition severity index (SIE) showed high t-values (23.83 and 34.78 respectively), reflecting a strong divergence between the isolates and the control. Average root length (MRL) and average plant size (MPH) showed negative t-values (-2.29 and -2.25), indicating a significant reduction in vegetative growth in inoculated plants compared with the control. MPW was also significantly affected ($t = 0.02$), although the difference seemed more moderate. Finally, SDR showed a very marked difference from the control ($t = 10.65$), confirming the pathogenic impact of the isolates on seedling survival. Taken together, these results confirm the significant pathogenicity of *Fusarium* spp. isolates towards quinoa, with a clear effect on symptom severity, seedling growth and early-stage mortality.

Relationship between radial growth and pathogenicity of *Fusarium* spp. isolates

Analysis of the relationship between mycelial growth and

pathogenicity of *Fusarium* spp. isolates revealed contrasting results depending on the infestation method used. Using the explanta deposition method (Figure 4a), a very weak and negative correlation was observed between colony diameter at 10 days post-incubation (10 JAI) and the incidence of isolate severity ($R^2 = 0.0008$). This suggests that greater mycelial growth is globally associated with a slight decrease in virulence expressed on quinoa seedlings. However, the low value of the coefficient of determination indicates that this relationship is not significant and that other factors probably influence severity. In contrast, with the inoculum deposition method (Figure 4b), the relationship between colony size and pathogen severity was slightly positive ($R^2 = 0.0106$), indicating an inverse trend. In this case, faster-growing isolates appear to be slightly more virulent, although the correlation remains very weak. These results suggest that the link between in vitro mycelial growth and in planta pathogenicity depends on the inoculation method, and that the virulence of isolates cannot be predicted solely from their radial growth.

DISCUSSION

Morphological analysis of *F. oxysporum* isolates from samples collected in Burkina Faso on PDA medium revealed considerable variability in both mycelial growth and sporulation. This diversity was reflected in significant differences in colony diameter, texture, coloration and sporulation capacity. Classification based on quantitative characteristics enabled us to structure the isolates into three distinct groups, essentially defined by radial growth and sporulation capacity. Interestingly, high-growth isolates were on average less sporulating, while low-growth isolates were more sporulating.

However, statistical analysis revealed a significant positive correlation between mycelial growth and spore

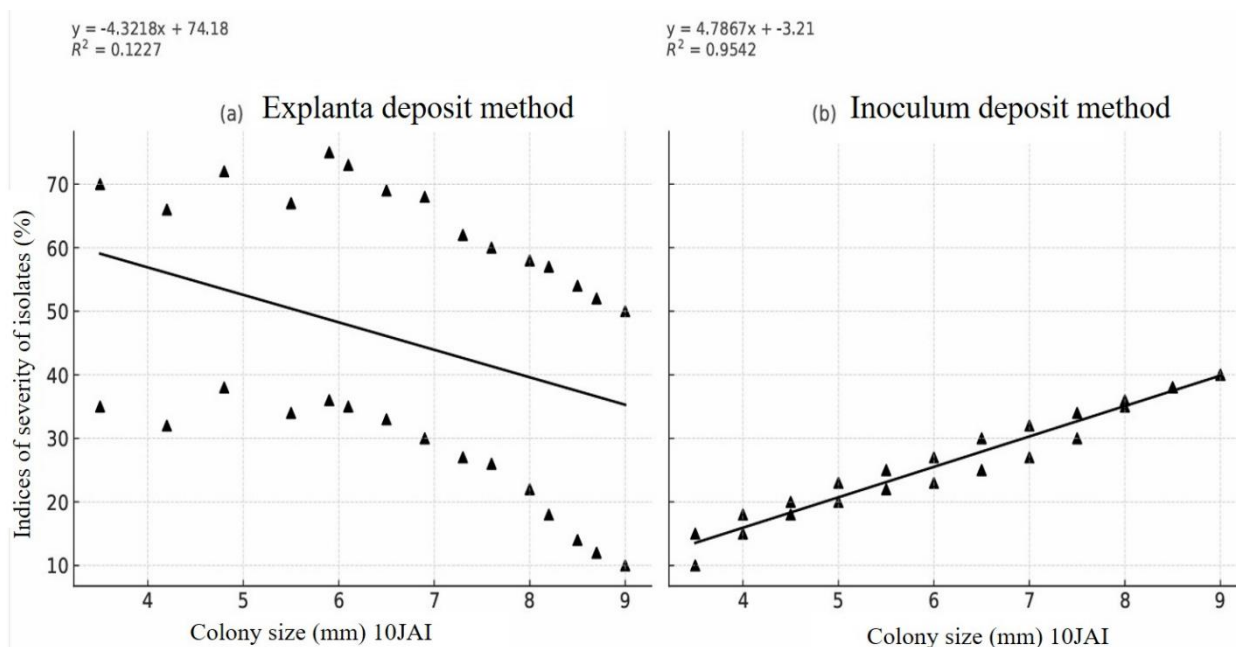


Figure 4. Correlation between radial growth and infectivity of *Fusarium* spp. isolates.

production ($p > 0.05$), which might seem contradictory.

This suggests that the overall trend is influenced by a few atypical isolates. Highly spore-forming isolates could benefit from an ecological advantage, giving them better survival in plant residues and soil, as well as a greater capacity for dissemination via storage organs. Conversely, rapid growth could promote efficient colonization of the host, enhancing infectivity.

These results are in line with those of Patil et al. (2005), Barhate et al. (2006) and Rafique et al. (2015), who reported a wide range of mycelial morphologies in *F. oxysporum*, from white to pinkish, and downy to dense textures. Similarly, Singh et al. (2006) observed irregular margins and whitish to pinkish coloration on PDA. This variability could be influenced by several factors, including the age of the culture, the number of subcultures, and the type of conidia produced (macro- or microconidia), as pointed out by Sedra and Lazrek (2011).

Morphological identification alone, however, cannot confirm the identity of isolates with any certainty, due to this high phenotypic variability. The molecular approach based on the ITS1/ITS4-Fu and EF1 α primers confirmed that 35 isolates belonged to the *Fusarium* genus, 32 of which were identified as *F. oxysporum*. The nine unconfirmed isolates could belong to other morphologically related fungal species, notably due to

frequent co-infections observed in certain localities (Soumouso, Lanfiéra, Koloko, Tiéfora).

Assessment of pathogenicity revealed marked variability in infectivity, depending on the isolation and the infestation method used. The explanta deposition method proved more sensitive, with a maximum severity index of 91.67%, compared with 66.67% for the direct inoculum deposition method, which identified several non-pathogenic isolates (KMA1, DJA2, IA2, DA1). However, certain isolates such as DRA2, IA1 and SAA2 stood out for their aggressiveness on several parameters. These observations confirm the work of Fonseca-Guerra et al. (2022) and Lai et al. (2020), who showed that inoculation methods significantly influence symptom expression.

On the other hand, a study by Miedaner et al. (2003) on 20 wheat varieties showed that spray inoculation was preferable for large-scale breeding, although both methods produced similar levels of disease. In this study, no significant correlation was detected between isolate sporulation and pathogenicity. On the other hand, a positive relationship between radial growth and aggressiveness was observed, suggesting that colonization speed could be a determining factor in virulence.

It is interesting to note that class III isolates, characterized by strong growth on PDA, were mostly

isolated from the northern and southern Sudanian agro-ecological zones. These warmer, more humid regions offer favorable conditions for the proliferation of phytopathogenic fungi. As a result, they could represent high-risk areas for quinoa crops, particularly in terms of food security.

Finally, *F. oxysporum* is recognized as one of the world's most destructive phytopathogenic fungi, affecting over 100 plant species (Zhang and Ma, 2017). According to Villa-Martínez et al. (2014), it can cause yield losses of up to 100% under optimal conditions. In addition to its agricultural effects, certain pathotypes belonging to the *F. oxysporum* complex (FOSC) have been implicated in human pathologies such as keratitis, onychomycosis or dermatitis (Batista et al., 2020; Veiga et al., 2018). Even more worryingly, Wang et al. (2020) have demonstrated that human pathogenic strains can colonize plants without causing visible symptoms, turning crops into silent reservoirs. This situation could favour the emergence of hypervirulent strains through genetic transfer between pathogenic and non-pathogenic isolates. In response to environmental stresses, *F. oxysporum* is also capable of producing cell wall degrading enzymes, effectors and mycotoxins, which play a crucial role in its pathogenicity (Zuriegat et al., 2021).

Conclusion

This study highlights the morphological and pathogenic diversity of *F. oxysporum* isolates associated with quinoa in Burkina Faso. The combined approach of morphological characterization and molecular identification revealed significant variability within the species, with no structuring according to the geographical origin of the isolates. Mycelial growth and spore production showed contrasting but statistically correlated behaviours, while pathogenicity varied according to isolate and inoculation method used.

These results underline the importance of considering both the biological characteristics of the pathogen and environmental conditions when assessing its impact. They provide an initial scientific basis for guiding quinoa breeding programs, developing integrated fungal disease management strategies, and promoting sustainable protection solutions adapted to the agroecological context of Burkina Faso.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interest.

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