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ORIGINAL ARTICLE

Curvularia muehlenbeckiae causing leaf spot on Johnson grass in Mexico

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Abstract

Johnson grass, a common weed in agricultural fields, is widely distributed in warm regions of the world. In Mexico, this grass grows mainly during the rainy season, along railways and roadsides, and in abandoned fields and urban communities. Significantly, it competes with a wide range of crops during the fall-winter season. Recently, a high incidence of severe leaf spot was observed on Johnson grass in northern Sinaloa, Mexico. The aims of this study were to identify the causal agent of this leaf spot based on morphology and molecular techniques and to determine the pathogenicity of the fungal isolates associated with the disease. The combination of morphological and phylogenetic analyses of the ITS and *GAPDH* allowed the identification of ten fungal isolates from Johnson grass as *Curvularia muehlenbeckiae.* Inoculation tests under greenhouse conditions demonstrated the pathogenicity of *C. muehlenbeckiae* on Johnson grass. Leaf spots displayed on the inoculated plants were similar to those observed under field conditions and in two independent inoculation tests. This is the first report implicating *C. muehlenbeckiae* as a causal agent of leaf spot on Johnson grass in México.

Keywords *Curvularia* · Pathogenicity · Molecular identification · *GAPDH* · Morphological analysis

Introduction

Johnson grass (*Sorghum halepense* L.) is native to India and is widely distributed in warm regions of the world (De Wet 1978). Competition of *Sorghum halepense* reduces yield up to 59 to 88% in soybean (*Glycine max* L.) (Williams and Hayes 1984), 80–100% in maize (*Zea mays* L.) (Mitskas et al. 2003; Barroso et al. 2016), 69% in sugarcane (*Saccharum officinarum*) (Dalley and Richard 2008), and 70% in cotton (*Gossypium hirsutum* L.) (Bridges and Chandler 1987). This weed is widely distributed in Mexico and competes with agricultural crops and grows along railways and roadsides, and in abandoned fields and urban communities (Ceseski et al. 2017). In Sinaloa, Mexico, this plant grows mainly during the rainy season (August and September).

Several foliar pathogens have been reported around the world on Johnson grass, including leaf blight (*Exserohilum turcicum*) (Del Serrone and Fornasari 1992). *Bipolaris cynodontis* (Marig.), *Curvularia lunata* (Wakk.) Boedijn, *C. geniculata* (Tracy and Earle) Boedijn, and *Exserohilum rostratum* (Drechs.) Leonard & Suggs have also been isolated from the same plant species (Pratt 2007).

In recent years, severe leaf spot has been observed on Johnson grass during the summer months in northern Sinaloa, Mexico. Leaf spot disease on Johnson grass has reached an incidence of up to 100%, with the foliar diseased area affected reaching from 30 to 70%. Preliminary observations indicate that the disease is highly destructive in rainy periods that accumulate from 0.5 to 1.5 in., with temperatures ranging between 27 and 38 °C.

The disease symptoms on the leaves begin as reddish-brown lesions that are 0.3 mm in diameter and expand to 15 mm in

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diameter. Symptoms on the leaves appear as dark brown oblong spots that subsequently coalesce to form irregular lesions on the leaf sheath, blade, and nodes of the cane. The lesions enlarge mainly in the lower portion of plants, occasionally presenting a light brown center. Similar symptoms on Johnson grass were previously reported in the USA (Pratt 2007), where the causal agents were identified as *Curvularia lunata* and *C. geniculata*. *C. lunata* and *C. dactyloctenicola* have been implicated as causal agents of leaf spot on sorghum in countries such as Pakistan, the USA, China, and Indonesia (Akram et al. 2014; Hidayat and Ramadhani 2019).

Since the etiology of leaf spot on Johnson grass remains unknown in Sinaloa, Mexico, the objectives of the present study were to (a) identify the fungus associated with leaf spot and (b) determine its pathogenicity on the same plant species.

Materials and methods

Sample collection and isolation of fungi associated with leaf spot on Johnson grass

Ten symptomatic plant samples were collected from ten sampling sites 2 km apart from each other, in the municipalities of Ahome and Guasave, Sinaloa, from October 28 through November 10, 2018 (Table 1). To determine the incidence and severity of leaf spot on Johnson grass, 10 leaves were randomly collected from the lower part of the canopy of 10 plants in each sampling site. The samples were placed in plastic bags and brought to the laboratory in an ice chest at 7–10 °C. The foliage area diseased (FAD) (in cm^2) was measured following previously described procedures (Zadoks and Schein 1979). The percentage of FAD was determined taking into consideration the total area of 10 leaves.

To isolate the fungi associated with leaf spot, lesions on the leaves were first swabbed with 70% ethanol, and then fragments of symptomatic tissue (5.0 mm) were disinfected in a 0.5% sodium hypochlorite solution for 2 min, washed three times in sterile distilled water, and dried on sterile Whatman No. 1 filter paper. Then, the fragments were plated on potato dextrose agar (PDA; Bioxon, Cuautitlan Izcalli, Estado de Mexico, Mexico) and incubated for 4 days at 25 ± 2 °C.

Ten isolates were transferred to water agar with sterilized Johnson grass leaves on top (WAJGL) and incubated for 10 days at 24 ± 1 °C. To obtain monosporic cultures, serial dilutions were prepared from pure cultures on WAJGL and spread in 1.6% water agar (WA). Individual spores were collected under a SPTI-ITH stereomicroscope (Fisher Scientific; Waltham, MA, USA) and transferred on PDA at the same temperature. Finally, the isolates were transferred to PDA slants; once the isolates colonized the medium, they were covered with sterile mineral oil and maintained at 15 ± 1 °C for subsequent study.

Molecular identification

DNA extraction and PCR amplification Genomic DNA was extracted from 10 monosporic cultures, according to the protocols of Manamgoda et al. (2012) and Tan et al. (2014). The internal transcribed spacer (ITS) region and partial sequence of the glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) gene were amplified using the ITS1/ITS4 (White et al. 1990) and gpd1/gpd2 (Berbee et al. 1999) primer sets, respectively. Each PCR mixture contained 1.0 μL of each primer, 1.25 μL of 10 mM dNTP, 2.5 μL of reaction buffer without $MgCl_2$, 0.25 μL of AmpliTaq-DNA polymerase, 0.75 μL of 10 mM $MgCl_2$, 17.25 μL of ultra-pure water, and 10 ng of template DNA in a final reaction volume of 25 μL. Amplification

Table 1 Localities and geographical coordinates of Johnson grass leaf sampling sites with leaf spot symptoms, in the municipalities of Ahome and Guasave, Sinaloa, from October 01 through November 10, 2018

Isolate	Location/municipality	Collection date	Coordinates		GenBank accession number	
			N	W	ITS	*GAPDH*
*Cm*7	Santa Rosa, Ahome	10/28/2018	25° 50′ 55″	108° 52′ 58″	MZ674442	MW239670
*Cm*10	Cerrillos, Ahome	10/28/2018	25° 53′ 18″	108° 54′ 17″	MZ674443	MW239671
*Cm*13	San Fernando, Guasave	11/06/2018	25° 32′ 56″	108° 34′ 23″	MZ674444	MW239672
*Cm*14	Campo Diaz, Guasave	11/06/2018	25° 30′ 52″	108° 30′ 35″	MZ674445	MW239673
*Cm*15	Bacahui, Guasave	11/06/2018	25° 35′ 05″	108° 38′ 24″	MZ674446	MW239674
*Cm*16	Bacahui II, Guasave	11/06/2018	25° 35′ 41″	108° 39′ 33″	MZ674447	MW239675
*Cm*17	Baturi, Guasave	11/06/2018	25° 39′ 29″	108° 46′ 39″	MZ674448	MW239676
*Cm*18	Corerepe, Guasave	11/06/2018	25° 38′ 20″	108° 44′ 31″	MZ674449	MW239677
*Cm*19	Bachoco, Guasave	11/10/2018	25° 41′ 43″	108° 47′ 59″	MZ674450	MW239678
*Cm*20	Valle Verde, Guasave	11/10/2018	25° 36′ 17″	108° 31′ 14″	MZ674451	MW239679

reactions were performed in a Verti 96-well Thermal Cycler (Thermo Fisher Scientific, Waltham, MA, USA) and a Labnet MultiGene 96-well Gradient Thermal Cycler (Labnet, Edison, NJ, USA) with the following program: initial denaturation at 95 °C for 2 min, followed by 35 cycles of denaturation at 95 °C for 1 min, annealing at 52 °C (*GAPHD*) and 55 °C (ITS) for 1 min, 45-s extension at 72 °C, and a final extension at 72 °C for 10 min. PCR products were purified using the QIAquick PCR purification Kit® (QIAGEN, Cat. No. 28106) according to the manufacturer's instructions, and quantified using a NanoDrop 2000c spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA).

Sequencing and phylogenetic analysis PCR products were sequenced unidirectionally with an ABI 3730xl sequencer (Applied Biosystems, Foster City, CA, USA) at the National Laboratory of Genomics for Biodiversity (Langebio) in Irapuato, Mexico. Sequences were edited in BioEdit version 7.0.5.3. (Hall 1999) and compared with sequences in the NCBI (National Center for Biotechnology Information) database using the BLAST-N software and the Megablast algorithm. All sequences were deposited in GenBank (Table 1). Sequences were aligned with 89 reference sequences of ex-epitype (^{ET}), ex-isotype (^{IsoT}), ex-isolectotype (^{IsoLT}), ex-paratype (^{PT}), ex-syntype (^{SynT}), and ex-type (^{T}) strains of *Curvularia* species and a sequence of *Bipolaris maydis* as an outgroup (Marin-Felix et al. 2020; Ferdinandez et al. 2021), with MUSCLE algorithm in MEGA X (Edgar 2004; Kumar et al. 2018), using the default parameters for gap opening (−400.00), gap extension (0.00), and UPGMA as cluster method. Multiple alignments of the ITS and *GAPDH* were concatenated and subjected to a partition analysis in PartitionFinder v 1.1.1 (Lanfear et al. 2012), using the branch lengths unlinked, greedy algorithm, and the Akaike Information Criterion (AIC). The phylogenetic analysis was performed in RAxML v 7.2.8 (Stamatakis 2006), using the GTRGAMMAI model for each partition identified by Partition Finder and 1000 rapid bootstraps. Finally, the inferred phylogram was visualized and edited in iTOL (Letunic and Bork 2016; https://itol.embl.de/) and Adobe Acrobat Pro (Adobe Inc.).

Morphological characterization of fungi associated with leaf spot on Johnson grass

Mycelial growth of ten fungal isolates was evaluated on PDA (four Petri plates for each isolate) and the average growth rate was obtained. Petri dishes were inoculated by placing a 5-mm-diameter mycelial plug taken from the margins of actively growing 5-day-old PDA cultures. Inoculated Petri dishes were incubated on a 10-h light/14-h dark photoperiod at 25 ± 2 °C. The frontal and reverse pigmentation of the colonies of each isolate was determined in 10-day-old cultures according to color standards and nomenclature (Ridgway 1912).

The conidia morphology of ten isolates was also determined on PDA. Plugs (5 mm in diameter) from monoconidial cultures were cut from the margins of 4-day-old colonies on PDA, transferred to the same media, and incubated for 10 days at 24 °C under a 10-h light/14-h dark regime. Fungal tissue was mounted in cotton blue dye in lactophenol (Parija and Prabhakar 1995), and the shape, number of septa, and length and width of 40 conidia from each isolate were measured at ×400 magnification with the aid of an ocular and stage micrometer, using a compound microscope (Labomed, Inc., Los Angeles, CA, USA).

Pathogenicity testing

Plant material Johnson grass seeds were collected in the experimental station of the National Institute of Forest, Agricultural and Livestock Research (abbreviated in Spanish as INIFAP), located in Juan José Ríos, Sinaloa, Mexico, in August 2019. The seeds were surface sterilized in 0.5% NaClO for 5 min and rinsed three times in sterile distilled water; subsequently, two seeds were sown per pot in the greenhouse in 28-cm-diameter plastic pots containing a sterile mixture of clay:loam:sand (28.1:27:44.9) at pH 7.0. Once the seeds germinated, plants were fertilized once per week with Miracle-Gro fertilizer (The Scotts Company LLC; Marysville, OH, USA) following the manufacturer's recommendations, and water was supplied as needed.

Inoculum preparation and inoculation For inoculum preparation, ten monoconidial fungal isolates were grown on WAJGL and incubated on a 10-h light/14-h dark photoperiod at 24 °C. After 12 days, conidia were collected by flooding the plates with sterile distilled water (containing 0.01% Tween-20) and scraping the agar surface with a sterile Drigalski spatula. The conidial suspension was filtered through three layers of cheese cloth to remove mycelia, and spores were counted using a hematocytometer and a compound light microscope. The suspension density was adjusted to 4×10^4 conidia/mL (Estrada and Sandoval 2004; Rodriguez et al. 2010).

Each conidial suspension was sprayed onto four healthy 67-day-old Johnson grass plants (at the flowering stage). Control plants were sprayed with sterile distilled water. After inoculation, plants were placed in clear polyethylene bags for 48 h to ensure 85% relative humidity (RH). Inoculated and control plants were arranged in a completely randomized design with four replications (4 plastic pots, each with two plants). Afterwards, plants were subjected to 85% RH for 12 h each day for six consecutive days. The severity of the disease caused by the isolates in Johnson grass was determined 15 days after inoculation. The FAD (in cm^2) was measured in ten leaves of each inoculated plant, following the previously described procedure reported by Zadoks and Schein (1979). The experiment was conducted twice in a greenhouse, in which the temperature

ranged from 25 to 31 °C in experiment 1, and 25 to 33 °C in experiment 2. To fulfill Koch's postulates, the fungus was isolated from the inoculated plants at the end of the experimental period, and its identity was confirmed by taking into account the colony morphology and conidial measurements of twenty conidia from each isolate.

Statistical analysis of data

In order to standardize the data, percentages of FAD were transformed into arcsine values as previously described (Gómez and Gómez 1984). The transformed data were analyzed using ANOVA, and the mean separation was achieved following Tukey's test ($P = 0.05$) (Little and Hills 1973). The analysis was performed with SAS (version 9.1, SAS Institute Inc., Cary, NC, USA). Since the analysis of variance reflected that the results were qualitatively different across experiments (i.e., there was a specimen × experiment interaction), the results for both experiments were included.

Results

Molecular identification

Comparison of the ITS sequences in the GenBank database showed a 100% query coverage and 100% identity with several strains of *C. lunata* (MK690419, MT163356, MN960349), *C. platzii* isolate L-558/2013 (MN540257) and strain BRIP27703b (MH414906), *C. hominis* strain B-36 (MH656705), *C. muehlenbeckiae* strain CY4-2 (MW221359), and *C. plantarum* isolate USJCC-0020 (MT410572); *GAPDH* sequences showed 99–100% query coverage and 99.79–100% identity with C. *muehlenbeckiae* strain CBS 144.63 (LT715806), UTHSC 08-2905 (LT715807), and SCUA-Saf-Vig-6La (MG975602). In addition, it showed high coverage (99–100%) and identity (98.50–99.15%) percentage with other *Curvularia* species such as *C. plantarum* USJCC-0020 (MT628902), *C. pisi* strain CBS 190.48 (KY905690), and *C. hominis* strain UTHSC 09-464 (LT715808).

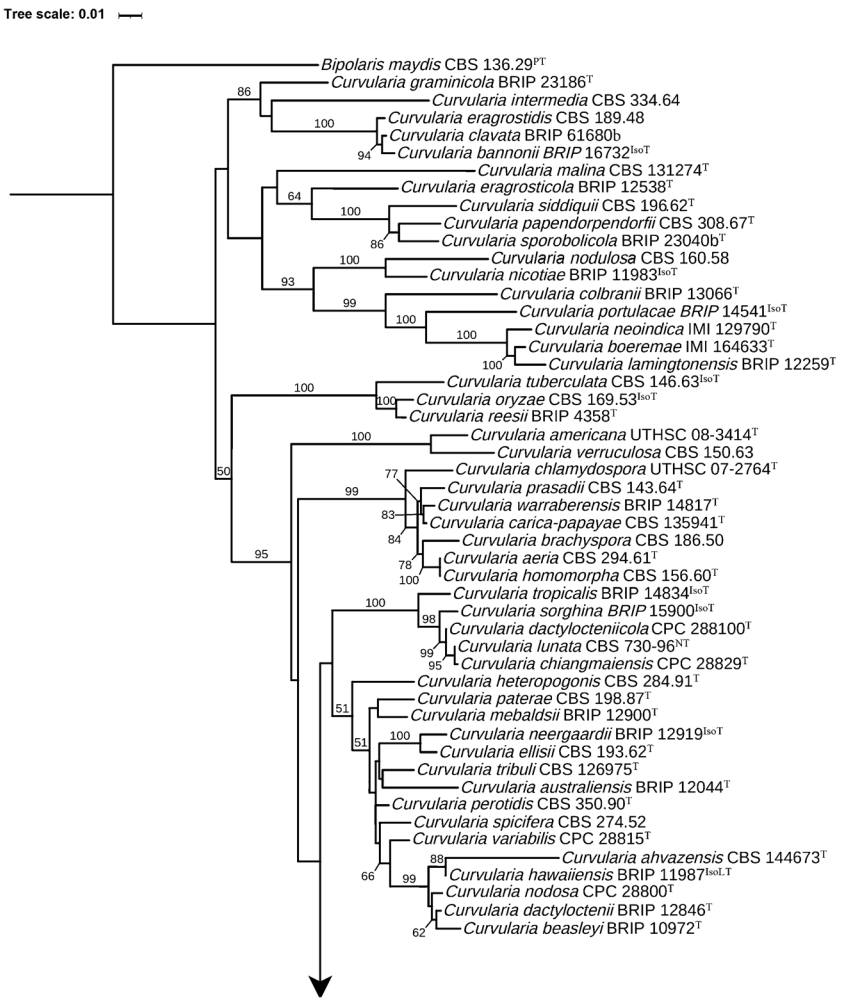

Fig. 1 Maximum likelihood phylogram obtained from the combined ITS and *GAPDH* sequences of *Curvularia* species. The tree was rooted to *Bipolaris maydis*. Isolates from the present study are shown in bold and red color. Bootstrap values above 50% are shown at the nodes. ^{ET}, ^{IsoT}, ^{IsoLT}, ^{PT}, ^{SynT}, and ^{T} indicate ex-epitype, ex-isotype, ex-isolectotype, ex-paratype, ex-syntype, and ex-type strains, respectively

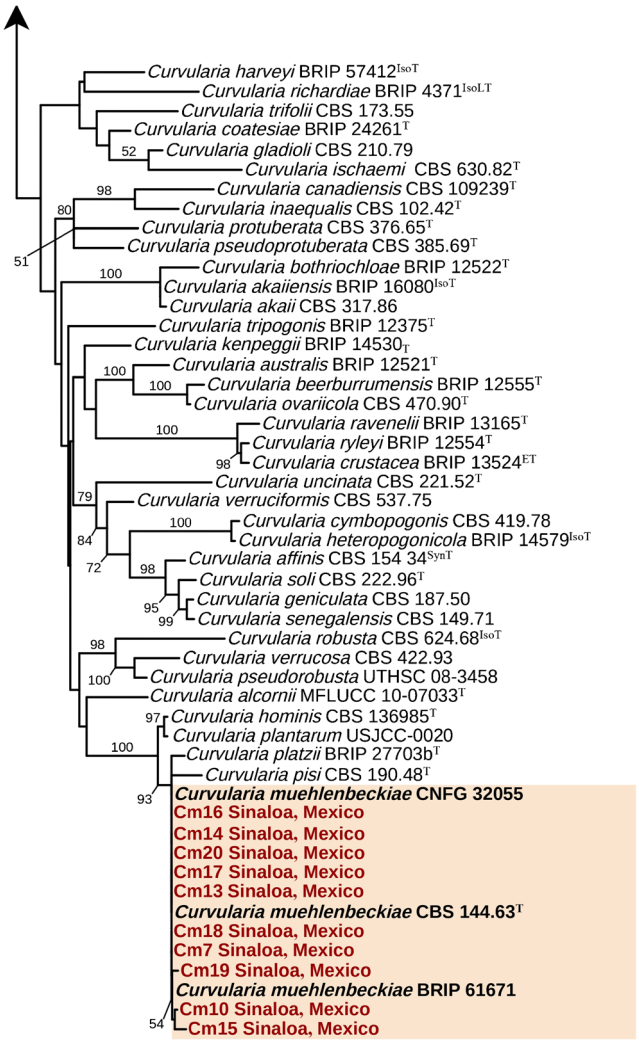

Fig. 1 (continued)

The combined dataset of the ITS and *GAPDH* was 1174 bp in length. PartitionFinder suggested a single partition of the data. The inferred phylogram (Fig. 1) shows that all isolates group within the *C. muehlenbeckiae/platzi/pisi* clade, supported by a high bootstrap value (93%).

Morphological characterization

Ten isolates (*Cm*7, *Cm*10, *Cm*13, *Cm*14, *Cm*15, *Cm*16, *Cm*17, *Cm*18, *Cm*19, and *Cm*20) showed cottony colonies with regular margins, and raised centers ranging from pale gray to ivory cream in color (Fig. 2a); the reverse of the colony was dark in color (Fig. 2b). The conidiophores of isolates *Cm*7, *Cm*10, *Cm*13, *Cm*14, *Cm*15, *Cm*16, *Cm*17, *Cm*18, *Cm*19, and *Cm*20 were septate, semi- to macronematous, simple to branched, straight or flexuous, geniculate towards the apex; cell walls were thicker than in vegetative hyphae, subhyaline to dark brown (Fig. 2c); the conidia were straight to more or less curved, ovoid, septate, pigmented with second cell swelling, (12.5) 17.5–20.0 (23.5) × (5.0) 8.7–10.0 (12.2) μm; the two middle cells were darker than the end cells with three transverse septa (Table 2; Fig. 2d). Mature conidia germinated apically (Fig. 2e).

Pathogenicity test

Seven of the tested isolates (*Cm*7, *Cm*10, *Cm*13, *Cm*16, *Cm*18, *Cm*19, and *Cm*20) were pathogenic on Johnson grass. Initial symptoms occurred 4 days after inoculation and consisted of minute reddish dark lesions. At day 8 after inoculation, lesions were enlarged in size and occurred along the main vein of leaves, reaching the leaf sheath to form irregular

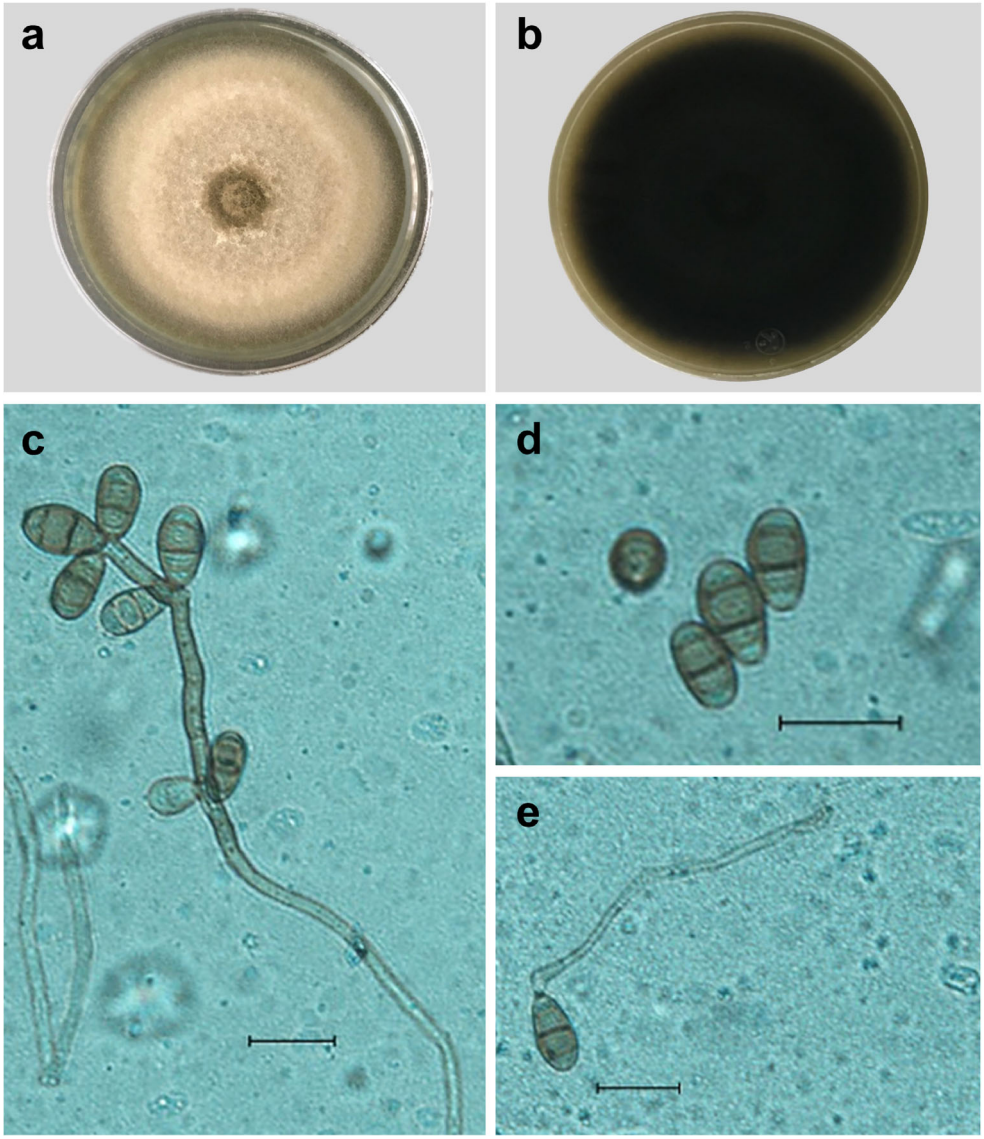

Fig. 2 Morphological characteristics of *Curvularia muehlenbeckiae* (isolate *Cm*7). **a** Colony appearance on PDA at 24 °C after 10 days; **b** pigmentation on a reverse plate; **c** conidia formation on conidiophores; **d** straight, to more or less curved, septate, middle cells darker than the end cells, pigmented conidia with second cell swelling; **e** germinated conidium. Scale bar = 20 μm

to oblong reddish-brown lesions (Fig. 3a) similar to those observed in natural infections under field conditions (Fig. 3b). There was no defoliation of the inoculated plants, as occurs under field conditions.

In experiment 1, the FAD caused by isolates *Cm*7, *Cm*10, *Cm*13, *Cm*16, and *Cm*19 varied from 32.3 to 47.1%. Furthermore, these isolates were significantly more virulent ($F = 121.9$; $P < 0.0001$) than isolates *Cm*18 and *Cm*20, which caused 32.3 and 37.7% of FAD, respectively. In experiment 2, isolates *Cm*10, *Cm*13, *Cm*18, and *Cm*20 were the most virulent, causing 52.3, 53.5, 62.6, and 57% of FAD, respectively. The FAD caused by these isolates was significantly different ($F = 126.2$; $P < 0.0001$) from the FAD caused by isolates *Cm*7, *Cm*16, and *Cm*19, which varied from 45.7 to 49.2% (Table 3). To fulfill Koch's postulates, the pathogen was reisolated from symptomatic leaves and its identity was confirmed by comparing the colony characteristics and conidial measurements of 20 conidia with those used in the inoculation tests.

Discussion

Under field conditions, the incidence of leaf spot on Johnson grass reached up to 100% and the FAD in the lower third of plants varied from 30 to 70%. Symptoms observed on this weed were similar to those caused by several species of *Curvularia* in other members of the family *Poaceae* in other parts of the world, such as sorghum (*S. bicolor*) (Akram et al. 2014), rice (*Oryza sativa*) (Estrada and Sandoval 2004;

Table 2 Morphometric characteristics of conidia of *Curvularia muehlenbeckiae* associated with leaf spot on Johnson grass, developed in PDA culture medium

Isolate	Length (μm)	Width (μm)	Ratio (L/W) (μm)	Transverse septa
*Cm*7	17.5–22.5[a]	7.5–11.2		
	20.1[b]	9.8	2.0	3
	1.4[c]	0.7		
*Cm*10	17.5–22.5	8.0–10.2		
	19.3	9.8	2.0	3
	1.3	0.4		
*Cm*13	17.0–22.5	7.5–11.2		
	19.7	9.8	2.0	3
	1.5	0.6		
*Cm*14	17.5–22.5	7.5–10.0		
	19.8	9.5	2.0	3
	1.4	0.7		
*Cm*15	17.0–22.5	7.5–10.7		
	18.8	9.3	2.0	3
	1.4	0.8		
*Cm*16	12.5–17.5	5.0–7.5		
	14.5	5.4	2.7	3
	1.4	0.8		
*Cm*17	16.2–22.5	7.5–10.7		
	19.6	9.8	2.0	3
	1.7	0.5		
*Cm*18	17.5–22.5	7.5–10.0		
	19.7	9.4	2.1	3
	1.3	0.9		
*Cm*19	17.5–22.5	7.5–10.5		
	19.6	9.6	2.0	3
	1.2	0.8		
*Cm*20	17.5–23.7	7.5–12.2		
	20.0	9.6	2.1	3
	1.5	0.9		
Minimum-maximum of 10 isolates	12.5–23.7	5.0–12.2	2.0	3
Mean of 10 isolates	19.1	9.2		
Standard deviation	2.1	1.4		

For each isolate, the first line represents the minimum and maximum lengths (a) of the asexual structures; the second line indicates the means (b) for 40 structures of each isolate; and the third line refers to the standard deviation (c) of the data

Azizah-Kusai et al. 2016), corn (*Zea mays*) (Garcia-Aroca et al. 2018), and king grass (*Pennisetum hydridum*) (Xu et al. 2018). The incidence of the leaf spot on Johnson grass in Sinaloa occurred when the accumulated rain during summer and mid-fall was 687.6 mm and temperature ranged from 25 to 38 °C (CONAGUA 2020). These weather conditions agree with previous reports of *C. lunata* causing leaf spot on corn in the USA (Munkvold and White 2016) and *Curvularia* sp. on the same crop in Brazil (Ribeiro-Chagas et al. 2020), as well as *Curvularia malina* on turf grasses in the southeastern USA (Tomaso-Peterson et al. 2016).

Species delimitation in this genus is complex because of the presence of cryptic species; however, recent multigene phylogenies have shown that the sequences of the ITS region and *GAPDH* gene can resolve the majority of species within the genus (Manamgoda et al. 2015; Marin-Felix et al. 2020). The obtained phylogram confirmed that *Curvularia* isolates from Johnson grass are closely related to *C. muehlenbeckiae*, *C. platzi*, and C. *pisi*, but species delimitation was not clear enough. Thus, morphological characteristics were essential for discrimination between species. The phenotypic characteristics of the colonies and morphology of the conidia of isolates *Cm*7, *Cm*10, *Cm*13, *Cm*14, *Cm*15, *Cm*16, *Cm*17, *Cm*18, *Cm*19, and

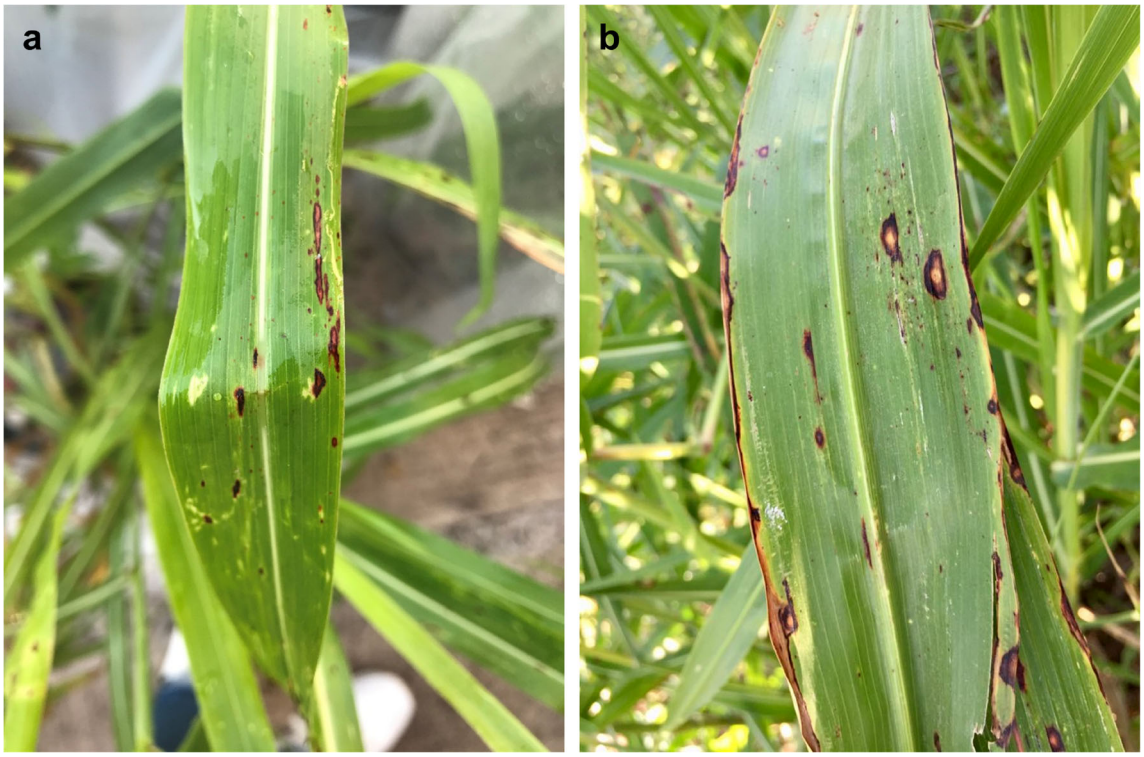

Fig. 3 Symptoms of leaf spot on Johnson grass. **a** Symptoms observed on a leaf caused by *Curvularia muehlenbeckiae* (isolate *Cm*7) in artificial inoculations in the greenhouse. **b** Symptoms produced from natural infections under field conditions

*Cm*20 were similar to those of *C. muehlenbeckiae* (Madrid et al. 2014; Nam et al. 2020). This fungal species is clearly distinct from other phylogenetically close species like *C. platzii* and *C. pisi*. Conidia of *C. platzii* are fusiform to narrowly clavate, whereas those of *C. muehlenbeckiae* are asymmetrical to more or less curved at the third cell from base (Madrid et al. 2014), like those observed in all Johnson grass isolates (Fig. 2d).

Table 3 Pathogenicity of *Curvularia muehlenbeckiae* on Johnson grass

Isolates	Disease severity (%)[z]	
	Experiment 1	Experiment 2
*Cm*7	39.4abc*	45.7c*
*Cm*10	38.8abc	52.3abc
*Cm*13	44.1ab	53.5abc
*Cm*16	47.1a	48.6bc
*Cm*18	32.3c	62.6a
*Cm*19	43.1ab	49.2bc
*Cm*20	37.7bc	57.0ab
Non-inoculated control	0.0d	0.0d
CV (%)	23.5	23.1

[z] Disease severity was determined in Johnson grass 15 days after inoculation

*Data within columns followed by common letter indicate no significant difference ($P = 0.05$) using Tukey's test

Conidia of *C. pisi* are similar in shape, but are larger (16–35 × 9–15.5 μm) than those produced by *C. muehlenbeckiae* (17–26 × 8.5–12 μm) (Marin-Felix et al. 2017). Conidia measurements of our isolates correspond to those of *C. muehlenbeckiae*. By combining the morphological and molecular data, we were able to identify all isolates as *C. muehlenbeckiae*. The seven tested isolates were pathogenic to Johnson grass, with variation in virulence among the isolates.

Despite the fact that *C. muehlenbeckiae* was originally reported as pathogenic in human beings, it has been implicated in leaf spot on *Muehlenbeckia* sp. in India (Madrid et al. 2014). Furthermore, it was reported to cause leaf spot on *Sorghum* sp. and *S. bicolor* in the USA and Japan (Manamgoda et al. 2015), while the present study is the first report of this species causing leaf spot on Johnson grass in Mexico. Other species of *Curvularia* such as *C. dactyloctenicola* have been reported on Egyptian grass (*Dactyloctenium aegyptium* (L.) Willd.) in Thailand (Marin-Felix et al. 2017) and on *S. bicolor* in Indonesia (Hidayat and Ramadhani 2019), as well as on rice (*Oryza sativa*) (Estrada and Sandoval 2004; Azizah-Kusai et al. 2016), corn (*Zea mays*) (Garcia-Aroca et al. 2018), and king grass (*Pennisetum hydridum*) (Xu et al. 2018) in other parts of the world, although these species were not found during our sampling of Johnson grass in Mexico.

This is the first report of *C. muehlenbeckiae* causing leaf spot in a member of the family *Poaceae* in Mexico. Studies on the identity of *Curvularia* species on members of the *Poaceae* family are in an early stage in Mexico. For example, *C. lunata* has been implicated in grain mold of sorghum (Montes-García et al. 2010) and leaf spot on corn (Ríos-Herrera et al. 2017). Therefore, future research should focus on determining whether Johnson grass is a potential source of inoculum for *C. muehlenbeckiae* infecting field crops such as corn and sorghum in Mexico.

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Author contribution R. Félix-Gastélum contributed to the conception and design of the study. Material preparation, sample collection, isolation, and morphology were performed by R. Félix-Gastélum and D. D. Olivas-Peraza. DNA sequencing, phylogenetic analysis, and sequence submission to GenBank were performed by D. D. Olivas-Peraza, K. Y. Leyva-Madrigal, and I. E. Maldonado-Mendoza. All the authors commented on the initial versions of the manuscript, and all the authors read and approved the final manuscript.

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Data availability All relevant molecular data has been deposited in GenBank (this has been already indicated in the main text).

Declarations

Conflict of interest The authors declare no competing interests.

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