Survey

Six Species of *Diaporthe* Associated with Phomopsis Stem Canker of Sunflower in Southern Pampean Region of Argentina

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Abstract

A survey of 67 commercial fields in 19 locations was conducted in the southern Pampean region of Argentina for Phomopsis stem canker of sunflower (*Helianthus annuus* L.) between 2014 and 2019. A total of 210 plants having typical symptoms of Phomopsis stem canker were randomly sampled, and fungal isolation was performed. Of the 187 isolates of *Diaporthe* that were recovered, 94% of the isolates showed morphological characteristics similar to *D. helianthi*, 3% to *D. gulyae*, 1% to *D. caulivora*, 1% to *D. sojae*, 0.5% to *D. kongii*, and 0.5% to *D. longicolla*. Following morphological characterization, the identity of the six morpho-species was confirmed by phylogenetic analyses of β-tubulin, translation elongation factor 1-α, and internal transcribed spacer gene regions. Koch's postulates were completed for the six fungi by inoculating one susceptible sunflower hybrid with one isolate each of the six species of *Diaporthe* using the stem-wound inoculation method. Seven days postinoculation, significant differences in disease severity were observed between the six isolates ($P < 0.0001$), with *D. helianthi* and *D. gulyae* isolates causing significantly greater disease severity. To our knowledge, this is the first report of *D. kongii*, *D. longicolla*, *D. caulivora*, and *D. sojae* associated with Phomopsis stem canker of sunflower in Argentina.

Keywords: Phomopsis stem canker, sunflower, *Diaporthe*, Argentina

Phomopsis stem canker (PSC) is a major yield-limiting disease of sunflower (*Helianthus annuus* L.) in the world (Harveson et al. 2016). Following the first report of the disease caused by *Diaporthe helianthi* in the former Yugoslavia (Muntañola-Cvetković et al. 1985), PSC has been reported in several sunflower-producing countries including Russia and Ukraine in Europe, the United States, South Africa, and Australia (Gulya et al. 1997; Lamarque and Perny 1985; Masirevic and Gulya 1992; Skripka et al. 1993; Voros et al. 1983; Yakutkin 1998).

Disease epidemics of PSC on sunflower in Australia (Thompson et al. 2011) and the United States (Mathew et al. 2015) revealed that multiple species of *Diaporthe* are involved in disease development. While Thompson et al. (2011) identified and described *D. kochmanii* Shivas et al. (syn. *D. sojae* [Lehman] Wehmeyer), *D. kongii* Shivas et al., and *D. gulyae* Shivas et al. causing PSC on sunflower in Australia, Mathew et al. (2015) reported *D. helianthi* and *D. gulyae* caused the disease in the United States. Subsequently, *D. novem* was also reported as pathogenic on sunflower and associated with the PSC outbreak in Australia (Thompson et al. 2018).

Argentina is the third largest producer of sunflower in the world, contributing approximately 7.3% of the total sunflower production in the world (USDA-NASS 2019). Commercial production of sunflower in Argentina has always been compromised by yield losses and harvest uncertainties due to diseases caused by fungi and factors leading to disease development (e.g., level of susceptibility and resistance to fungal pathogens present in commercial hybrids, presence of fungal inoculum in commercial fields, weather conditions, production practices, etc.) (Pereyra and Escande 1994). In Argentina, species of *Diaporthe* were historically identified based on their association with the host, and the first report of PSC listed *D. helianthi* as the causal agent (Fálico de Alcaraz et al. 1994).

Since this report, PSC has been present at low to no incidence in the country. Between 2003 and 2005, monitoring for PSC was attempted by Huguet (2006) in the east-central part of Argentina. This included inspection of field trials planted with commercial hybrids obtained from the Argentine Sunflower Association (ASAGIR) and seed companies. These hybrids were evaluated for PSC from flowering growth stage (R5) to physiological maturity (R9) of sunflower (Schneiter and Miller 1981), but no disease was observed (Huguet 2006). In addition, between 2010 and 2017, commercial hybrids were evaluated by Facultad de Ciencias Agropecuarias (UNER, Entre Ríos, Argentina) for resistance to PSC in field trials in the Entre Ríos province, and it was observed that genotypes responded differentially to the disease among years (average disease incidence of 4.16%; Cáceres and Musante 2018). The differential response among hybrids to PSC was possibly because of differences in environmental factors, levels of fungal inocula, genetic differences among hybrids, and the diversity of species of *Diaporthe* causing the disease. To address if multiple species of *Diaporthe* were causing PSC in field trials in Entre Ríos province, fungal isolations were made from the diseased plants, and multiple species of *Diaporthe* were tentatively identified using molecular tools (Burgos et al. 2015; Cáceres and Musante 2018).

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In 2015, PSC re-emerged in the southern Pampean region of Argentina, where more than 40% of the country's sunflower production is concentrated (Corró-Molas et al. 2016; Ivancovich and Lavilla 2016). The grain yield loss ranged between 17 and 44%, and the oilseed content loss was estimated to be 15% from the PSC disease epidemic (Ghironi et al. 2018). Currently, options to manage PSC in Argentina are limited. Although there are some hybrids available with partial resistance to PSC, yield losses are still observed when such hybrids are used in commercial fields. Preventive application of foliar fungicides at mid- to late-vegetative growth stages of sunflower development may help manage PSC (Corró-Molas et al. 2019). However, fungicide applications may not be effective after the PSC symptoms appear on the sunflower plants. Recent research characterizing species of *Diaporthe* causing PSC using morphology as well as analyses of translation elongation factor 1-α (EF1-α) and internal transcribed spacer (ITS) gene regions confirmed the presence of *D. helianthi* and, for the first time in Argentina, of *D. gulyae* (Mancebo et al. 2019).

The objective of this research is to report the species of *Diaporthe* associated with PSC on sunflower in the southern Pampean region of Argentina using morphology; phylogenetic analyses of β-tubulin, EF1-α, and ITS gene regions; and pathogenicity under controlled conditions.

Collection of *Diaporthe* Isolates

A survey of 67 production fields was performed in 19 locations (36 to 40° S and 57 to 63° W) in the southern Pampean region of Argentina for PSC between 2014 and 2019. The incidence of PSC was observed to be between 2 and 30% in these fields. A total of 210 plants with brown discoloration on the stem at the insertion of the petiole were randomly sampled.

Fungal isolations were made from the margins of stem lesions from the sampled plants. The stems were cut into pieces (5 to 10 mm), surface disinfected with 1% sodium hypochlorite for 1 min, and washed with 70% ethanol for 1 min. Following surface disinfection, the pieces were plated on potato dextrose agar acidified with lactic acid 0.02% (pH 4.5, PDAL) to inhibit bacterial growth. The PDAL plates were incubated at 25°C for 7 to 10 days under a 12-h photoperiod, and hyphal tips of white to gray colonies resembling the cultural characteristics of *Diaporthe* were transferred to fresh PDAL plates. The resulting PDAL plates were incubated for 30 days at 25°C under a 12-h photoperiod.

Morphological Identification of *Diaporthe* Isolates

Fungal structures (pycnidia and conidia) were mounted on glass slides and examined using an optical microscope (10× magnification). A putative identification of isolates was made based on comparison with the taxonomic description for species of *Diaporthe* (Hanlin 1990; Santos et al. 2011; Thompson et al. 2011). Of the 187 isolates examined, 176 isolates showed similar morphological characteristics as *D. helianthi* (β-conidia; 29.0 to 32.3 × 0.5 to 2.0 μm) and five isolates as *D. gulyae* (α-conidia; 6.6 to 9.9 × 3.3 to 4.9 μm). However, six isolates fitted the taxonomic description of either *D. kongii*, *D. sojae*, *D. caulivora* (Athow & Caldwell) Santos et al., or *D. longicolla* (Hobbs) Santos et al. (Table 1).

DNA Isolation, PCR Amplification, and DNA Sequencing

DNA was extracted from one isolate selected as morphologically typical of each of the four species of *Diaporthe* reported in the present work, and they were identified as isolates 1, 2, 3, and 6 (Isol1, Isol2, Isol3, and Isol6, respectively). In the analysis were also included isolates 4 (Isol4) and 5 (Isol5), corresponding to *D. helianthi* and *D. gulyae*, respectively, previously described in Mancebo et al. (2019). Mycelial discs of the four isolates were cut from the margin of actively growing colonies and transferred to 150 ml of potato dextrose liquid medium. After growing those cultures at 25°C for 15 days, mycelia were separated by filtration, dried under sterile conditions, and stored at –20°C. Dried mycelia were transferred to an autoclaved mortar to which liquid nitrogen was already added and allowed to evaporate. The mycelia were ground to fine powder and transferred to sterile microcentrifuge tubes (1.5 ml) for DNA extraction. Genomic DNA was extracted with the Wizard Genomic DNA Purification kit (Promega, Madison, WI), according to the manufacturer's instructions.

The ITS gene region of the isolates was amplified using ITS1 and ITS4 primers (White et al. 1990) in a 25.0-μl mixture, which contained 2.0 μl (10 ng/μl) of DNA, 0.75 μl (10.0 μM) of forward

TABLE 1
Morphological characteristics of species of *Diaporthe* identified in this study

Sample location[y]	Morphological description	Tentative identification[z]
Pieres (1): Isol1	Conidiomata pycnidial, scattered, subglobose, up to 3 mm diameter, occasionally with ostiolate beaks up to 1 mm. α-Conidia, hyaline, 9.9 to 13.2 μm (mean, *n* = 10). β-Conidia occasionally produced on sunflower seed culture medium. Colonies in potato dextrose agar ropey with tufted aerial white mycelium. It becomes grayish and occasionally greenish.	*D. kongii*
Pieres (2): Isol2	Globose perithecia, black, scattered in high numbers within black ectostromatic areas. Necks >400 μm, filiform, with hairy and dilated tip. Asci 42.9 to 59.4 × 9.9 μm. Ascospores 9.9 to 10.89 × 3.3 μm (mean, *n* = 10). β-Conidia 26.4 to 34 × 3.3 μm, and α-conidia 6.6 to 9.9 × 3.3 μm (mean, *n* = 10).	*D. sojae*
Orense (1); Pieres (1): Isol3	Globose perithecia, single or clustered in groups of two to three. Black, straight necks. Asci 36.3 to 42.9 × 9.9 μm (mean, *n* = 40), ellipsoid, widest at center and rounded toward the apices, eight spores. Ascospores 13.2 to 16.5 × 3.3 to 4.95 μm (mean, *n* = 10), hyaline, ellipsoid to fusoid, guttules. Anamorph not seen.	*D. caulivora*
Pieres (1): Isol6	Frequent stroma extended from the center of the colony to the margins, continued. Pycnidial necks prominent >400 μm, α-conidia unicellular, ellipsoid to ovoid, 9.9 to 13.2 × 3.3 to 4.95 μm (mean, *n* = 10), hyaline, biguttulate, exuding from the pycnidial ostiole in a yellowish, translucent drop. β-Conidia not seen.	*D. longicolla*

[y] In parentheses is the number of isolates sampled from each location.
[z] Based on Muntañola-Cvetković et al. (1985), Santos et al. (2011), and Thompson et al. (2011).

primer, 0.75 µl (10.0 µM) of reverse primer, 12.5 µl of Master Mix (Qiagen) containing Taq DNA Polymerase, and 9.0 µl of nuclease-free water. The EF1-α and β-tubulin genes were amplified using the primer pairs EF1-728F/EF1-986R (Carbone and Kohn 1999) and Bt2a/Bt2b (Glass and Donaldson 1995), respectively, in a 25.0-µl PCR mixture, as described previously for ITS. The PCR fragment sizes obtained were approximately 580 bp for ITS, 350 bp for EF1-α, and 530 bp for β-tubulin. The ITS and EF1-α from Isol4 and

TABLE 2
Reference sequences of species of *Diaporthe* for phylogenetic analyses[z]

Diaporthe species	Host	Isolate/culture collection	Abbreviation	GenBank accessions		
				ITS	β-Tubulin	EF1-α
D. caulivora	*G. max*	17-DIA-016	Dcau17DIA016	MK942652	MK941318	MK941273
		16-DIA-068	Dcau16DIA068	MK942656	MK941317	MK941275
		17-DIA-085	Dcau17DIA085	MK942655	MK941316	MK941274
		17-DIA-066	Dcau17DIA066	MK942657	MK941315	MK941278
		17-DIA-033	Dcau17DIA033	MK942654	MK941314	MK941277
	H. annuus	**Isolate 3**	**Isol3**	**MT636145**	**MT636156**	**MT636151**
D. endophytica	*G. max*	CBS 133811	Dend133811	KC343065	KC344033	KC343791
		LGMF948	DendLGMF948	KC343072	KC344040	KC343798
D. goulteri	*H. annuus*	BRIP 55657a	Dgou55657a	KJ197290	KJ197270	KJ197252
D. gulyae	*H. annuus*	MF-Ha17-043	DgulMFHa17043	MK024253	MK033489	MK039421
		MF-Ha17-042	DgulMFHa17042	MK024252	MK033488	MK039420
		MFLUCC 17-1026	Dgul171026	KY964223	KY964107	KY964179
		Isolate 5a	Isol5a	MK105835	**MT636158**	MK110012
		Isolate 5b	Isol5b	MK105836	**MT636159**	MK110013
D. helianthi	*H. annuus*	CBS 592.81	Dhel592.81	AY705842	KC344083	KC343841
		CBS 344.94	Dhel344.94	KC343114	KC344082	KC343840
		Isolate 4	Isol4	MK105837	**MT636157**	MK110014
D. kongii	*H. annuus*	17-DIA-077	Dkon17DIA077	MK942659	MK941320	MK941280
		BRIP 54031	DkonBRIP54031	NR_111616	KJ197272	–
		Isolate 1	**Isol1a**	**MT636147**	**MT636153**	–
			Isol1b	**MT636148**	**MT636154**	**MT636149**
D. longicolla	*G. max*	LDSG3-2	DlonLDSG32	MN960195	MN974482	MN974483
		17-DIA-128	Dlon17DIA128	MK942662	MK941335	MK941283
		17-DIA-018	Dlon17DIA018	MK942663	MK941323	MK941284
		17-DIA-040	Dlon17DIA040	MK942667	MK941326	MK941288
		FAU657	DlonFAU657	KJ590727	KJ610882	KJ590766
	H. annuus	**Isolate 6**	**Isol6**	**MT636144**	**MT636160**	**MT636152**
D. masirevicii	*G. max* / *H. annuus*	DM-GS	DmasDMGS	MF682435	MF668289	MF668290
		BRIP 54118b	Dmas54118b	KJ197280	KJ197260	KJ197242
		BRIP 57330	Dmas57330	KJ197275	KJ197255	KJ197237
		BRIP 54256	Dmas54256	KJ197277	KJ197256	KJ197238
		BRIP 57892a	Dmas57892a	KJ197276	KJ197257	KJ197239
D. novem	*G. max*	KARE1378	DnovKARE1378	MN168883	MN318151	MN318144
		EFA 461	DnovEFA 461	MH050430	MH051285	MH051279
		CPC 28169	Dnov28169	MF418429	MF418589	MF418508
D. sackstonii	*H. annuus*	BRIP 54669b	Dsac54669b	KJ197287	KJ197267	KJ197249
D. serafiniae	*H. annuus*	Phom225	DserPhom225	KY511313	KY511344	MH708545
		BRIP 54136	Dser54136	KJ197273	KJ197253	KJ197235
D. sojae	*G. max*	PSCG 530	Dsoj530	MK626943	MK691310	MK654892
		AR3602	DsojAR3602	KJ590714	KJ610870	KJ590757
		FAU637	DsojFAU637	KJ590720	KJ610876	KJ590763
		FAU635	DsojFAU635	KJ590719	KJ610875	KJ590762
		FAU604	DsojFAU604	KJ590716	KJ610872	KJ590759
		FAU499	DsojFAU499	KJ590717	KJ610873	KJ590760
	H. annuus	**Isolate 2**	**Isol2**	**MT636146**	**MT636155**	**MT636150**
Valsa ambiens (outgroup)	–	CFCC 89622	Vamb	KR045616	KR045657	KU710911

[z] Accessions of the sequences derived from this study are indicated in bold. *G. max* = *Glycine max*; *H. annuus* = *Helianthus annuus*; EF1-α = translation elongation factor 1-α; and ITS = internal transcribed spacer.

Isol5 were taken from deposited sequences in GenBank, whereas the β-tubulin gene region was amplified from genomic DNA isolated and stored by Mancebo et al. (2019). The DNA samples were sequenced (Functional Biosciences, Madison, WI) using the same primer pairs.

All sequences generated in this study were deposited in the National Center for Biotechnology Information (NCBI, Bethesda, MD; https://www.ncbi.nlm.nih.gov/) under the accession numbers MT636144 to MT636160 (Table 2).

Molecular Phylogenetic Analyses

Phylogenetic reconstruction of concatenated and individual gene trees was performed using the maximum likelihood method in MEGA version X (Kumar et al. 2018) using the reference sequences available in GenBank. The evolutionary history of the taxa was represented by the bootstrap consensus trees inferred from 1,000 replicates. The bootstraps were calculated as percentage of replicate trees in which the associated taxa clustered together in the bootstrap test. The branches corresponding to partitions reproduced in less than 50% of bootstrap replicates are collapsed (Felsenstein 1985). Generated ITS, β-tubulin, and EF1-α sequences were analyzed with available *Diaporthe* sequences coming from the same isolate in order to carry out the concatenated analysis (Table 2). For rooting, *Valsa ambiens* (Pers.) Fr. (synonym *Cytospora populina* [Pers.] Rabenh.) was used as the outgroup for all trees.

The ITS tree showed that Isol1 belonged to the *D. kongii* clade (bootstrap 87%), Isol2 to *D. sojae* (bootstrap 97%), Isol3 to *D. caulivora* (bootstrap 100%), and Isol6 to *D. longicolla* (bootstrap 92%) (Fig. 1). As expected, Isol4 and Isol5 grouped with *D. helianthi* and *D. gulyae*, respectively. The EF1-α (Fig. 2) tree showed a similar topology as the ITS tree for Isol2 (*D. sojae*; bootstrap 97%), Isol3 (*D. caulivora*; bootstrap 100%), and Isol6 (*D. longicolla*; bootstrap 99%). The EF1-α and β-tubulin (Fig. 3) trees did not clarify the identity of Isol1, because EF1-α grouped it with *D. kongii*, *D. masirevicii*, and *D. endophytica* (bootstrap 77%; Fig. 2), and the β-tubulin tree grouped Isol1 and Isol2 in a polyphyletic clade with *D. kongii*, *D. masirevicii*, *D. endophytica*, and *D. sojae* (bootstrap 99%; Fig. 3).

A maximum likelihood consensus tree was developed by concatenating the sequences of the three genes, and this clarified the identity of the four isolates as follows: Isol1 (*D. kongii*; bootstrap 70%), Isol2 (*D. sojae*; bootstrap 99%), Isol3 (*D. caulivora*; bootstrap 100%), and Isol6 (*D. longicolla*; bootstrap 100%). As

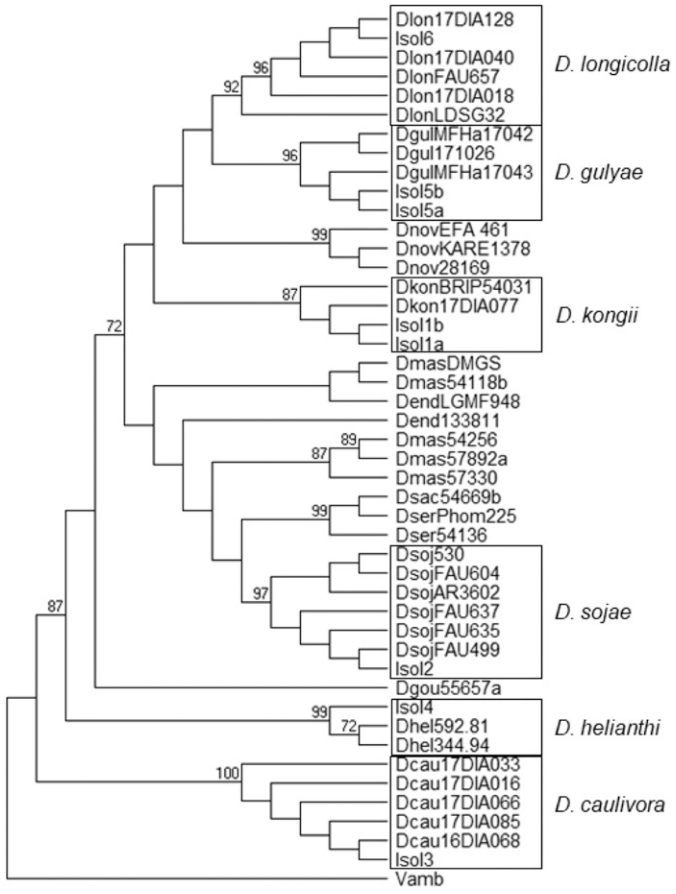

FIGURE 1

Bootstrap consensus tree generated from the analysis of the internal transcribed spacer (ITS) gene region by using the maximum likelihood method. Bootstrap values above 70% are shown. Isol1, Isol2, Isol3, and Isol6 correspond to the ITS sequences from each of the four new isolates characterized in this study. Isol4 and Isol5 correspond to *Diaporthe helianthi* and *D. gulyae*, respectively, described in Mancebo et al. (2019). The ITS sequences of different species of *Diaporthe* are indicated in Table 2. *Valsa ambiens* (Vamb) was used as the outgroup.

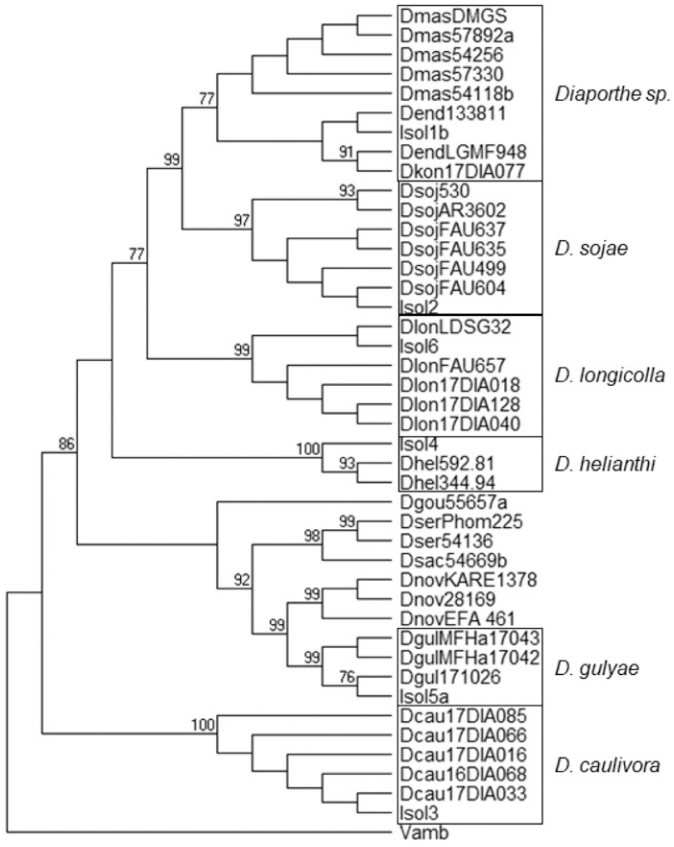

FIGURE 2

Bootstrap consensus tree generated from the analysis of the translation elongation factor 1-α (EF1-α) gene region by using the maximum likelihood method. Bootstrap values above 70% are shown. Isol1, Isol2, Isol3, and Isol6 correspond to the EF1-α sequences from each of the four new isolates characterized in this study. Isol4 and Isol5 correspond to *Diaporthe helianthi* and *D. gulyae*, respectively, described in Mancebo et al. (2019). The EF1-α sequences of different species of *Diaporthe* are indicated in Table 2. *Valsa ambiens* (Vamb) was used as the outgroup.

described by Mancebo et al. (2019), Isol4 and Isol5 grouped with *D. helianthi* and *D. gulyae*, respectively (bootstrap 100% for both isolates; Fig. 4).

Pathogenicity of Isolates of *Diaporthe*

The pathogenicity of Isol1, Isol2, Isol3, and Isol6 was evaluated by inoculating plants of a susceptible experimental hybrid (Advanta H1) at the vegetative phenological stage V4 (four leaves) to V6 (six leaves) of crop development (Schneiter and Miller 1981). Isolates of *D. helianthi* and *D. gulyae* sampled by Mancebo et al. (2019) were included as positive controls.

A randomized complete block design with eight replicates and four pots per isolate and per block (one plant per pot) was used. The pots were placed in a growth chamber set at 12 h of light at 25°C/12 h of dark at 20°C (14,000 lm/m^2 of light intensity) and a relative humidity of 85%. The stems were wounded with a micropipette tip on the second node, and a mycelial plug was placed on the wound. Following the placement of the mycelial plug of 9 mm diameter, the wound was wrapped with a film (Parafilm) to avoid desiccation (Mathew et al. 2015). For control plants, sterile PDAL discs were placed on plant wounds. The experiment was repeated twice for each isolate. Seven days postinoculation, outside lesion length (cm) and plant height (cm) were measured. Disease severity was calculated as the percentage of diseased stems (lesion length/plant height × 100). Data were transformed by applying arcsine square root for disease severity. Analyses of variance and mean comparison test (Tukey) ($\alpha = 0.05$) were performed using the InfoStat Software version 2015 (Di Rienzo et al. 2015).

All isolates caused internal and external discoloration of the stem of the plants. Overall, the appearance of the lesion caused by the isolates representing *D. helianthi*, *D. gulyae*, *D. caulivora*, *D. sojae*, *D. kongii*, and *D. longicolla* was similar, so it was difficult to differentiate them. Additionally, each isolate of *D. helianthi*, *D. gulyae*, and *D. caulivora* caused small necrotic spots (typical toxin effect on upper leaves as descried by Mazars et al. 1990) and lodging of the stems. The fungi were successfully reisolated from stems, and Koch's postulates were completed. Significant differences in disease severity were observed among the six isolates ($P < 0.0001$). The isolates of *D. helianthi* and *D. gulyae* caused significantly greater disease severity, whereas isolates of *D. caulivora* and *D. longicolla* caused

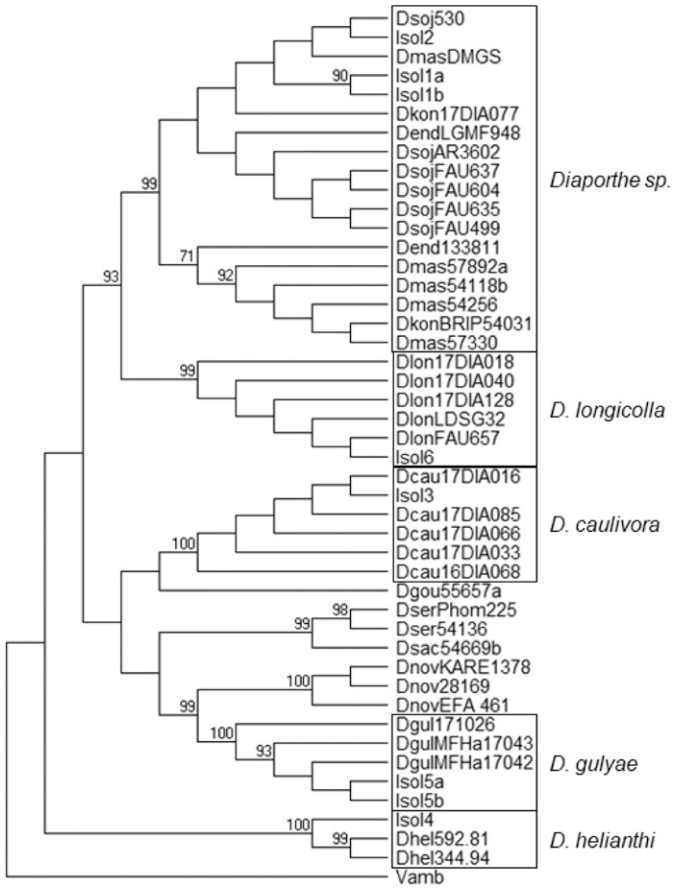

FIGURE 3

Bootstrap consensus tree generated from the analysis of β-tubulin gene region by using the maximum likelihood method. Bootstrap values above 70% are shown. Isol1, Isol2, Isol3, and Isol6 correspond to the β-tubulin sequences from each of the four new isolates characterized in this study. Isol4 and Isol5 correspond to *Diaporthe helianthi* and *D. gulyae*, respectively, described in Mancebo et al. (2019). The β-tubulin sequences of different species of *Diaporthe* are indicated in Table 2. *Valsa ambiens* (Vamb) was used as the outgroup.

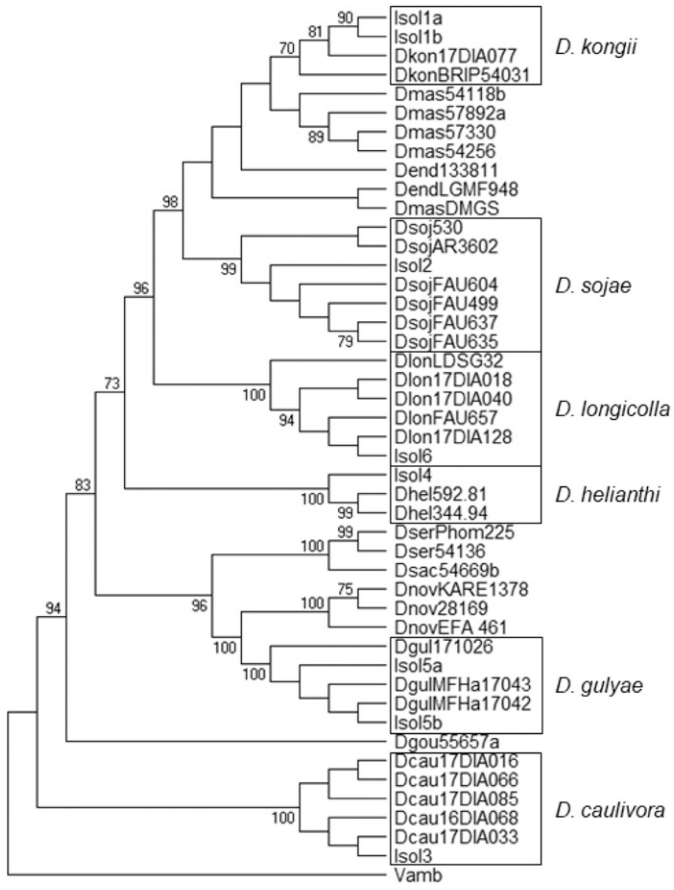

FIGURE 4

Bootstrap consensus tree generated from the concatenated analysis of the internal transcribed spacer, translation elongation factor 1-α, and β-tubulin gene regions by using the maximum likelihood method. Bootstrap values above 70% are shown. Isol1, Isol2, Isol3, and Isol6 correspond to the sequences from each of the four new isolates characterized in this study (in the cases of isolate sequences with "a" and "b" versions, they correspond to sequencing replicates). Isol4 and Isol5 correspond to *Diaporthe helianthi* and *D. gulyae*, respectively, described in Mancebo et al. (2019). The reference sequences of different species of *Diaporthe* are indicated in Table 2. *Valsa ambiens* (Vamb) was used as the outgroup.

TABLE 3
Disease severity of the different isolates of *Diaporthe* evaluated on a susceptible sunflower hybrid (Advanta H1) using stem-wound inoculation method (Mathew et al. 2015) under controlled conditions

Treatment	Fungal species	Mean disease severity (%)
Noninoculated control		0.00 a
Isol2	*D. sojae*	3.08 ab
Isol1	*D. kongii*	4.69 ab
Isol6	*D. longicolla*	6.57 b
Isol3	*D. caulivora*	16.60 c
D. gulyae (Isol5)		52.06 de
D. helianthi (Isol4)		68.56 e
Tukey's HSD		6.37

significantly lower disease severity. Severity of *D. kongii* and *D. sojae* isolates was not significantly different from the control (Table 3) plants inoculated with a sterile PDA plug.

Significance of This Study

This study demonstrates that six species of *Diaporthe* are associated with PSC of sunflower in the southern Pampean region in Argentina, and these include *D. helianthi*, *D. gulyae*, *D. kongii*, *D. sojae*, *D. caulivora*, and *D. longicolla*. Although only one isolate representing each of the six species was used in the pathogenicity study, the isolates of *D. helianthi* and *D. gulyae* were determined to be the most aggressive, in agreement with data reported in Mathew et al. (2015). In contrast, isolates of *D. sojae*, *D. kongii*, and *D. longicolla* are at best minor pathogens, possibly opportunistic colonizers. To the best of our knowledge, this is the first report of *D. kongii*, *D. sojae*, *D. caulivora*, and *D. longicolla* associated with PSC of sunflower in Argentina.

Among the six species, *D. gulyae*, *D. kongii*, *D. sojae*, *D. caulivora*, and *D. longicolla* cause disease in soybean (*Glycine max* L.) (Mathew et al. 2018a; Santos et al. 2011). Identification of these species on sunflower indicates the possibility of cross-pathogenicity of these fungi between the two crops. This is not surprising, because there are several localities in the southern Pampean region of Argentina where soybean and sunflower are planted side-by-side or in rotation on no-till ground, as per the data published by the Ministry of Agriculture, Livestock and Fisheries of Argentina (MAGyP 2019). Species of *Diaporthe* are known to survive in previous crop and weed residues (Mathew et al. 2018b; Thompson et al. 2015), and adoption of no-till practices could favor development of PSC in commercial sunflower fields. The findings of this study suggest that Argentine production practices should be reviewed to support the sustainable production of sunflower and soybean in areas where both crops are produced.

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