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ORIGINAL ARTICLE

Characterization and pathogenicity of *Fusarium* species causing sugar beet root rot in Morocco

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Abstract

The sugar beet crop ranks second globally among the plant species grown mainly for sugar production. In Morocco, the area under sugar beet farming occupies approximately 57,000 ha yielding more than 3 million tons of roots. However, sugar beet root-tip rot (RTR) caused by *Fusarium* spp. dramatically reduces the anticipated yields, the purity of the resulting juice, and the sugar concentration. The current study aimed at identifying and characterizing the *Fusarium* species responsible for the root rot in sugar beet grown in the Khenifra-Beni Mellal region of Morocco. In this survey, 69 isolates of *Fusarium* were sampled from sugar beet roots showing typical symptoms of root rot from 2019 to 2021. After screening based on the pathogenicity test, 28 isolates were selected and identified based on morphological features and sequence analyses of the ribosomal internal transcribed spacer (ITS) region and translation elongation factor 1 α 34 (*TEF-1 α*). *Fusarium oxysporum* was the most frequently identified species, followed closely by *F. solani*, *F. equiseti*, *F. nygmai*, *F. brachygibbosum*, *F. proliferatum*, *F. culmorum*, and *F. falciforme*. Six weeks after inoculations under greenhouse conditions, the studied isolates caused internal vascular discoloration and tip rot of sugar beet roots, with disease incidences ranging from 37.5% to 100.0% and a disease index between 30.3% and 70.5%. Isolates belonging to *F. solani* were the most aggressive. Moreover, the majority of isolates significantly reduced plant growth. To our knowledge, this research article is the first report of *Fusarium* species inducing RTR in sugar beet in Morocco.

KEYWORDS
Beta vulgaris, *Fusarium*, ITS, root-tip rot, sugar beet, *TEF1*, vascular internal discoloration

1 | INTRODUCTION

The sugar beet (*Beta vulgaris* L.) is an annual crop that grows specifically in temperate regions. This plant is used to produce a wide variety of products, mainly sugar, ethanol, animal feed, and other products used in the chemical and pharmaceutical industries (Tomaszewska et al., 2018). Sugar beet is cultivated in diverse countries such as Brazil, France, Germany, India, Russia, and the USA (FAOSTAT, 2021). In 2019, around 57,172 ha were reserved for sugar beet production in Morocco, with production yields reaching an

estimated 3.7 million tonnes (FAOSTAT, 2021). In the Khenifra-Beni Mellal region (a large area in central Morocco), 15,000 ha are normally used for planting this crop, which contributes 26% to national production (ORMVAT, 2021). Despite its economic importance in this area, the sugar beet crop is frequently threatened by several pests and pathogens which reduces its yield and quality. Root rot is considered to be one of the main diseases affecting sugar beet in the Khenifra-Beni Mellal region. In fact, sugar beet root rot leads to significant losses in crop yield and quality (Farhaoui et al., 2022). It is caused by numerous soil-borne pathogens, namely *Sclerotium rolfsii* (Farooq et al., 2011; Fidah, 1995; Paul et al., 2021), *Rhizoctonia solani* (Bartholomäus et al., 2017; Buddemeyer & Märländer, 2005), and *Fusarium* spp. (Hanson et al., 2018; Hanson & Hill, 2004; Harveson & Rush, 1997; Ruppel, 1991).

The genus *Fusarium* is undoubtedly one of the widely studied fungal taxa in the world of fungi and can infect virtually all agriculturally important crops worldwide (Leslie et al., 2006). Numerous species of *Fusarium* were isolated from rotting roots of sugar beet, with these pathogens being either primary, secondary, or storage-related pathogens (Christ et al., 2011; Nitschke et al., 2009). *Fusarium*-induced root rot in sugar beet leads to a significant reduction in yield, juice purity, and sugar concentration (Cao et al., 2018; Hanson & Jacobsen, 2009). Several mycotoxins are normally secreted by *Fusarium* spp., and they are detrimental to the health of humans (Rai et al., 2020). In fact, many surveys revealed that roots of sugar beets are sometimes contaminated by mycotoxins produced specifically by *Fusarium* species (Boudra et al., 2015; Christ et al., 2011). Due to the economic damage, toxigenic ability, and biodiversity of *Fusarium* species responsible for the root rot in sugar beet, precise characterization of causative agents is essential.

Based on multi-locus phylogenetic analyses, the genus *Fusarium* comprises an estimated 300 species or more, which are grouped into 22 distinct complexes of *Fusarium* species (Aoki et al., 2014; Ezrari et al., 2021; Geiser et al., 2021). Notably, four distinct species complexes of *Fusarium* possess the higher number of phytopathogenic species: *F. oxysporum* species complex (FOSC), whose members are responsible for internal vascular wilt and root rot and comprise more than 100 formae speciales (ff.spp.); *F. solani* species complex (FSSC), the species induce root and foot rot in numerous hosts; *F. fujikuroi* species complex (FFSC), whose members can contaminate various kinds of cereal with fumonisin mycotoxins; *F. graminearum* species complex (FGSC), whose pathogens induce head blight of barley and wheat plants (Aoki et al., 2014). The phylogenetic lineages in the *F. incarnatum-F. equiseti* species complex (FIESC) were formally described as species, but new lineages have been reported (Lima et al., 2021; Xia et al., 2019). Isolates belonging to *F. brachygibbosum*, which is a species of *F. sambucinum* species complex (FSAMSC; Ezrari et al., 2021; Rabaaoui et al., 2021), were isolated from sugar beets showing root rot symptoms in China (Cao et al., 2018).

Worldwide, sugar beet root-tip rot (RTR) induced by *Fusarium* spp. constitutes an economically important fungal disease (Cao et al., 2018; Hanson & Jacobsen, 2006; Harveson & Rush, 1998; Ruppel, 1991). Scanty literature chronicles the negative impacts of sugar beet RTR disease in Morocco, including the Khenifra-Beni Mellal region. *Fusarium oxysporum* was the only species isolated from sugar beet plants cultivated in the Gharb region (north-west of the country; Chenaoui et al., 2017). Its identification was limited only to morphological traits, so characterization was incompletely definitive. In addition, the pathogenicity of isolates on the sugar beet plant was not tested. A recent study reported the incidence of *F. equiseti* and *F. fujikuroi* in some fields within the Beni Mellal area. However, their direct impact on sugar beet health remains largely unknown (Aallam et al., 2021).

Generally, the pathogen *F oxysporum* was reported to be the most frequently encountered species of the genus *Fusarium* inducing root diseases in sugar beet (Hanson et al., 2009; Hanson & Jacobsen, 2006; Harveson & Rush, 1997; Lai et al., 2020). Sugar beet root rot induced by the pathogen *F. oxysporum* f.sp. *radicis-betae* was reported first in America by Martyn et al. (1989). This disease is associated with IVD, foliar yellowing, chlorosis, wilting, and necrosis. Foliar burning gradually spreads from the tips to the center of the leaves. In addition, RTR was associated with infection by this fungal pathogen (Harveson et al., 2009). However, *F. oxysporum* f.sp. *betae*, a fungus causing Fusarium yellows, is normally characterized by root vascular discoloration and interveinal yellowing of the leaves. Other species of the *Fusarium* taxon have also been described as causative agents of root rot in sugar beet. Francis and Luterbacher (2003) documented that *F. culmorum* can induce root necrosis in this crop in the UK. In the USA, *F. solani* (Hanson & Lewellen, 2007) and *F. equiseti* (Khan et al., 2021) have been reported to be causative agents of root rots in sugar beet. In addition, the pathogen *F. andiyazi* can induce RTR in sugar beet seedlings in Egypt (Taha, 2020). In China, Cao et al. (2018) reported that *F. brachygibbosum*, *F. nygamai*, *F. proliferatum F. redolens*, and *F. tricinctum* have also been isolated from diseased roots of sugar beet. Ruppel (1991) reported that several diseases showing symptoms of leaf attack are associated with root rot diseases in sugar beet. For example, Fusarium yellows generated by the pathogens *F. solani*, *F. roseum*, *F. acuminatum*, and *F. avenaceum* can show rots in tip roots. Similarly, Secor et al. (2014) documented that Fusarium yellow disease, induced by *F. secorum*, caused yellowing of leaves as well as vascular necrosis in the petioles and roots of sugar beet.

Isolates belonging to *F. solani* ((Martius) Appel and Wollenweber emend. Snyder and Hansen) cause rot symptoms including RTR in sugar beet plants (Cao et al., 2018; Ruppel, 1991). The complex of *F. solani* includes several species that all cumulatively belong to the FSSC (Sandoval-Denis et al., 2018). Based on phylogenetic data, a research group proposed to classify FSSC among the species that cluster under the genus *Neocosmospora* (Lombard et al., 2015). However, many scientists express their strong opinions that FSSC should be included in the genus *Fusarium*, as this classification is considered to be the best taxonomic and practical choice available (Geiser et al., 2021). So, through the current study, the concept of *F. solani* will be employed.

The morphological identification of species belonging to the genus *Fusarium* requires considerable expertise in taxonomy, takes a

lot of time, and often constitutes a real problem for the researcher. This identification appears almost impossible to properly discriminate closely related species. Therefore, molecular characterization appears necessary for a quick sensitive, and dependable diagnosis of *Fusarium* species. Several genomic sequences, namely the rDNA ITS, β-tubulin, calmodulin, and *TEF1*, were evaluated for their importance in identifying and distinguishing isolates belonging to the group *Fusarium* spp. (Geiser et al., 2004; Hill et al., 2011). In fact, molecular characterization based on the combined regions of *TEF1* and ITS has effectively been utilized for the diagnosis of *Fusarium* species (Arif et al., 2012; Mohamed Zubi et al., 2021).

Evidently, there exists a vast knowledge gap concerning the different *Fusarium* species that are responsible for causing sugar beet RTR in the Khenifra-Beni Mellal region and their impact on this plant's health. However, this knowledge appears important for epidemiological studies, making it possible to better comprehend the taxonomy and pathogenicity of each species and therefore to adopt relevant management methods to control the resulting crop diseases. In this regard, the current investigation sought to identify *Fusarium* species that are responsible for causing RTR in sugar beet from the Khenifra-Beni Mellal region by conducting genetic analyses of *TEF1* and ITS. The isolates were additionally evaluated in vivo for their ability to cause root rots and disrupt the normal growth of the plant of sugar beet.

2 | MATERIALS AND METHODS

2.1 | Collection and survey of sugar beet farms

During 2019, 2020, and 2021 growing seasons, sugar beet fields in various areas of the Khenifra-Beni Mellal region were surveyed for the existence of key Fusarium root rot symptoms such as RTR, IVD as well as wilting and drying of foliage (Figure 1). Plants of sugar beet displaying typical symptoms of RTR and IVD were aseptically collected from fields in several sugar beet growing areas of this region (Figure 2). Samples gathered from each field were carefully stored in plastic bags at 4°C awaiting fungal isolation.

2.2 | Isolation of fungi from infected field samples

Plants were received at the Plant and Environmental Protection Laboratory of the National School of Agriculture (ENA-Meknes). The infected roots were first washed with running tap water in order to eliminate all adhering soil and additional debris. Afterwards, minute fragments ($4 \times 4\,mm^2$) were incised from the necrotic root tissue, surface sterilized for 4 min in a 2.5% sodium hypochlorite solution (NaClO), rinsed thrice using sterile distilled water (SDW), and then dried with a sterilized Whatman filter paper (Azil et al., 2021). Four fragments of tissue cut from the root of each sample were then placed on the surface of potato dextrose agar (PDA) medium supplemented with 50 μg/mL chloramphenicol and 90 μg/mL streptomycin sulfate. All the Petri dishes were thereafter incubated in the darkness at 25°C for 72–96 h. Afterwards, the resulting fungal colonies that grew on the tissue samples were transferred and subsequently subcultured onto freshly prepared PDA media. Different types of fungal isolates were recovered from each piece of root tissue; however, only those that exhibited *Fusarium* characteristics were selected and transferred onto new PDA media for the purposes of single-spore purification (Leslie et al., 2006). After a 5-day incubation at 24°C, a whole of 69 single-spored isolates of *Fusarium* were isolated and carefully maintained by serial transfer on PDA. All isolates were maintained in 25% glycerol and then stored in the Plant and Environmental Protection Laboratory (ENA-Meknes) microorganism collection. A preliminary greenhouse pathogenicity test was performed for the 69 isolates using 6-week-old sugar beet seedlings (Barossa variety). Non-pathogenic isolates were excluded. Isolates

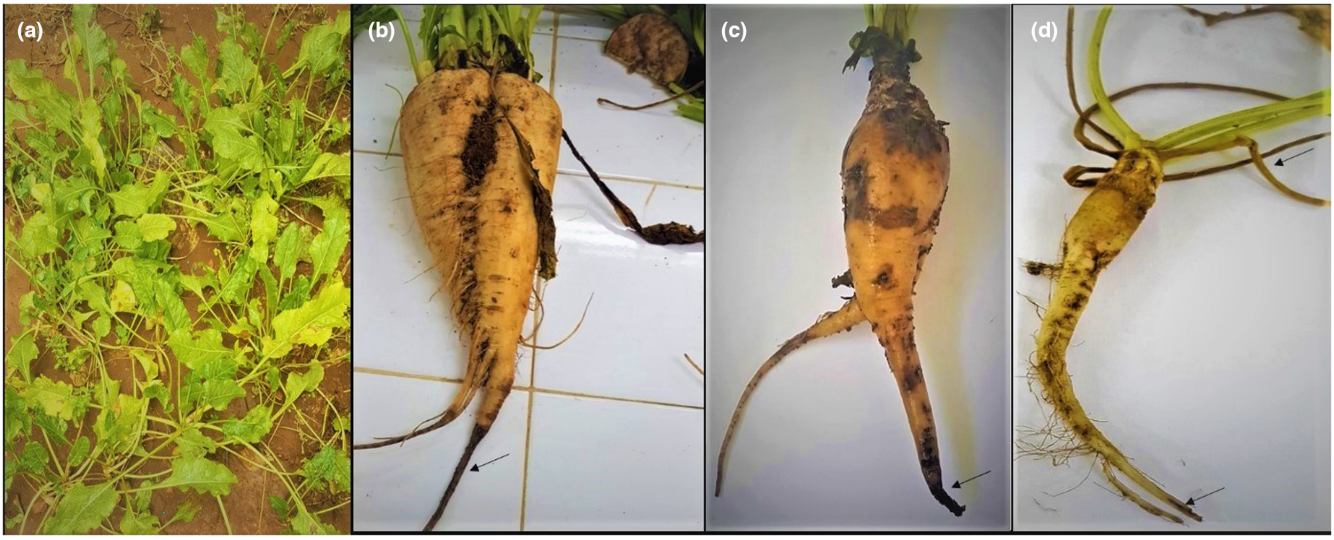

FIGURE 1 Infected sugar beets showing several symptoms related to root rot caused by *Fusarium*. Infected plants in the field with wilted foliage (a). Symptoms include RTR (taproot and secondary roots) (b, c), IVD (d), and leaf wilt (b and d).

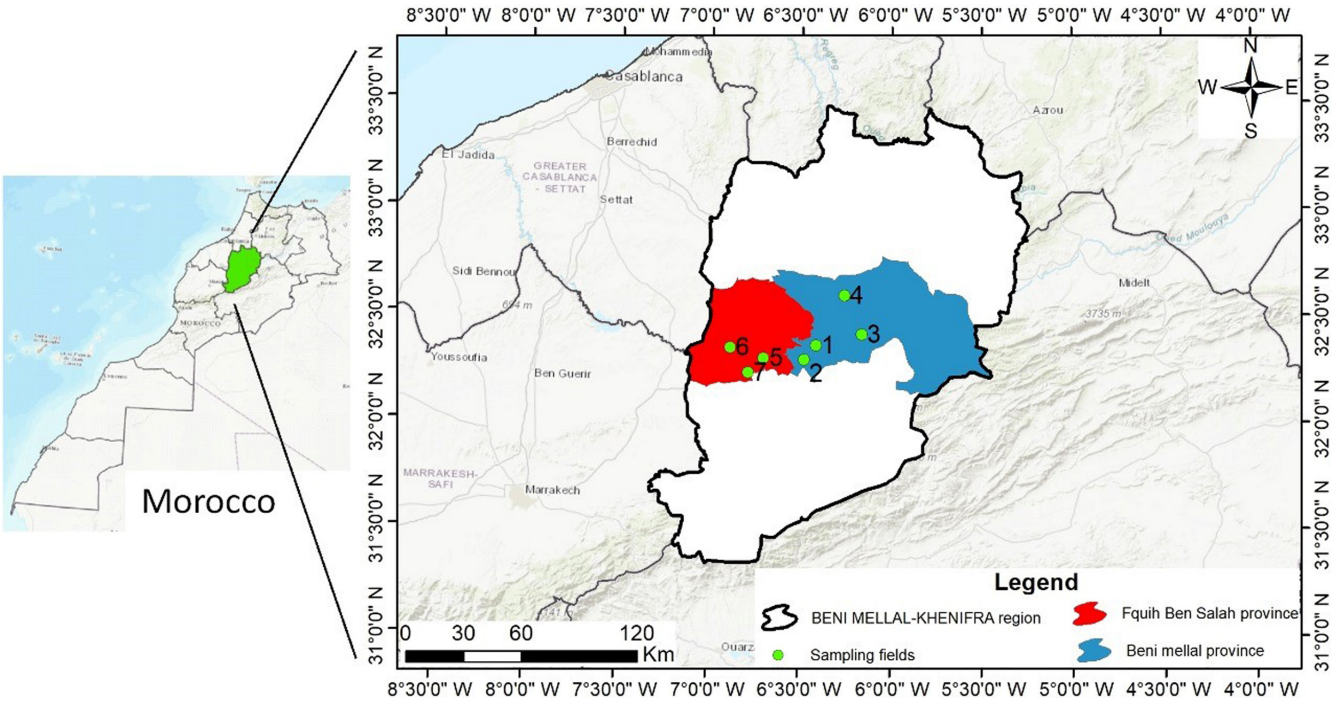

FIGURE 2 Map of Morocco showing the localization of region where samples of sugar beet plants showing root rot symptoms were collected, prepared using ArcGIS software 10.3.1; 1: Sidi jabber, 2: Ouled Mbarek, 3: Taghzirt, 4: Kasba Tadla, 5: Souk Essebt, 6: Dar Ould Zidouh, 7: Khlalta.

showing different aspects on PDA (colour and shape of colonies) and which exhibited their pathogenicity for sugar beet plants were chosen. Finally, 28 isolates were selected for the rest of this study.

2.3 | Morphological characterization

Purified cultures of the 28 representative isolates displaying various morphological characters and representing various species and geographical regions (Table 1) were incubated on a PDA medium at 25°C in the darkness. Morphological identification of *Fusarium* isolates was conducted following the method previously described by Leslie et al. (2006). Briefly, the major characteristics that were assessed included macroscopic traits (colony colour, presence and appearance of aerial mycelium) and microscopic characteristics (existence of chlamydospores and micro/ macroconidia and their arrangement). Microscopic photos of spores (chlamydospores and micro/macroconidia) of each species of *Fusarium* were captured. The overall length of chlamydospores and micro/macroconidia was measured while using a microscope BX51 (Olympus) equipped with a camera (Olympus C-5060 associated with Touch-Scope Integrated powerful software). The imaging results were then compared with those from earlier researches.

2.4 | DNA extraction and sequencing

DNA was extracted from fungal mycelium obtained from 5-day-old cultures of selected isolates growing on PDA utilizing the Doyle and Doyle (1990) method (Ezrari et al., 2021). The obtained DNA was dried, re-suspended in 50 µL of SDW and stored at −20°C for future use. Two primer sets were used to identify *Fusarium* strains assessed. These primers were *TEF1* (EF-728F/EF-986R) (Mahmooli et al., 2013) and ITS (ITS1/ITS4) (White et al., 1990; Table S1). The 25 µL PCR mixture used in each PCR reaction consisted of 2.5 µL PCR buffer (dNTPs [10 mM], $MgCl_2$ [50 mM]), 1 µL each of primer (10 µM), 0.2 µL Taq DNA Polymerase (5 U/µL) (DreamTaq DNA Polymerase), 2.5 µL of fungal DNA, then the PCR grade water was added to complete the volume of the final reaction.

PCR amplification of the *TEF1* region was performed respecting the PCR program described by Carbone and Kohn (1999). For the ITS region, the amplification was carried out respecting the PCR program described previously by Ezrari et al. (2021). The amplified PCR products were visualized on 1.5% agarose gel by electrophoresis, stained with EtBr and visualized with a UV transilluminator. The PCR product was then sequenced in Genetics STAB Vida Inc Laboratory (Lisbon, Portugal) utilizing Sanger dideoxy sequencing method. The obtained sequences were edited and aligned utilizing DNAMAN sequence analysis software (version 7.212, Lynnon Corp.). The same software was used in the manual editing of the individual data sets of *TEF1* and rDNA ITS sequences; all apparent errors were addressed. Sequences were deposited in GenBank with the accession numbers as shown in Table 1. For each isolate, species identity was determined utilizing BLAST (Basic Local Alignment Search Tool) where the analysis of each fungal sequence was clustered with the most closely related species at NCBI-BLAST.

TABLE 1 Detailed information on the origin, year of sampling, and GenBank accession numbers of *Fusarium* species isolates recuperated from sugar beet plants showing root rot symptoms.

Strain	Species	Location		Accession number		Sampling year
		Town	Province	ITS	EF1-α	
FSS1	*F. oxysporum*	Souk Essebt	Fquih Ben Saleh	OM876886	ON365735	2020
FSS2	*F. oxysporum*	Souk Essebt	Fquih Ben Saleh	OM876890	ON365741	2019
FSD1	*F. oxysporum*	Douar Oulad Zeidouh	Fquih Ben Saleh	OM876888	ON365733	2020
FSD2	*F. oxysporum*	Dar Ould Zidouh	Fquih Ben Saleh	OM876904	ON365736	2021
BMO	*F. oxysporum*	Ouled Mbarek	Beni Mellal	OM876889	ON365734	2021
BMT1	*F. oxysporum*	Taghzirt	Beni Mellal	OM876877	ON365737	2021
BMT2	*F. oxysporum*	Taghzirt	Beni Mellal	OM876879	ON365738	2020
BMT3	*F. oxysporum*	Taghzirt	Beni Mellal	OM876884	ON365739	2021
BMS2	*F. oxysporum*	Sidi Jaber	Beni Mellal	OM876885	ON365740	2021
BMS3	*F. oxysporum*	Sidi Jaber	Beni Mellal	OM876897	ON210987	2021
BMS4	*F. solani*	Sidi Jaber	Beni Mellal	OM876892	ON398342	2021
FSF	*F. solani*	Fquih Ben Saleh	Fquih Ben Saleh	OM876898	ON365745	2020
FSS3	*F. solani*	Souk Essebt	Fquih Ben Saleh	OM876891	ON409226	2020
FSK1	*F. solani*	Khlalta	Fquih Ben Saleh	OM876905	ON398344	2021
FSD3	*F. solani*	Dar Ould Zidouh	Fquih Ben Saleh	OM876878	ON398343	2020
FSS4	*F. falciforme*	Souk Essebt	Fquih Ben Saleh	OM876900	ON365746	2020
FSS6	*F. equiseti*	Souk Essebt	Fquih Ben Saleh	OM876903	ON381738	2021
FSS5	*F. equiseti*	Souk Essebt	Fquih Ben Saleh	OM876893	ON381737	2021
BMK3	*F. equiseti*	Kasbah Tadla	Kasbah Tadla	OM876882	ON381287	2021
FSK4	*F. equiseti*	Khlalta	Fquih Ben Saleh	OM876894	ON381739	2019
FSD4	*F. nygamai*	Dar Ould Zidouh	Fquih Ben Saleh	OM876883	ON365744	2020
FSK2	*F. nygamai*	Khlalta	Fquih Ben Saleh	OM876901	ON365743	2020
FSK3	*F. nygamai*	Khlalta	Fquih Ben Saleh	OM876902	ON365742	2020
BMK1	*F. brachygobusum*	Kasbah Tadla	Kasbah Tadla	OM876895	ON381740	2021
BMK2	*F. brachygobusum*	Kasbah Tadla	Kasbah Tadla	OM876896	ON398341	2021
BMS1	*F. brachygobusum*	Sidi Jaber	Beni Mellal	ON642071	ON783853	2021
BMT5	*F. culmorum*	Taghzirt	Beni Mellal	OM876880	ON381742	2021
BMT4	*F. proliferatum*	Taghzirt	Beni Mellal	OM876899	ON381741	2020

Sequences were aligned through Clustal W by aligning the most closely related sequences and then gradually adding in the more distantly related sequences. This approach helps to improve the accuracy of the alignment by taking into account the evolutionary relationships among the sequences. The dendrogram was generated employing the Kimura 2-parameter model using the maximum likelihood method to estimate the best-fit evolutionary model and the optimal tree topology. This method provides a powerful approach for inferring the evolutionary relationships among the sequences and is commonly used in phylogenetic analysis. Phylogenetic analysis based on a combination of two sequences (*TEF1* and ITS) was conducted with MEGA 5 software (version 5.2.2) in order to evaluate the relationships between fungal isolates, and while the dendrogram was generated utilizing the maximum likelihood method and Kimura 2-parameter model (Figure 3). Fifteen other reference *Fusarium* species extracted from GenBank were used in the building of the phylogenetic tree. The latter was assessed using bootstrap analysis based on 1000 replicates.

2.5 | *Fusarium* pathogenicity test

The 28 selected *Fusarium* isolates were additionally used in performing a pathogenicity test on sugar beet seedlings (Barossa variety) following the method of Hill et al. (2011) with a slight modification. 14-day-old fungal cultures were utilized in the preparation of the inocula. For each isolate, conidia were harvested by adding 10 mL of SDW to the Petri dish, and the resulting conidial suspension was adjusted to concentrations of 1×10^6 conidia/mL utilizing a Neubauer hemacytometer.

Sugar beet seeds were placed in small sterile plastic pots filled with a mixture of peat and field soil (1:1, v/v). The mixture was

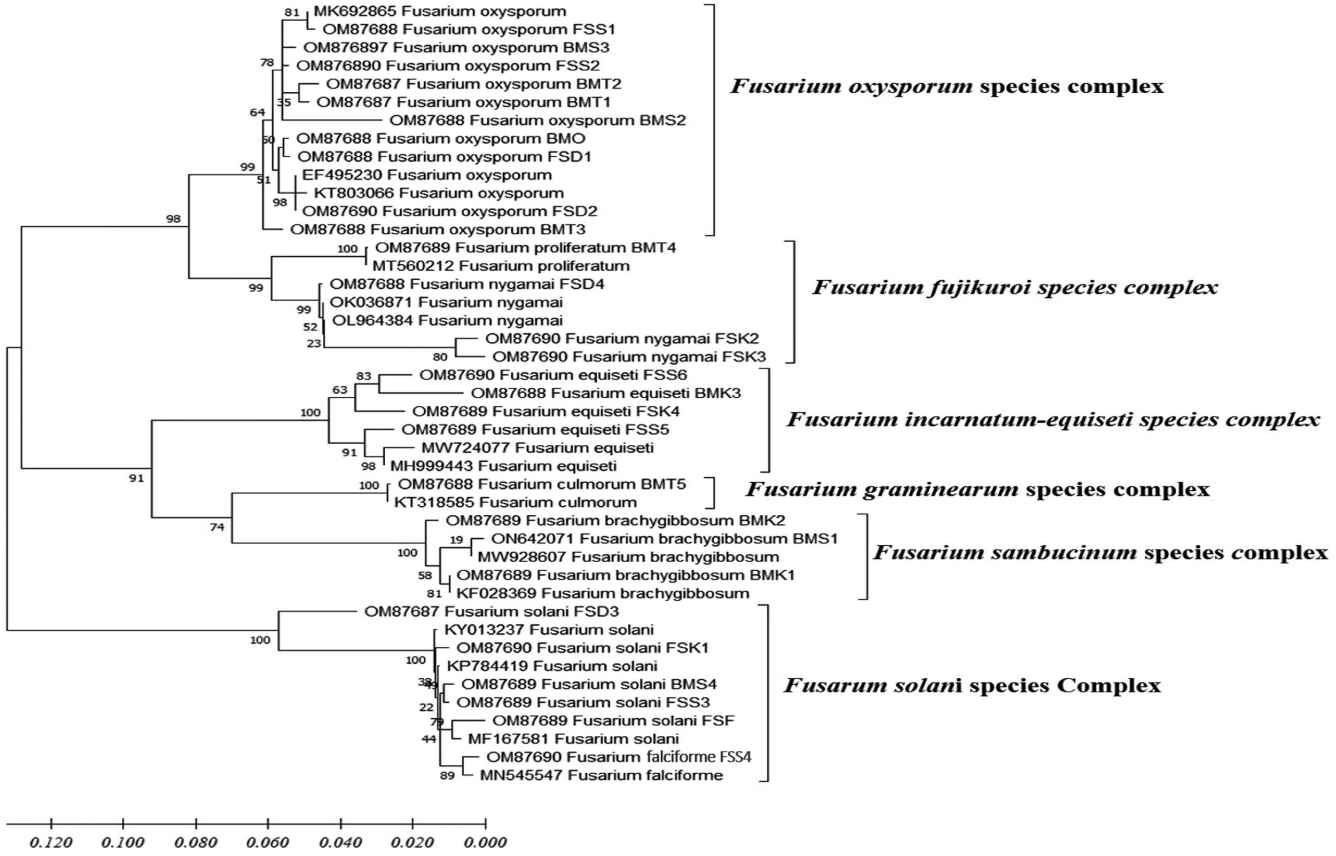

FIGURE 3 Phylogenetic tree of *Fusarium* spp based on the gene sequences showing the relationship of *Fusarium* spp analysed in this study to other *Fusarium* spp. The phylogenetic tree was reconstructed based on both elongation factor (TEF1) and internal transcribed spacer (ITS) sequences using the maximum likelihood method on MEGA ver. 5. The tree topology was assessed by 1000 bootstrap replicates. The isolates that were obtained in this study were followed by their strain code whereby they were recorded in GenBank. The numbers at the nodes expressed bootstrap values.

previously sterilized for 4 h at 121°C. The pots were later taken to the greenhouse and watered every day to maintain vigorous growth. This operation makes it possible to acquire a satisfactory number of plants at a similar stage of growth. After 6 weeks of sowing, 16 healthy sugar beet seedlings were selected for use with each selected *Fusarium* isolate. Gently, plants were uprooted from their substrate, shaken down to remove excess soil and their roots were later placed in a conidial suspension (10^6 conidia/mL) for 20 min with periodic shaking. The treated plants were then replanted in new pots containing 6 kg of sterile soil. Sugar beet plants soaked in potato dextrose (PD) served as a negative control. For each treatment, four replicates per isolate were used and each replicate comprised four plants. The pots were incubated in a greenhouse (25°C), watered with tap water twice or thrice a week, and regularly examined for signs of the disease. After 6 weeks of incubation, the plants were harvested and examined for symptoms of root rot and IVD. For each plant, the disease severity was determined by adopting a scale ranging from 0 to 4 according to the percentage of necrosis or discoloration of the root vascular tissue (Harveson & Rush, 1998; Table S2). In addition, the disease incidence value and the disease index value were determined according to the following formulas (Cao et al., 2018):

$$\text{Disease incidence} = 100 \times (p_1 + p_2 + p_3 + p_4) / P,$$

$$\text{Disease index} = 100 \times (0p_0 + 1p_1 + 2p_2 + 3p_3 + 4p_4) / 4P,$$

where p_0–p_4 represents the number of plants that correspond to each class and P is the total number of inoculated plants.

After measuring root length and shoot length, plant fresh mass was recorded. Next, the sample was put in an oven at 65°C, after which the plant and root dry weight (RDW) were measured once a constant weight of the samples was achieved. The pathogenicity tests were achieved twice over time. The causative agents were re-isolated, and their microscopic morphology was compared to those inoculated initially according to Koch's postulates.

2.6 | Statistical analyses

The tests in this study were achieved in a completely randomized design. Datasets obtained were first assessed for normality (Shapiro–Wilks test) and homogeneity of variance (Bartlett's test) before being subjected to an analysis of variance using the statistical software SPSS (version 20, IBM SPSS Statistics 20). When the effect

TABLE 2 Detailed information on colony appearance and morphological characteristics of microconidia, macroconidia, and chlamydospores of *Fusarium* species isolates obtained from sugar beet showing root-tip rot.

	Colony		Microconidia			Macroconidia			Chlamydospore
Specie	Pigment colour	Aerial growth appearance	Shape	Septa	Size (μm)	Shape	Septa	Size (μm)	
F. oxysporum	Pink, white, violet	Cotton, wool dense	Reniform, oval, elliptical	0–1	5.1–19.1×2.4–6.5	Curved to straight	3	18.9–37.8×4.1–7.9	Singly or in pairs 7.5–9.1 μm
F. solani	White, cream	Fibrous	Reniform, oval	0–3	8.7–19.8×2.9–4.8	Slightly curved to straight	3–5	24.9–44.7×3.2–6.1	Intercalary or terminal, single or in pairs, globular, 5.8–8.7 μm
F. falciforme			Fusiform	0–2	8.5–(5.5)–2.8×2.1–(2.3)–2.7	Falcate/oval	3–4	34.8–(42.7)–47.5×5.1–(5.3)–5.9	Singly, in pairs, or chains
F. equiseti	White then turns greenish-yellow	Cotton	Absent	—	—	Long, slender, curved at the ends	4–7	27.8–50.4×3.2–5.5	Singly, in clumps or in chains 5.8–13.1 μm
F. brachygibbosum	White becoming pink with age	Wool, cotton	Ovoid, straight	0–1	7.2–15.7×3.3–4.3	Curved	3–5	26.4–41.2×4.1–4.9	Intercalary or terminal 5.8–11.4 μm
F. nygamai	White becoming violet	Wool, floccose	Obovoid with ellipsoidal base	0–2	5.8–14.5×2.6–4.5	Slender, straight to slightly curved	3–5	25.1–44.6×3.1–5.3	Terminal or intercalary, solitary or in pairs 6–14.9 μm
F. culmorum	Orange to red	Dense and white	Absent	—	—	Curved dorsally	3–5	24.9–36.5×4.2 to 7.2	Oval to globose 5.4–10.1 μm
F. proliferatum	White, orange	Floccose, cotton	Pyriform or Club shaped with a truncate base	0–1	3.1–11.9×1.9–4.9	Slender, straight, thin-walled	0–5	18.8–47.8×2.7–4.9	Absent

was found to be significant, the least significant difference test was achieved to separate the means at a significance level of $p \leq .05$.

3 | RESULTS

3.1 | Symptoms of Fusarium root rot on the plants

Infected sugar beet plants displayed specific symptoms of root rot. In the field, the disease manifests in the form of yellowing chlorosis, scorching, and wilting which spreads gradually from the leaf margins to the main rib (Figure 1). Root symptoms cover taproot and secondary root tip rot (Figure 1b,c), IVD, and increased lignification of the root (Figure 1d).

3.2 | Morphological identification of the causal pathogen of RTR

Based on morphological features, the findings showed that the causative agent of RTR was *Fusarium* spp. Indeed, eight species belonging to the *Fusarium* genus have been identified from infected sugar beet roots, which were *F. oxysporum* (Figure S1), *F. solani* (Figure S2), *F. equiseti* (Figure S3), *F. brachygobusum* (Figure S4), *F. nygamai* (Figure S5), *F. falciforme* (Figure S6), *F. proliferatum* (Figure S7), and *F. culmorum* (Figure S8).

Selected isolates showed morphological characteristics that concur with the preceding descriptions of each *Fusarium* species (Table 2). Studied strains generated conidia in aerial mycelium and sporodochium with abundant production of macroconidia in PDA medium. Isolates belonging to *F. equiseti* and *F. culmorum* were the only ones that did not produce macroconidia. After 20 days of incubation, all the isolates produced chlamydospores except the *F. proliferatum* isolate.

Fungal strains belonging to FOSC varied the greatest in terms of morphology when cultured on PDA medium (Figure S1). *Fusarium oxysporum* BMO strain colonies were violet (or white) with plenty of aerial mycelium. The reverse side of the cultured colonies was violet (or pink). Macroconidia were noticed as straight, long, elliptical, tapered ends, thin-walled, and with a mean 3 septa. The basal cell was notched or foot-shaped. The size of macroconidia varied from 18.9 × 4.1 µm to 37.8 × 7.9 µm. Microconidia were organized on monophialides structures and were commonly observed to be abundant in reniform, oval, or elliptical shapes with 0–1 septae. Chlamydospores were produced after 2 weeks of incubation on PDA medium. They were abundant, generally rounded, intercalary, or terminal, present in either single or pairs, globose in form and measured 7.5–9.10 µm. *Fusarium* isolates of FSSC displayed fast-growing colonies (Figures S2 and S6). *Fusarium solani* FSS3 colonies on PDA were cream to white and have plenty of aerial mycelium, the undersurface exhibiting a dark purple colour. Macroconidia were generally thick-walled with 3–5 septate and characterized by notched or rounded basal cells. Apical cells were short, rounded, and blunt. The macroconidia size was 24.9–44.7 × 3.2–6.1 µm. Microconidia were oval, sometimes reniform and formed on scarified and elongated conidiophores. These structures were mostly 0-septate and measured 8.7–19.8 × 2.9–4.8 µm. Chlamydospores occurred singly or in pairs. They were globular or oval, abundant, rounded, terminal, or intercalary, with a smooth or wrinkled wall, and measured 5.8–8.7 µm. Colonies of *F. brachygibbosum* (Figure S4) isolates were initially white to pink in colour and then turn yellow with abundant aerial mycelium. Spherical chlamydospores were produced in abundance. They measured 5.8–11.4 µm and were terminal or intercalary, single, and in chains. Macroconidia measured 26.4–41.2 × 4.1–4.9 µm and were curved with 3–5 septa. They had slightly foot-shaped basal cells and slightly hook-shaped apical cells. Produced microconidia were slightly curved, occasionally ovoid and generally unseptate (rarely uniseptate). They measured 7.2–15.7 × 3.3–4.3 µm. Fungal isolates of *F. equiseti* (Figure S3) produced colonies that were white at first and then turned greenish-yellow. Macroconidia were characterized by significant dorsiventral curvature and 4–7 septa measuring 27.8–50.4 × 3.2–5.5 27.8–50.4 × 3.2–5.5 µm. They had foot-shaped basal cells and tapered apical cells. Chlamydospores were produced in abundance, measured 5.8–13.1 µm, and formed singly, in clumps or chains. *Fusarium nygamai* isolates showed white colonies turning violet with age (Figure S5). Macroconidia were thin, with 3–5 septa, transparent, straight to slightly curved shape, and 25.1–44.6 × 3.1–5.3 µm in size. Microconidia were produced in abundance, the majority were obovoid with an ellipsoidal base. They had 0–2 septae and measured 5.8–14.5 × 2.6–4.5 µm. Generally, chlamydospores occurred after 2 weeks of incubation. They had rough or smooth-walled chlamydospores, measured 6–14.9 µm, and were either singly or in pairs. The *F. culmorum* isolate showed rapid growth (1.9 cm/day) and produced abundant macroconidia (Figure S8). Colonies were initially pale orange and turned dark brownish to reddish later. Produced macroconidia were curved dorsally and thick-walled with 2–4 septa. They were 24.9–36.5 × 4.2–7.2 µm with notched foot cells and blunt apical cells. Chlamydospores rarely appeared after 14 days of incubation and were oval to globose. The colony of the *F. proliferatum* isolate revealed a growth rate equal to 2.75 mm/day on PDA medium at 25°C. Aerial mycelium was almost white-orange and floccose (Figure S7). Microconidia were produced in abundance and were pyriform or club-shaped with a truncate base. Produced macroconidia were slender, straight, thin-walled, and hyaline. They usually had 0–5 septa and measured 18.8–47.8 × 2.7–4.9 µm.

3.3 | Phylogenetic analysis

Apart from the morphological characteristics, the studied *Fusarium* isolates were further confirmed by molecular sequencing using the *TEF1* and the rDNA ITS regions. Thus, based on molecular characterization, the distribution of the 28 isolates studied on the various species of *Fusarium* was performed. As shown in Table 1, 10 isolates belonged to *F. oxysporum*, which was then the most isolated

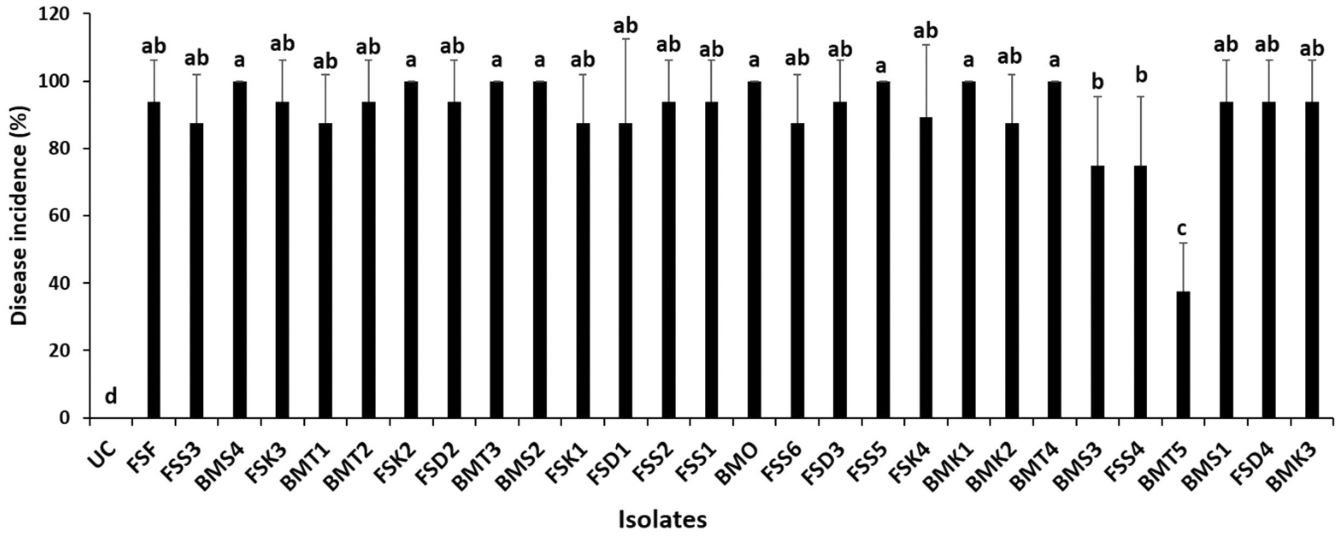

FIGURE 4 Root rot disease incidence caused by different *Fusarium* strains. Treatments having the same letter are not significantly different according to the Duncan test ($p < .05$). UC, untreated control.

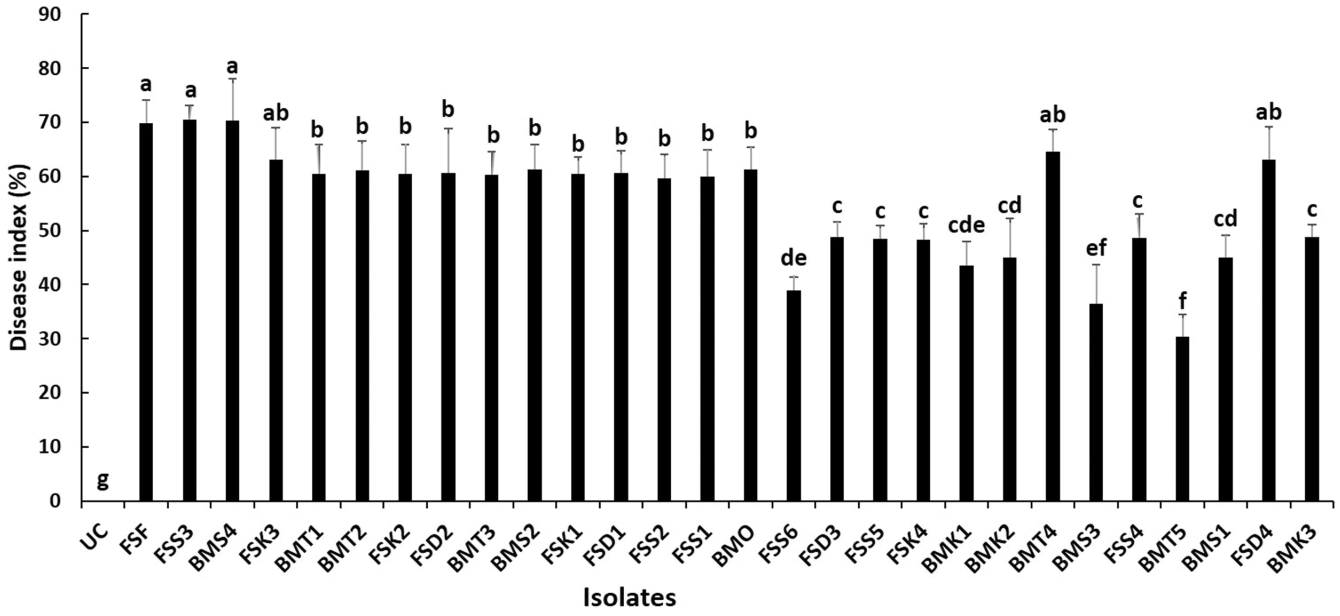

FIGURE 5 Root rot disease index caused by different *Fusarium* isolates. Treatments having the same letter are not significantly different according to the Duncan test ($p < .05$). UC, untreated control.

at a greater frequency when contrasted to other species, followed by *F. solani* (five isolates) and *F. equiseti* (four isolates). *Fusarium nygamai* and *F. brachygibbosum* were represented by three isolates for each species, while the other three species (*F. proliferatum*, *F. falciform*, and *F. culmorum*) were represented by only one isolate for each taxon. The combined morphological criteria and the phylogenetic analysis by combining the rDNA ITS and *TEF1* regions displayed that the isolates of *Fusarium* spp. were responsible for sugar beet RTR.

Sequence data acquired for *TEF1* α and ITS from 28 of the selected isolates, as well as sequences of 23 other reference *Fusarium* species retrieved from the database of the GenBank, were exploited to construct a phylogenetic tree (Figure 3). Analysis of phylogenetic data allowed us to classify the 28 isolates into eight species grouped within FOSC, FSSC, FIESC, FFSC, FIESC, and FSAMSC. More than a third of isolates are members of the FOSC ($n = 10$). Six isolates belong to the FSSC group, one of which is identified as *F. falciforme* based on *TEF1* regions. Three isolates were identified as *F. brachygibbosum*, strains of this species belong to *F. sambucinum* species complex (FSAMSC). Among the identified isolates, four are defined as *F. equiseti* and clustered with strains belonging to FIESC. In this study, FFSC was presented by four isolates, three of which belong to *F. nygamai* and 1 to *F. proliferatum*. BMT5 is the only strain that belongs to *F. culmorum* (FGSC).

TABLE 3 Shoot length, root length, plants fresh and dry weights, and root dry weight of sugar beet 6 weeks postinoculation with *Fusarium* spp.

	Parameters evaluated				
			Plant weight (g)		
Isolates	Shoot length (cm)	Root length (cm)	Fresh	Dry	Root dry weight
Uninoculated control	27.61 ± 1.5^{a}	9.04 ± 1.52^{ab}	19.24 ± 1.64^{a}	2.85 ± 0.33^{ab}	0.77 ± 0.11^{ab}
FSS1	25.35 ± 3.84^{abc}	7.08 ± 1.49^{ab}	16.14 ± 2.33^{cd}	2.29 ± 0.15^{bc}	0.53 ± 0.1^{bc}
FSS2	25.4 ± 0.76^{abc}	7.81 ± 1.07^{ab}	16.17 ± 1.36^{cd}	2.42 ± 0.08^{abc}	0.59 ± 0.17^{abc}
FSD1	24.44 ± 2.72^{abc}	7.39 ± 0.87^{ab}	16.27 ± 1.7^{bcd}	2.45 ± 0.08^{abc}	0.63 ± 0.14^{abc}
FSD2	24.14 ± 1.91^{abc}	7.65 ± 2.01^{ab}	$16.27 \pm 2.1b^{cd}$	2.42 ± 0.3^{abc}	0.64 ± 0.1^{abc}
BMO	24.19 ± 2.63^{abc}	7.33 ± 1.59^{ab}	16.22 ± 0.14^{bcd}	2.37 ± 0.05^{abc}	0.64 ± 0.14^{abc}
BMT1	24.12 ± 3.03^{abc}	6.66 ± 0.86^{b}	15.78 ± 0.94^{cd}	2.35 ± 0.35^{abc}	0.65 ± 0.14^{abc}
BMT2	26.73 ± 3.05^{abc}	7.66 ± 0.97^{ab}	16.21 ± 2.07^{bcd}	2.45 ± 0.21^{abc}	0.7 ± 0.13^{abc}
BMT3	24.69 ± 1.72^{abc}	6.96 ± 1.24^{ab}	15.73 ± 2.19^{cd}	2.36 ± 0.52^{abc}	0.61 ± 0.06^{abc}
BMS2	24.94 ± 2.97^{abc}	8.06 ± 1.18^{ab}	16.28 ± 2.26^{bcd}	2.26 ± 0.48^{c}	0.66 ± 0.2^{abc}
BMS3	27.32 ± 3.55^{ab}	8.91 ± 1.41^{ab}	18.98 ± 1.55^{ab}	2.83 ± 0.41^{ab}	0.76 ± 0.06^{abc}
BMS4	22.52 ± 3.41^{bc}	7.09 ± 11.29^{ab}	15.9 ± 1.86^{cd}	2.46 ± 0.49^{abc}	0.53 ± 0.2^{bc}
FSF	22.36 ± 1.41^{bc}	6.9 ± 1.2^{ab}	15.67 ± 1.19^{cd}	2.31 ± 0.31^{abc}	0.53 ± 0.11^{bc}
FSS3	22.25 ± 1.83^{c}	6.78 ± 1.14^{ab}	15.57 ± 1.84^{d}	2.23 ± 0.58^{c}	0.52 ± 0.11^{c}
FSK1	25.88 ± 4.5^{abc}	7.06 ± 1.33^{ab}	16.2 ± 2.04^{bcd}	2.36 ± 0.31^{abc}	0.68 ± 0.13^{abc}
FSD3	27.24 ± 1.68^{abc}	7.65 ± 0.95^{ab}	17.57 ± 1.84^{abcd}	2.58 ± 0.25^{abc}	0.69 ± 0.06^{abc}
FSS4	27.61 ± 0.95^{a}	9.07 ± 1.35^{a}	18.48 ± 2.03^{abc}	2.84 ± 0.44^{ab}	0.75 ± 0.12^{abc}
FSS6	26.56 ± 1.83^{abc}	8.8 ± 0.88^{ab}	17.04 ± 1.01^{abcd}	2.48 ± 0.17^{abc}	0.65 ± 0.12^{abc}
FSS5	26.99 ± 1.87^{abc}	8.47 ± 2.36^{ab}	17.81 ± 1.06^{abcd}	2.65 ± 0.1^{abc}	$0.0.53 \pm 0.18^{bc}$
BMK3	27.17 ± 2.67^{abc}	8.95 ± 2.55^{ab}	19.79 ± 1.58^{abcd}	2.46 ± 0.25^{abc}	0.73 ± 0.38^{abc}
FSK4	26.8 ± 1.24^{abc}	7.37 ± 0.78^{ab}	17.92 ± 1.41^{abcd}	2.65 ± 0.04^{abc}	0.73 ± 0.11^{abc}
FSD4	27.94 ± 3.36^{a}	9.09 ± 1.33^{a}	16.08 ± 1.98^{bc}	2.31 ± 0.21^{abc}	$0.67 \pm 0.19a^{bc}$
FSK2	26.69 ± 0.88^{abc}	8.14 ± 1.47^{ab}	15.9 ± 1.83^{cd}	2.41 ± 0.13^{abc}	0.59 ± 0.13^{abc}
FSK3	24.44 ± 4.24^{abc}	7.84 ± 0.8^{ab}	16.12 ± 1.07^{cd}	2.33 ± 0.17^{abc}	0.66 ± 0.19^{abc}
BMK1	26.86 ± 1.73^{abc}	8.55 ± 1.17^{ab}	17.81 ± 0.89^{abcd}	2.67 ± 0.2^{abc}	0.71 ± 0.12^{abc}
BMK2	26.18 ± 3.28^{abc}	7.55 ± 1.75^{ab}	17.88 ± 1.25^{abcd}	262 ± 0.3^{abc}	0.78 ± 0.17^{a}
BMS1	27.27 ± 5.19^{ab}	8.49 ± 0.71^{ab}	17.2 ± 0.59^{abcd}	2.49 ± 0.35^{abc}	0.65 ± 0.04^{abc}
BMT5	27.02 ± 3.68^{abc}	8.83 ± 1.66^{ab}	19.29 ± 0.65^{a}	2.86 ± 0.28^{a}	0.72 ± 0.2^{abc}
BMT4	25.47 ± 4.08^{abc}	7.53 ± 1.52^{ab}	17.24 ± 1.63^{abcd}	259 ± 0.29^{abc}	0.67 ± 0.09^{abc}

Note: Data represent mean ± standard deviation (SD). In each column, values followed by the same letter are not significantly different according to the Duncan test at $p < .05$.

3.4 | Pathogenicity results

3.4.1 | Incidence and index diseases rate

The ability of all selected isolates to induce RTR in the sugar beet plant was evaluated. Findings displayed that all the selected isolates were pathogenic when inoculated in plants. All selected isolates induced RTR symptoms, identical to natural infections, on the crops by 6 weeks after experimental inoculation. First, inoculated plants exhibited IVD on the root (Figure S1c). Then, root rot most often begins from the tip of the root and progressed in the form of a black spot along with the infected parts (Figure S3c). In severely infected roots, black rot appears on their surface and a significant part of the root has been necrosed and destroyed (Figure S2c). Some plants showed shorter shoots, leaf chlorosis, and yellowing gradually extend from the edges of the leaves to the main rib (Figure S5b). Their root systems were reduced in size compared to control seedlings that had been placed in sterile PDA medium and remained completely healthy. Fungal isolates were re-isolated from the rotten roots, and identification was done using the species previously utilized in the initial inoculation of the seedling, fulfilling Koch's postulates. The symptoms mentioned above were not detected in uninoculated plants.

Statistical analyses showed significant differences in the disease incidence rate (Figure 4). Eight isolates (BMS4, FSk2, BMT3, BMS2, BMO, FSS5, BMK1, and BMT5) belonging to six species (*F. brachygobusum*, *F. solani*, *F. nygamai*, *F. oxysporum*, *F. equiseti*, and *F. culmorum*) revealed an incidence rate equal to 100%. The lowest value (37.5%) was recorded by the BMT5 isolate which belongs to *F. culmorum*. Statistical differences ($p < .05$) were observed in symptoms intensity when sugar beet seedlings were inoculated with different *Fusarium* isolates (Figure 5). Mean disease index values for the 28 *Fusarium* isolates ranged between 30.33 and 70.46. FSF, FSS3, and BMS4 isolates, all belonging to the *F. solani* group, were the most highly aggressive isolates. However, like the incidence rate, *F. culmorum* BMT5 isolate recorded the lowest disease severity value. In general, isolates belonging *to F equiseti* were less aggressive compared to those of *F solani* and *F oxysporum*, whereas two *F. nygamai* isolates were more virulent compared to all *F. oxysporum* isolates.

3.4.2 | Effect of fungal isolates on plant growth parameters

Table 3 shows information concerning shoot length, root length, root weight, and fresh and dry weight per sugar beet plant *6* weeks after inoculation. Statistical analyses showed significant differences regarding the effect of studied isolates on plant growth parameters. All isolates studied except FSD4 and FSS4 caused a significant decrease in shoot length. *Fusarium solani* FSS3 recorded the lowest value with a reduction rate equal to 19.42%. The average root length of control plants was 9.04 ± 1.52 cm, which significantly differed from plants inoculated with BMT1 isolates belonging to *F. oxysporum* (6.66 ± 0.86 cm). Our results also showed that plant fresh weight (PFW) and plant dry weight (PDW) were significantly decreased in plants artificially inoculated with *Fusarium* species comparable to control plants. Obtained PFW and PDW values ranged from 19.29 ± 2.03 to 15.75 ± 1.84 g and from 2.68 ± 0.28 to 2.23 ± 0.58 g, respectively, whereas the average PFW and PDW of uninoculated plants were 19.24 ± 1.64 g and 2.85 ± 0.33 g, respectively. All tested isolates, except BMK2, showed a significant decrease in RDW. Plants infected with *F. solani* FSS3 displayed a reduction in RDW by 32.74% compared with uninfected plants. In addition, this isolate showed the greatest reduction in both PFW and PDW by 17.08% and 21.76%, respectively, compared with the uninoculated control. All these results reveal an evident variation between different isolates and species.

4 | DISCUSSION

Sugar beet is the principal industrial crop grown in the extensive agricultural lands of the Khenifra-Beni Mellal region (Farhaoui et al., 2023). RTR was detected in a variety of sugar beet-growing regions in central Morocco, including Beni Mellal, Kasbah Tadla, and Fquih Ben Saleh provinces. Despite root rot disease of sugar beets being first reported by Martyn et al. (1989) in Texas, very few studies have investigated this disease in detail in Morocco. For the first time, the present investigation studied in detail both morphological and molecular characterization of *Fusarium* spp. causing RTR in sugar beet in Morocco and evaluated the pathogenicity of studied strains under greenhouse conditions. *Fusarium* strains isolated and evaluated in the present study showed their ability to induce RTR and IVD in sugar beet. Similarly, Cao et al. (2018) mentioned that sugar beet plants inoculated with *Fusarium* species exhibited external and internal symptoms of the disease. Selected strains were successfully identified based on morphological features as well as ITS rDNA and *TEF1* sequence analyses. Obtained results demonstrated a greater *Fusarium* species diversity compared to previously published reports (Chenaoui et al., 2017; Hanson & Hill, 2004; Harveson & Rush, 1998). The current study details the first report revealing that *F. solani*, *F. equiseti*, *F. proliferatum*, *F. brachygibbosum*, *F. falciforme*, *F. culmorum*, and *F. nygamai* are causative agents of root rot in sugar beets grown in Morocco. In fact, *Fusarium* species have been reported as causative agents of sugar beet RTR in other sugar beet growing regions around the world like the USA, China, Egypt, and the United Kingdom (Cao et al., 2012; Hanson & Hill, 2004; Harveson & Rush, 1997; Taha, 2020).

According to the current research, *F. oxysporum* was the highly isolated pathogen from sugar beet plants showing RTR, which concurs with the results of previous studies (Cao et al., 2018). In the literature, two *F. oxysporum* formae speciales on sugar beet have been described. *Fusarium oxysporum* f.sp. *betae* which can cause Fusarium yellows and internal discoloration of the root, while *F. oxysporum* f.sp. *radicis-betae* induces visible black rot at the root tip surface. Studied *Fusarium* strains were isolated from plants showing root rot. Then, *F. oxysporum* isolates evaluated in this study may be strains of *F. oxysporum* f.sp. *radici-betae*. According to a study carried out by Lombard et al. (2019), the formae speciales of FOSC are defined by the accessory chromosome obtained via horizontal gene transfer and should not be confused with the limits of species within this species complex. Using multi-locus phylogenetic inference and subtle morphological differences, 21 phylogenetic species were designed within the FOSC. Based on the criteria proposed by Lombard et al. (2019), *Fusarium* isolates belonging to the FOSC from our investigation could be included in *Fusarium carminascens*, which belongs to phylogenetic clade III of the FOSC.

In our current survey, *F. solani* showed the highest virulence compared to the other species assessed, which is compatible with the results of previous investigations (Cao et al., 2018). *Fusarium* species are known for their great ability to affect several crops in Morocco, including date palm (Tantaoui et al., 1996), citrus (Jaouad et al., 2020), and zucchini (Ezrari et al., 2020), which are especially infected by *F. oxysporum*, *F. solani*, and *F equiseti*, respectively. In addition, *F. oxysporum* was reported as an important pathogen for wheat (Qostal et al., 2019) and tomato (Taghdi et al., 2015) crops in many regions of Morocco. *Fusarium solani* was also isolated from

dried branches of olive trees that grew in western Morocco (Chliyeh et al., 2017). In the Khenifra-Beni Mellal region, the crops most commonly used in rotation with sugar beet are wheat (Ennouari et al., 2013) and barley (Haikel et al., 1986). Strains of *Fusarium* spp. isolated from diseased sugar beet roots were also found pathogenic for barley (Vanova et al., 2004) and wheat (Burlakoti et al., 2007; Christ et al., 2011). Similarly, Tillmann et al. (2017) demonstrate that the risk of crown and foot rot on wheat seedlings increases significantly when sugar beets are grown in rotation with this cereal crop. Thus, cross-pathogenicity trials between *Fusarium* spp. causing sugar beet root rot and routine rotational crops should be performed to determine which plants can be employed in a crop rotation and dish up as non-hosts or lowered-risk plants.

Fusarium species infection may vary with plant age (Rispail et al., 2015). Hence, *F. oxysporum* is known to be associated with various soybean diseases in the initial stage of growth. However, during the reproductive stages, *F. solani* was mentioned to be the most frequently isolated species (Farias & Griffin, 1989; Killebrew et al., 1993). In sugar beets, *Fusarium* can induce both root rot and seedling mortality. *Fusarium camptocearas*, *F. lateritium*, and *F. xylarioides* are the main causative agents of damping-off (Abo-Elnaga, 2012), while root rot is generally generated by *F. equiseti*, *F. solani*, and *F. oxysporum* (Cao et al., 2018).

It was noted that root rot observed after 6 weeks of inoculation was associated with a decrease in seedling growth parameters. Following these results, Harveson and Rush (1998) display that sugar beet seedlings inoculated with *F. oxysporum* showed a significant reduction in PDWs and heights compared to uninoculated controls. Similarly, Jorgenson (1970) indicated that reduced fresh and dry root weights of sugar beet seedlings were observed in soil naturally infested by *F. oxysporum*. Minimal previous studies are available concerning an association between *F. brachygibbosum*, *F. equiseti*, *F. solani*, *F. nygamai*, *F. proliferatum*, and *F. culmorum* and reduced growth parameters in sugar beet. Nevertheless, this report revealed for the first time that other *Fusarium* spp. than *F oxysporum* can reduce sugar beet seedling growth and vigour. Indeed, recent studies showed that *F. solani*, *F. brachygibbosum*, *F. equiseti*, and *F. falciforme* can induce a reduction in stem height, root length, and weight of citrus (Ezrari et al., 2021). In addition, other research demonstrated that root rot of sorghum induced by *F. nygamai* was associated with a reduction in shoot and root length and dry mass of seedlings relative to uninoculated plants (Al-Juboory & Juber, 2011). Root rot generated by *F. proliferatum* reduces seedling growth and vigour in soybean (Chang et al., 2015) and onions (Carrieri et al., 2013).

Managing plant diseases induced by *Fusarium* is a challenge for growers. Thus, expanding our understanding regarding the diversity of *Fusarium* species involved in RTR and their effect on sugar beet growth is essential if we want to sufficiently manage the disease. Generally, the main target in root disease management of sugar beet plants in Morocco has been *S. rolfsii* and *F. oxysporum* (Farhaoui et al., 2023). The findings of this survey displayed that *F. solani*, *F. equiseti*, *F brachygibbosum*, and *F. nygamai* were sufficiently abundant enough to require greater attention, mainly to conceive adequate management strategies and select the most appropriate fungicides. Several *Fusarium* species produce toxins that can cause human mycotoxicoses (Barac, 2019; Mahanty et al., 2017; Naiker & Odhav, 2004). Therefore, future studies should assess the toxicological menace in sugar beet products based on *Fusarium* species diversity.

5 | CONCLUSIONS

Seven species including *F. solani*, *F. equiseti*, *F. nygamai*, *F. brachygibbosum*, *F. proliferatum*, *F. falciforme*, and *F. culmorum* were observed to be associated with RTR and IVD disease of sugar beet in Morocco for the first time. Isolates belonging to *F. solani* showed the highest virulence among fungal isolates assessed. However, *F. oxysporum* was the species that isolated with the greatest frequency in this survey. Our finding also demonstrated that RTR induced by *Fusarium* was associated with a decline in plant height, root length, and fresh and dry weights of plants infected with fungal isolates in comparison with control plants. Findings of this investigation, therefore, furnish the theoretical basis for integrated management of RTR in the Khenifra-Beni Mellal region of Morocco. Our results are also valuable for future research such as the search for epidemiology, diversity, and effect of the *Fusarium* responsible for seedling mortality and RTP in sugar beet. Moreover, resistance against the prevalent *Fusarium* responsible for sugar beet root rot requires to be more exhaustively studied in future investigations.

CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest to declare that are relevant to the content of this article.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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