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Fusarium solani and *F. oxysporum*: Etiological agents of damping off in crambe

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ABSTRACT: The association of *Fusarium* sp. with agricultural seeds is a limiting factor for the uniform establishment of the crop. The aim of this work was to identify six isolates of *Fusarium* at the species level, based on morphological and molecular markers (TEF-1α and βtub); and to verify the pathogenicity of these isolates to the seeds of *Crambe abyssinica* (crambe). The pathogenicity test was carried out through the contact of the seeds with the fungal culture for 48 hours, followed by sowing in sand. The variables evaluated were: percentage of emergence at 30 days and damping off. The *Fusarium* species identified were *F. solani* (FCa/3, FCa/7.2 and FCa/10) and *F. oxysporum* (FCa/4, FCa/2 and FCa/8). The FCa/3, FCa/4 and FCa/7.2 isolates were pathogenic to crambe seedlings, causing reduction in the percentage of emergence and damping off.

Key words: Btub; *Crambe abyssinica*; fungi; macroconidia; TEF-1α

Fusarium solani e *F. oxysporum*: Agentes etiológicos de damping off em crambe

RESUMO: A associação de *Fusarium* sp. com sementes agrícolas é um fator limitante para o estabelecimento uniforme da cultura. Objetivou-se com este trabalho identificar seis isolados de *Fusarium* em nível de espécie, com base em marcadores morfológicos e moleculares (TEF-1α e βtub); e verificar a patogenicidade desses isolados à sementes de *Crambe abyssinica* (crambe). O teste de patogenicidade foi realizado através do contato das sementes com a cultura fúngica por 48 h, seguida de semeadura em areia. As variáveis avaliadas foram: porcentagem de emergência aos 30 dias e tombamento de plântulas. As espécies de *Fusarium* identificadas foram *F. solani* (FCa/3, FCa/7.2 e FCa/10) e *F. oxysporum* (FCa/4, FCa/2 e FCa/8), sendo que os isolados FCa/3, FCa/4 e FCa/7.2 mostraram-se patogênicos ao crambe, causando redução no percentual de emergência e tombamento de plântulas.

Palavras-chave: Btub; *Crambe abyssinica*; fungos; macroconídios; TEF-1α

Introduction

Crambe (*Crambe abyssinica* Hochst. Ex R.E. Fries) has potential for the production of biodiesel and may be an option for crop rotation in practically all regions of Brazil (Pilau et al., 2012). Belonging to the botanical family Brassicaceae, this plant originates in the Mediterranean region (Souza et al., 2009). It tolerates drought and low temperatures and easily adapts to both tropical and subtropical regions (Reginato et al., 2013). Crambe seeds contain between 26 and 38% of oil and a high concentration of proteins (Masetto et al., 2013).

As in other agricultural crops, several diseases can affect crambe, with the seeds being an efficient form of dissemination of phytopathogens. In this sense, a complex of soil and seed pathogens, whose occurrence can be combined or isolated, can cause damping off of pre- or post-emergence of seedlings (Chitarra et al., 2009), damaging the establishment of crambe cultures in the field.

Among the etiological agents that can affect the crambe, fungi of the genus *Fusarium* play an important role. Represented by several species, these pathogens can cause damping off, wilting, root rot, and diseases of the storage organs (Bedendo, 2011), and their dissemination and transmission can occur through seeds. Moers et al. (2012) accompanying the development of crambe plants in west of Parana, observed the incidence of damping off in the young phase of the crop (50 days after sowing), and *Fusarium* sp. was identified as a pathogen.

Considering the importance of the characterization of *Fusarium* spp. related to damping off in crambe cultivation, the use of morphological and molecular characters is essential for species differentiation. Some morphological characteristics can separate and group *Fusarium* isolates, signaling possible differences and similarities at the genetic level. The sequencing of regions such as TEF-1α (Lazarotto et al., 2014) and βtub (Maciel et al., 2013) provides reliability for the identification of complexes of *Fusarium* species.

In this context, the objective of the present study was: *i*) to verify the pathogenicity of *Fusarium* spp. on crambe; *ii*) to identify, through morphological markers, the isolates of *Fusarium* spp. pathogenic to crambe; and *iii*) to characterize the pathogenic isolates through TEF-1α and βtub sequences.

Material and Methods

Symptoms of collar rot were observed in the crambe cv. 'FMT Brilhante'. Samples of symptomatic plants were collected and fragments of the collar of these plants were disinfested with 70% ethanol for 1 min, 1% sodium hypochlorite for 1 min, and washed in sterile distilled water three times. Posteriorly, the fragments were placed in Gerbox boxes with sterilized and moistened filter paper and subsequently incubated for 48 h at 25 ± 2°C, under a 12 h photoperiod, in a BOD incubator.

The stereoscopic and optical microscopes were used to identify reproductive structures of *Fusarium* sp. The presence of the fungus was also observed in symptomatic seedlings in a germination test conducted with crambe seeds of the same cultivar. Six isolates of *Fusarium* sp. were obtained: FCa/2, FCa/3, FCa/4, and FCa/7.2 from the root fragments, and FCa/8 and FCa/10 from crambe seeds.

The pathogenicity of *Fusarium* isolates in crambe seeds was determined by inoculating the seeds with the pathogens. Initially, the seeds were sterilized with a solution of 70% (v/v) alcohol for 30 s and then with a solution of sodium hypochlorite (1% v/v) for 1 minute. They were subsequently washed with sterile distilled water and dried on sterile filter paper. After the incubation period of the fungus (seven days at 25 ± 2 °C with a photoperiod of 12 h light/dark), inoculation was carried out by keeping the seeds in contact with the fungal culture for 36 h at 25 ± 2 °C and 12 h light/dark photoperiod (when there was radicular protrusion on the first seed). For the control, seeds were exposed only to the Potato-Dextrose-Agar (PDA) medium under the same conditions.

After inoculation, the test of emergence in sand was performed, in which the seeds were placed in plastic boxes (11 × 11 × 3.5 cm) containing sifted sand as a substrate; the sand was sterilized by autoclaving for 2 h (with interval of 24 h) 1 atm and 120 °C. The material remained incubated in a temperature-controlled room at 25 ± 2 °C and with manual irrigation where necessary. During the experiment, the presence or absence of symptoms of damping off (pre- and post-emergence) was recorded and, at 30 days after sowing (DAS), seedling emergence was evaluated.

After the pathogenicity tests, only the isolates with pathogenic potential were identified morphologically and molecularly. To obtain pure cultures and for subsequent identification, the structures of *Fusarium* sp. were removed from the symptomatic tissues to PDA culture medium. Monosporic cultures were obtained according to the methodology of Leslie & Summerell (2006) and stored in the mycological collection of the Laboratory of Plant Pathology, Federal University of Santa Maria, Santa Maria, RS, Brazil.

For morphological characterization, sterilized leaf fragments (~3–5 mm^2) of carnation (*Dianthus caryophyllus*) were infected with colonies of the pure isolates on 2% culture medium agar (Nelson et al., 1983; Ventura, 1999; Maciel et al., 2017) and incubated for 14 days at 25 ± 2 °C under 12 h light/dark photoperiod, for scaling and photography of reproductive structures. After this period, the microstructures of the fungus were observed in a stereoscopic and optical microscope and the species were identified according to identification keys (Gerlach & Nirenberg, 1982; Nelson et al., 1983; Leslie & Summerell, 2006).

For molecular characterization of the fungal isolates, mycelium and spores were collected from the cultures grown on potato-dextrose (PD) medium for 10 days at 25 ± 2 °C under 12 h light/dark photoperiod. The DNA from the pathogen was extracted following the Doyle & Doyle (1990) protocol. In the polymerase chain reaction (PCR), we used the primer pair EF1-T (ATGGGTAAGGARGACAAGAC) and EF1-

1567R (ACHGTRCCRATACCACCRATCTT) (Rehner & Buckley, 2005); Btub-F 35 (AAGGGHCAYTAYACYGARGG) and Btub-R (CATGTTGGACTCDGCCTC) (Developed by Instituto Biológico - São Paulo, SP).

The reaction totaled 25 µL, containing approximately 30 µg DNA, 10X buffer (10 mM Tris-HCl, pH 8,3; 50 mM KCl; 0,1% tween 10; 2,5 mM $MgCl_2$), 2,5 µM dNTP mixer, 20 nM $MgCl_2$, 25 ηmol of each oligonucleotide primers (Biogen), 5 U Taq DNA polymerase (Invitrogen™ Life Technologies), and autoclaved MiliQ water to bring the solution up to volume. The reactions were carried out in a thermal cycler MJ Research, INC. PTC - 100MT, under the following cycling conditions: 94 °C for 2 min, 30 cycles of denaturation at 94 °C for 45 s, 55 °C for 30 s, 72 °C for 35 s, and 72 °C for 10 min. At the end of the reaction, the PCR products were stored at 4 °C. A negative control, without DNA, was included in the PCR amplifications. The PCR products obtained were purified using polyethylene glycol 8000 (PEG 8000 – 13%). In the sequencing reactions for the coding regions of TEF-1α and βtub, 1,100 bp and 900 bp fragments were used, respectively.

The obtained sequences were compared to those of *Fusarium*, available in the GenBank. The sequences from the GenBank that showed the highest scores were selected and aligned with the sequences of the amplified products by the ClustalW algorithm (Thompson et al., 1994). Furthermore, phylogenetic analysis was conducted, adopting the Neighbor-joining method with 5,000 replicates in the MEGA program version 6.0 (Tamura et al., 2013). The similarity of the nucleotide sequences between the isolates was calculated using the Basic Local Alignment Search Tool-BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi). For the construction of the dendrograms, the sequences of *Fusarium* spp., presented in Tables 1 and 2, were used.

The trials were conducted in a completely randomized design composed of six treatments (*Fusarium* sp. isolates) distributed in four replicates each; each repetition consisted of one plastic box containing 25 seeds. Comparison on means was performed by Tukey's test at 5% probability using the software package SISVAR 5.0 (Ferreira, 2010).

Results and Discussion

The isolates FCa/3, FCa/4, and FCa/7.2 were pathogenic to crambe, reducing the seed germination percentage and causing pre- and post-emergence damping off (Figure 1). The effects were significantly different from those in the control (Table 3).

Mendonça et al. (2012) identified *Fusarium*, *Penicillium*, *Alternaria*, *Aspergillus*, *Cladosporium,* and *Curvularia*, associated with crambe seeds, through the sanity test in BDA culture medium. Yet in this culture, Cruz et al. (2013) verified the presence of the genera *Cladosporium*, *Fusarium*, *Penicillium,* and *Alternaria* in seeds through the blotter test.

Moers et al. (2012) identified *Fusarium* sp. as the etiological agent of damping off in crambe in the western region of Paraná. Pathogens that cause these diseases are aggressive and unspecific; in contact with host tissue, they release enzymes capable of destroying plant tissue and lead to seedling to death in a short time (Bedendo, 2011).

Table 1. Access codes on GenBank for *Fusarium* spp. (TEF-1a region) used in this study.

Source	Isolated	Acess Codes	Reference
China	*Fusarium solani* FS-01403	KJ572787	Guan & Gao (2014a)
Spain	*Fusarium solani* 2205 I	HQ731053	Jimenez-Fernandes et al., (2011)
China	*Fusarium solani* YQC-C4	KF939495	Cong & Yang (2013)
United States of America	*Fusarium solani* NRRL 52798	JF740866	O'Donnell et al. (2012)
United States of America	*Fusarium solani* NRRL 52778	JF740846	O'Donnell et al. (2012)
China	*Fusarium oxysporum* FO – 02911	KJ127285	Guan & Gao (2014b)
China	*Fusarium oxysporum* FSY – 0935	JQ965456	Zhang & Gao (2012a)
China	*Fusarium oxysporum* FXM – 0830	JQ965439	Zhang & Gao (2012b)
China	*Fusarium oxysporum* F0418	JQ965441	Zhang & Gao (2012c)
India	*Fusarium oxysporum* NRRL 32158	FJ538245	Gurjar et al. (2009)
Poland	*Fusarium equiseti* KF1017	JF966252	Stepien et al. (2012)

Table 2. Access codes on GenBank for *Fusarium* spp. (B-tubulin region) used in this study.

Source	Isolated	Acess Codes	Reference
South Korea	*Fusarium solani* MAFF 7445	DQ092481	Lee & Min (2005)
Argentina	*Fusarium solani* MR386	GQ121903	Casasnovas et al. (2009a)
Argentina	*Fusarium solani* MR140	GQ121892	Casasnovas et al. (2009b)
South Africa	*Fusarium solani* FCC3782	DQ220245	Bogale et al. (2005)
Japan	*Fusarium solani* NRBC8505	AB674261	Watanabe et al. (2011)
China	*Fusarium oxysporum* FFS0814	JQ965464	Zhang & Gao (2012d)
Colombia	*Fusarium oxysporum* Pil5a	JX465152	Rodriguez et al. (2012)
Malaysia	*Fusarium oxysporum* DEB5	KF918544	Wafa & Latiffah (2013a)
Malaysia	*Fusarium oxysporum* DE3	KJ001537	Wafa & Latiffah (2013b)
Malaysia	*Fusarium oxysporum* DEB27	KJ001541	Wafa & Latiffah (2013c)
China	*Fusarium acuminatum* FA-02622	KJ572784	Wang et al. (2016)

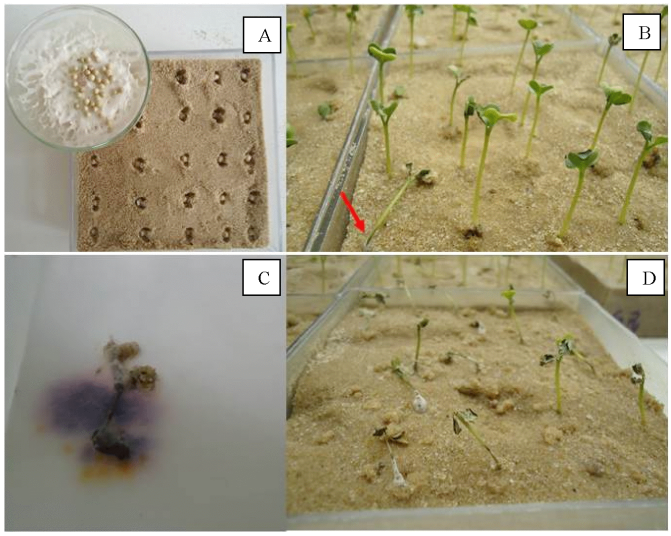

Figure 1. *Fusarium* spp. *vs. Crambe abyssinica*: installation of the sand pathogenicity test (A); symptoms in post-emergence seedlings (B and D); and pathogen recovery on filter paper (C).

Table 3. Total emergence of crambe seedlings, cv. 'FMS Brilhante', at 30 days after sowing (DAS) and pre and post-emergence damping off in seeds inoculated with *Fusarium* spp. through the method of direct contact with the fungal culture.

Fusarium sp. isolates	Seedlings emergence (%)	Damping off (%)	
		Pre emergence	Post emergence
FCa/2	84 ab[1]	5 [ns]	11 ab**
FCa/3	71 ab	12	17 ab
FCa/4	60 b	21	19 a
FCa/7.2	62 b	26	12 ab
FCa/8	87 ab	7	6 bc
FCa/10	87 ab	2	11 ab
Control	90 a	10	0 c

[1]Means followed by the same letter in the column do not differ statistically from each other, by the Tukey test at 5% probability. [ns] not significant.

The genus *Fusarium* is associated with vascular wilt in other species of the Brassicaceae family, with *F. oxysporum* being identified in Argentina (Gaetán, 2005) and causing productivity losses in canola.

The moisture and temperature conditions used in the seedling emergence test favor the development of phytopathogens that are seed external or internal. Two isolates obtained from seeds (FCa/8 and FCa/10) had no pathogenic potential, with high values of seedling emergence (87%).

Based on the morphological characteristics, the isolates FCa/3 and FCa/7.2 were allocated in the Martiella section, while the isolate FCa/4 was classified in the Elegans section. These sections are differentiated through the morphological characteristics of the species (Gerlach & Nirenberg, 1982; Nelson et al., 1983) and provide guidance during the process of morphological identification. The isolates FCa/3 and FCa/7.2 as well as FCa/4 were identified as *Fusarium solani* and *Fusarium oxysporum*, respectively. However, the confirmation of species became possible with molecular identification through the sequencing of TEF-1α and βtub regions (Figures 2 and 3).

Regarding the morphological characterization of *Fusarium* sp., in the isolates FCa/3 and FCa/7.2, characterized as *F. solani*, the macroconidia formed in sporodochia could be observed in FCA medium, being relatively short and thin and with the number of septa varying between 3 and 4. The shape of the apical cell is rounded, while the basal cell is pedicellate, with a notched or rounded end. In this species, microconidia are abundant, organized in "false heads", with a shape varying between oval, reniform, ellipsoid, or fusiform. Chlamydospores were abundant and may be intercalated in the hyphae or their lateral branches (Leslie & Summerell, 2006).

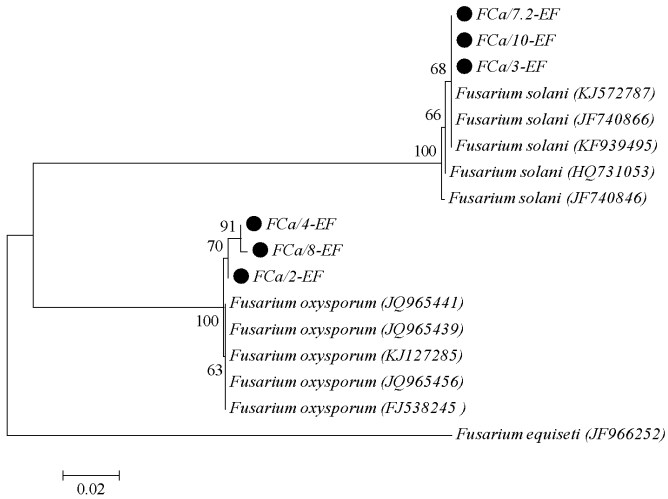

Figure 2. Phylogenetic dendrogram based on the Neighbor-Joining method derived from the sequences of the TEF-1α region, based on 5000 bootstrap replicates of the isolates FCa/7.2, FCa/10, FCa/3 (*Fusarium solani*); and FCa/2, FCa/4 and FCa/8 (*Fusarium oxysporum*) and sequences obtained in GenBank from different *Fusarium* spp.

Table 4. Morphological characteristics of the isolates (I) of *Fusarium* sp. pathogenic to crambe, obtained from symptomatic plants.

I	D* (mm)	MGR** (mm/day)	Macroconidia			Pigmentation of the colony in PDA medium[1]		Chlamydospores[2,3]
			L[†] (µm)	W[††] (µm)	Septos	Colony	Aerial mycelium	
FCa/3	63,89	9,13	39,57	5,68	3	Orange cream	white	+
FCa/4	75,61	10,80	30,44	5,32	1 ou 3	Dark purple	white	+
FCa/7.2	72,11	10,30	43,72	5,49	3 ou 4	Light cream	white	+

* Maximum Petri dish size: 80 mm; D (mm): colony diameter at seventh day; ** MGR (mm/ day): Micelial Growth Rate, calculated from the colony diameter at the end of the evaluation / day of the last evaluation; [†] L (µm): length; [††] W (µm): width; [1] Potato-Dextrose-Agar culture medium; [2] Carnation leaf-Agar culture medium; [3] Presence (+) and absence (-).

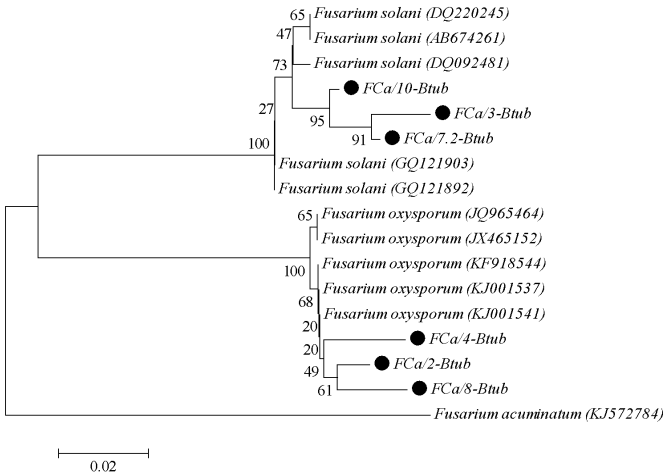

Figure 3. Phylogenetic dendrogram based on the Neighbor-Joining method derived from the sequences of the βtub region, based on 5000 bootstrap replicates of the isolates FCa/7.2, FCa/10, FCa/3 (*Fusarium solani*); and FCa/2, FCa/4 and FCa/8 (*Fusarium oxysporum*) and sequences obtained in GenBank from different *Fusarium* spp.

The pigmentation of the colony in PDA medium varied between orange cream (FCa/3) and light cream (FCa/7.2), with a whitish aerial mycelium for both. After seven days of incubation, final colony size was 6.38 and 7.21 cm for the isolates FCa/3 and FCa/7.2, respectively. Pathogenic *F. solani* isolates submitted to the same conditions of temperature and luminosity showed an average growth of 6.78 cm after seven days of incubation in PDA medium (Silva & Teixeira, 2012).

For the FCa/4 isolate (*F. oxysporum*), macroconidia were short (30.44 x 5.32 µm), with a pedicellate basal cell. The apical cell was short and hook-shaped. The pigmentation in PDA culture medium corresponded to dark purple, with a mean colony size of 7.56 cm after seven days of incubation. Gupta et al. (2010) reported that *Fusarium oxysporum* f.sp. *psidii* and *Fusarium solani* showed a maximum size of 7.25 cm at a temperature of 28 °C after seven days of incubation.

Through amplification, sequencing, and comparison with other sequences of the TEF-1α and βtub regions available in GenBank, phylogenetic dendrograms were constructed for *Fusarium* sp. (Figures 2 and 3).

The isolates FCa/7.2, FCa/10, and FCa/3 were allocated with proximity to *Fusarium solani*, while FCa/4, FCa/8, and FCa/2 are compatible with *F. oxysporum*, independently of the sequenced region. These results corroborate with the morphological characterization carried out for isolates pathogenic to crambe.

The molecular markers ITS and TEF-1α are considered fast and efficient tools for the discrimination and identification of *Fusarium* spp. (Arif et al., 2012). Walker et al. (2016) concluded the identification of *Fusarium acuminatum* and *F. verticillioides*, pathogenic to *Cordia americana*, based on these regions. Maciel et al. (2017) also used these tools to characterize isolates of *Fusarium* sp., pathogenic to *Pinus elliottii*. Thus, markers based on the TEF-1 α and βtub regions enable fast and accurate characterization of fungi of the genus *Fusarium*, complementing the identification of species based on morphological characters.

Conclusions

The isolates of *Fusarium* spp. identified from crambe seedlings with damping off symptoms, based on morphological and molecular characteristics, are compatible with *Fusarium solani* (FCa/3, FCa/7.2, and FCa/10) and *F. oxysporum* (FCa/4, FCa/2, and FCa/8).

The isolates FCa/3, FCa/4, and FCa/7.2 are pathogenic to *Crambe abyssinica*, causing a reduction in seedling emergence.

Literature Cited

Arif, M.; Chawla, S.; Zaidi, N. W.; Rayar, J. K.; Variar, M.; Singh, U. S. Development of specific primers for genus *Fusarium* and *F. solani* using rDNA sub-unit and transcription elongation factor (TEF-1a) gene. African Journal of Biotechnology, v. 11, n. 2, p. 444-447, 2012. https://doi.org/10.5897/AJB10.489.

Bedendo, I. P. Damping-off. In: Amorim, L.; Rezende, J. A. M.; Bergamin filho, A. Manual de fitopatologia: princípios e conceitos. Piracicaba: Agronômica Ceres, 2011. v. 1, p. 435-441.

Bogale, M.; Wingfield, B. D.; Wingfield, M. J.; Steenkamp, E. T. *Fusarium solani* strain FCC3782 beta-tubulin gene, partial cds - GenBank: DQ220245.1. 2005. https://www.ncbi.nlm.nih.gov/nuccore/DQ220245.1?report=GenBank. 05 Mar. 2017.

Casasnovas, F.; Reynoso, M. M.; Torres, A. M.; Chulze, S. N. *Fusarium solani* strain MR386 beta-tubulin gene, partial cds - GenBank: GQ121903.1. 2009a. https://www.ncbi.nlm.nih.gov/nuccore/GQ121903. 05 Mar. 2017.

Casasnovas, F.; Reynoso, M. M.; Torres, A. M.; Chulze, S. N. *Fusarium solani* strain MR140 beta-tubulin gene, partial cds - GenBank: GQ121892.1. 2009b. https://www.ncbi.nlm.nih.gov/nuccore/GQ121892. 05 Mar. 2017.

Chitarra, L. G.; Goulart, A. C. P.; Zorato, M. de F. Tratamento de sementes de algodoeiro com fungicidas no controle de patógenos causadores de tombamento de plântulas. Revista Brasileira de Sementes, v. 31, n. 1, p. 168-176, 2009. https://doi.org/10.1590/S0101-31222009000100019.

Cong, L.; Yang, Q. *Fusarium solani* strain YQC-C4 translation elongation factor 1-alpha (tef1) gene, partial cds - GenBank: KF939495.1. 2013. https://www.ncbi.nlm.nih.gov/nuccore/KF939495. 03 Mar. 2017.

Cruz, S. M.; Nery, M. C.; Rocha, A. de S.; Von Pinho, É. V. de R.; Andrade, P. C. de R.; Dias, D. C. F. dos S. Vigor tests for evaluation of crambe (*Crambe abyssinica* Hochst) seed quality. Journal of Seed Science, v. 35, n. 4, p. 485-494, 2013. https://doi.org/10.1590/S2317-15372013000400010.

Doyle, J. J.; Doyle, J. L. Isolation of plant DNA from fresh tissue. Focus, v. 12, p. 13-18, 1990.

Ferreira, D. F. Sisvar versão 5.0 (Biud 75). Sistemas de análises de variância para dados balanceados: Programa de análises estatísticas e planejamento de experimentos. Lavras, Universidade Federal de Lavras, 2010.

Gaetán, S. A. Occurrence of *Fusarium* Wilt on Canola Caused by *Fusarium oxysporum* f. sp. *conglutinans* in Argentina. Plant Disease, v. 89, n. 4, p. 432. 2005. https://doi.org/10.1094/PD-89-0432C.

Gerlach, W.; Nirenberg, H. The genus *Fusarium* – a pictorial atlas. Berlin: Biologische Bundesanstaltfür Land – und. Forstwirtschoft. 1982, 406 p.

Guan, Y. M.; Gao, J. *Fusarium oxysporum* strain FO-02911 translation elongation factor 1 alpha (EF1alpha) gene, partial cds - GenBank: KJ127285.1. 2014b. https://www.ncbi.nlm.nih.gov/nuccore/KJ127285. 03 Mar. 2017.

Guan, Y. M.; Gao, J. *Fusarium solani* strain FS-01403 translation elongation factor 1-alpha (tef1) gene, partial cds - GenBank: KJ572787.1. 2014a. https://www.ncbi.nlm.nih.gov/nuccore/KJ572787. 03 Mar. 2017.

Gupta, V. K.; Misra, A. K.; Gaur, R. K. Growth characteristics of *Fusarium* spp. causing wilt disease in *Psidium guajava* L. in India. Journal of Plant Protection Research, v. 50, n. 4, p. 452-462, 2010. https://doi.org/10.2478/v10045-010-0076-3.

Gurjar, G.; Barve, M.; Giri, A.; Gupta, V. Identification of Indian pathogenic races of *Fusarium oxysporum* f. sp. *ciceris* with gene specific, ITS and random markers. Mycologia, v. 101, n. 4, p. 484-495, 2009. https://doi.org/10.3852/08-085.

Jimenez-Fernandez, D.; Navas-Cortes, J. A.; Montes-Borrego, M.; Jimenez-Diaz, R. M.; Landa, B. B. Molecular and Pathogenic Characterization of *Fusarium redolens*, a New Causal Agent of Fusarium Yellows in Chickpea. Plant Disease, v. 95, n. 7, p. 860-870, 2011. https://doi.org/10.1094/PDIS-12-10-0946.

Lazarotto, M.; Milanesi, P. M.; Muniz, M. F. B.; reiniger, L. R. S.; Beltrame, R.; Harakava, R.; Blume, E. Morphological and molecular characterization of *Fusarium* spp. pathogenic to pecan tree in Brazil. Genetics and Molecular Research, v. 13, n. 4, p. 9390-9402, 2014. https://doi.org/10.4238/2014.November.11.5.

Lee, H. J.; Min, B. R. *Fusarium solani* strain MAFF 7475 beta-tubulin gene, partial sequence - GenBank: DQ092481.1. 2005. https://www.ncbi.nlm.nih.gov/nuccore/DQ092481. 05 Mar. 2017.

Leslie, J. F.; Summerell, B. A. The *Fusarium* laboratory manual. 1.ed. USA: Blackwell Publishing, 2006. 388p.

Maciel, C. G.; Muniz, M. F. B.; Milanesi, P. M.; Lazarotto, M.; Blume, E.; Reiniger, L. R. S.; Hamann, F. A. First report of *Fusarium sambucinum* associated on *Pinus elliottii* seeds in Brazil. Plant Disease, v. 97, n. 7, p. 995, 2013. https://doi.org/10.1094/PDIS-11-12-1045-PDN.

Maciel, C. G.; Walker, C.; Santos, R. F. dos; Muniz, M. F. B.; Brum, D. L. *Fusarium oxysporum* and *F. verticillioides* associated with damping-off in *Pinus* spp. Revista Ciência Agronômica, v. 48, n. 1 p. 134-141, 2017. https://doi.org/10.5935/1806-6690.20170015.

Masetto, T. E.; Ribeiro, D. M.; Rezende, R. K. S. Germinação de sementes de *Urochloa ruziziensis* em função da disponibilidade hídrica do substrato e teor de água das sementes. Pesquisa Agropecuária Tropical, v. 43, n. 4, p. 385-391, 2013. https://www.revistas.ufg.br/pat/article/view/22634/15520. 29 Mar. 2017.

Mendonça, R. F.; Colodetti, T., Cruz, T.P., Tomaz, M.A., Rodrigues, C., Jesus Junior, W.C. Detecção de fungos associados a sementes de crambe (*Crambe abyssinica*) em meio ágar sólido. Tropical Plant Pathology, v. 38 (Suplemento), p.769, 2012.

Moers, E. M.; Kuhn, O. J.; Gonçalves J. R., A.C.; Franzener, G.; Stangarlin, J.R. Levantamento de doenças na cultura do crambe (*Crambe abyssinica* Hochst) na região oeste do Paraná. Scientia Agraria Paranaensis, v. 11, n. 1, p. 35-48, 2012. https://doi.org/10.1818/sap.v11i1.5664.

Nelson, P. E.; Tousson, T. A.; Marasas, W. F. O. *Fusarium* Species: an Illustrated Manual for Identification. Philadelphia: Pennsylvania State University Press, 1983. 193p.

O'Donnell, K.; Humber, R. A.; Geiser, D. M.; Kang, S.; Park, B.; Robert, V. A.; Crous, P. W.; Johnston, P. R.; Aoki, T.; Rooney, A. P.; Rehner,S.A. Phylogenetic diversity of insecticolous fusaria inferred from multilocus DNA sequence data and their molecular identification via FUSARIUM-ID and Fusarium MLST. Mycologia, v. 104, n. 2, p. 427-445, 2012. https://doi.org/10.3852/11-179.

Pilau, F. G.; Somavilla, L.; Battisti, R.; Schwerz, L.; Kulczynski, S. M. Germinação de sementes de crambe em diferentes temperaturas e substratos. Semina*:* Ciências Agrárias, v. 33, n. 5, p. 1825-1830, 2012. https://doi.org/10.5433/1679-0359.2012v33n5p1825.

Reginato, P.; Souza, C.M.A. de; Silva, C.J. da; Rafull, L.Z.L. Desempenho agronômico e qualidade de sementes de crambe em diferentes épocas e profundidades de semeadura. Pesquisa Agropecuária Brasileira, v.48, n.10, p.1410-1413, 2013. https://doi.org/10.1590/S0100-204X2013001000013.

Rehner, S. A.; Buckley, E. A *Beauveria* phylogeny inferred from nuclear ITS and EF1-a sequences: evidence for cryptic diversification and links to *Cordyceps* teleomorphs. Mycologia, v. 97, n.1, p. 84-98, 2005. http://cordyceps.us/files/Rehner_Buckley_2005.pdf. 31 Mar. 2017.

Rodriguez, E.; Gonzalez, C.; Pulido, V.; Barrero, L. S. *Fusarium oxysporum*: Causal agent of vascular wilt in cape gooseberry (*Phisalys peruviana*). *Fusarium oxysporum* isolate PiI5a beta-tubulin (btub) gene, partial cds. GenBank Acession: JX465152. 2012. https://www.ncbi.nlm.nih.gov/nuccore/JX465152. 06 Mar. 2017.

Silva, J. L.; Teixeira, R. N. V. Esporulação e crescimento micelial de *Fusarium solani* em diferentes meios de cultura e regimes de luminosidade. Agro@mbiente On-line, v. 6, n. 1, p. 47-52, 2012. https://doi.org/10.18227/1982-8470ragro.v6i1.604.

Souza, A. D. V.; Fávaro, S. P.; Ítavo, L. C. V.; Roscoe, R. Caracterização química de sementes e tortas de pinhão manso, nabo forrageiro e crambe. Pesquisa Agropecuária Brasileira, v. 44, n.10, p. 1328-1335, 2009. https://doi.org/10.1590/S0100-204X2009001000017.

Stepien, L.; Gromadzka, K.; Chelkowski,J. Polymorphism of mycotoxin biosynthetic genes among *Fusarium equiseti* isolates from Italy and Poland. Journal of Applied Genetics, v. 53, n. 2, p. 227-236, 2012. https://doi.org/10.1007/s13353-012-0085-1.

Tamura, K.; Stecher, G.; Peterson, D.; Filipski, A.; Kumar, S. Mega 6: Molecular Evolutionary Genetics Analysis Version 6.0. Molecular Biology and Evolution, v. 30, n.12, p. 2725-2729, 2013. https://doi.org/10.1093/molbev/mst197.

Thompson, J. D.; Higgins, D. G.; Gibson, T. J. Clustal W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specifc gap penalties and weight matrix choice. Nucleic Acids Research, v. 22, n. 22, p. 4673-4680, 1994. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC308517. 28 Mar. 2017.

Ventura, J. A. Taxonomia de *Fusarium* e seus segredos: Parte I - História, meios e procedimentos de cultivo. Revisão Anual de Patologia de Plantas, v. 7, n.1, p. 271-298, 1999.

Wafa, Z.; Latiffah, Z. *Fusarium oxysporum* strain DEB5 translation elongation factor (TEF) gene, partial cds - GenBank: KF918544.1. 2013a. https://www.ncbi.nlm.nih.gov/nuccore/KF918544. 03 Mar. 2017.

Wafa, Z.; Latiffah, Z. *Fusarium oxysporum* isolate DE3 beta-tubulin gene, partial cds - GenBank: KJ001537.1. 2013b. https://www.ncbi.nlm.nih.gov/nuccore/KJ001537. 06 Mar. 2017.

Wafa, Z.; Latiffah, Z. *Fusarium oxysporum* isolate DEB27 beta-tubulin gene, partial cds - GenBank: KJ001541.1. 2013c. https://www.ncbi.nlm.nih.gov/nuccore/KJ001541. 06 Mar. 2017.

Walker, C.; Maciel, C. G.; Milanesi, P. M.; Muniz, M. F. B.; Mezzomo, R.; Pollet, C. Caracterização morfológica, molecular e patogenicidade de *Fusarium acuminatum* e *Fusarium verticillioides* a *Cordia americana.* Ciência Florestal, v. 26, n. 2, p. 463-473, 2016. https://doi.org/10.5902/1980509822747.

Wang, Y.; Guan, Y. M.; Lu, B. H.; Gao, J. First report of ginseng (*Panax ginseng*) root rot caused by *Fusarium acuminatum* in China. Plant Disease, v. 100, n. 2, p. 525, 2016. https://doi.org/10.1094/PDIS-03-15-0273-PDN.

Watanabe, M.; Yonezawa, T.; Lee, K. I.; Kumagai, S.; Sugita-Konishi, Y.; Goto, K.; Hara-Kudo, Y. Molecular phylogeny of the higher and lower taxonomy of the *Fusarium* genus and differences in the evolutionary histories of multiple genes. BMC Evolutionry Biology, v. 11, p. 322-338, 2011. https://doi.org/10.1186/1471-2148-11-322.

Zhang, S.; Gao, Z. G. *Fusarium oxysporum* isolate FSY0935 translation elongation factor 1 alpha (EF1a) gene, partial cds - GenBank: JQ965456.1. 2012a. https://www.ncbi.nlm.nih.gov/nuccore/JQ965456. 02 Mar. 2017.

Zhang, S.; Gao, Z. G. *Fusarium oxysporum* isolate FXM0830 translation elongation factor 1 alpha (EF1a) gene, partial cds - GenBank: JQ965439.1. 2012b. https://www.ncbi.nlm.nih.gov/nuccore/JQ965439. 02 Mar. 2017.

Zhang, S.; Gao, Z. G. *Fusarium oxysporum* isolate F0418 translation elongation factor 1 alpha (EF1a) gene, partial cds - GenBank: JQ965441.1. 2012c. https://www.ncbi.nlm.nih.gov/nuccore/JQ965441. 02 Mar. 2017.

Zhang, S.; Gao, Z. G. *Fusarium oxysporum* isolate FFS0814 beta-tubulin gene, partial cds - GenBank: JQ965464.1. 2012b. https://www.ncbi.nlm.nih.gov/nuccore/JQ965464. 03 Mar. 2017.