ABRATES

Journal of Seed Science

ISSN 2317-1545
www.abrates.org.br/revista

ARTICLE

Journal of Seed Science, v.44, e202244004, 2022

http://dx.doi.org/10.1590/2317-1545v44256994

Buckwheat seed quality and pathogenicity of *Fusarium* spp. in plants

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ABSTRACT: Buckwheat is a species with great economic and production potential, which has gained increasing importance. This study aimed to determine the physical, physiological, and sanitary quality of samples from seed lots, and to evaluate the pathogenicity of *Fusarium* spp. in buckwheat plants. Physical and physiological quality was evaluated by the thousand-seed weight, moisture content, germination test (5th and 7th days), seedling length, and seedling dry mass, while sanitary quality was determined by the health test on filter paper, with the seeds subjected to asepsis and without asepsis. Isolates of *Fusarium* spp. were obtained from symptomatic seedlings in the paper roll germination test. To identify and characterize fungal isolates, morphological and molecular approaches were used. Pathogenicity was determined on healthy plants in a controlled environment. The lots showed high physiological quality in the germination evaluation (5th and 7th days). There was a high incidence of *Fusarium* spp. in all lots, which can be reduced with seed asepsis. The isolates were identified as *Fusarium incarnatum-equiseti* species complex, and were pathogenic to buckwheat plants.

Index terms: damping-off, *Fagopyrum esculentum* Moench, germination, seed health.

RESUMO: O trigo mourisco é uma espécie com grande potencial econômico e de produção, a qual tem ganhado cada vez mais importância. O objetivo deste estudo foi determinar a qualidade física, fisiológica e sanitária de amostras provenientes de lotes de sementes, e avaliar a patogenicidade de *Fusarium* spp. em plantas de trigo mourisco. A qualidade física e fisiológica foi avaliada pelo peso de mil sementes, grau de umidade, teste de germinação (5° e 7° dias), comprimento de plântula e massa seca de plântula, enquanto que a qualidade sanitária foi determinada pelo teste de sanidade em papel filtro, com as sementes submetidas à assepsia e sem assepsia. Os isolados de *Fusarium* spp. foram obtidos de plântulas sintomáticas no teste de germinação em rolo de papel. Para a identificação e caracterização dos isolados fúngicos foram utilizadas abordagens morfológicas e moleculares. A patogenicidade foi realizada em plantas sadias em ambiente controlado. Os lotes apresentaram alta qualidade fisiológica na avaliação de germinação (5° e 7° dias). Houve alta incidência de *Fusarium* spp. em todos os lotes, a qual pode ser diminuída com a assepsia das sementes. Os isolados foram identificados como complexo de espécies de *Fusarium incarnatum-equiseti*, e foram patogênicos às plantas de trigo mourisco.

Termos para indexação: tombamento, *Fagopyrum esculentum* Moench, germinação; sanidade.

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Received: 10/05/2021.
Accepted: 01/10/2022.

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INTRODUCTION

Buckwheat (*Fagopyrum esculentum* Moench) belongs to the *Polygonaceae* family, *Fagopyrum* genus, and originated in China and Central Asia (Zhang et al., 2012). It is a dicotyledonous plant of herbaceous habit, with indeterminate growth and a short cycle. The genus has 15 species, distributed into annual and perennial, and the most cultivated are the annual species *Fagopyrum esculentum* and *F. tataricum* (Cai et al., 2016). It is a rustic plant with the potential to develop under different soil and temperature conditions. Among the main countries that produce and consume buckwheat are China, Japan, Italy, Canada, India, Ukraine, and Brazil (Cai et al., 2016).

The grain is rich in protein of excellent quality and its starch has special properties that are different from other cereals because there is no gluten, an important characteristic for the celiac diet (Gao et al., 2016). It is a species that has been widely studied, with potential for the production and commercialization of its products.

Regarding good cultivation practices, seed quality is one of the main factors to be taken into account to aim for good seedling establishment in the field and, consequently, good grain yield. With this, germination is the main characteristic of determining the maximum potential of seed lots. It evaluates the percentage of normal seedlings under optimal development conditions (Brasil, 2009b; Pereira et al., 2011). However, when referring to the quality of seed lots, it is known that it can be affected by genetic, physical, physiological, and sanitary factors (Peske et al., 2012).

The association of phytopathogenic microorganisms with seeds compromises their quality in all production stages. Fungi are considered the main microorganisms, causing various types of damage from the establishment of seedlings in the field to the storage of seeds (Medeiros et al., 2019). As a result, seeds are considered a means of survival and dissemination of several pathogens that cause diseases of economic importance (Santos et al., 2016).

Preliminary observations have already indicated the presence of *Fusarium* spp. in buckwheat seed lots from southern Brazil. In studies evaluating the occurrence of diseases in buckwheat, Morral and McKenzie (1975) identified *Fusarium* spp. causing root rot in buckwheat in the state of Saskatoon, Canada. Later, Nyvall (1989) reported *Fusarium oxysporum* as a cause of wilt in buckwheat seedlings, resulting in losses of 50% in the initial establishment of the crop. Thus, this study aimed to determine the physical, physiological, and sanitary quality of seed lots and to evaluate the pathogenicity of *Fusarium* spp. in buckwheat plants.

MATERIAL AND METHODS

Four representative samples of buckwheat seed lots, two from Paraná and two from Rio Grande do Sul (Table 1) were used. The cultivar IPR 91 has early cycle (70 days), while the cultivar IPR 92 has long cycle (95 days).

Determination of physical and physiological quality

Thousand-seed weight: determined by following the methodology described in the manual of Rules for Seed Testing (RAS) (Brasil, 2009b).

Table 1. Characterization of representative samples of buckwheat seed lots from different localities.

Sample	Cultivar	Origin	Harvest year
1	IPR 91- Baili	IAPAR®* - PR	2018
2	IPR 92- Altar	IAPAR®- PR	2018
3	IPR 91- Baili	Sementes com Vigor® - RS	2018
4	IPR 92- Altar	Sementes Pozza® - RS	2018

* IAPAR: Agronomic Institute of Paraná.

Moisture content: determined by the oven method at 105 ± 3 °C for 24 hours, using two working samples with 4.5 +/- 0.5 g (Brasil, 2009b).

Germination test (PGT): four replications of 50 seeds of each lot were sown in a roll of germination paper moistened with distilled water in the proportion of 2.5 times the dry paper mass. The rolls were kept in BOD (Biochemical Oxygen Demand)-type germination chamber with constant light and alternating temperature of 20-30 °C, corresponding 16 h at 20 °C and 8 h at 30 °C. Germination evaluation was performed on the 5th and 7th days after setting up the test (Brasil, 2009b), and the results were expressed as a percentage.

Seedling length: four replications of 20 seeds of each lot were sown in two unmatched rows in the upper third of the germination paper and maintained under the same condition as the PGT. On the 5th day after sowing, the lengths of the shoots and primary root of ten normal seedlings of each replication were measured (Krzyzanowski et al., 2020);

Seedling fresh and dry mass: for these evaluations, ten normal seedlings of each replication of the seedling length test were selected. Fresh mass was obtained by weighing on a scale with precision of 0.001 g, while the dry mass was obtained after drying the material in a forced ventilation oven at 65 ± 5 °C for 48 h (Krzyzanowski et al., 2020).

Determination of sanitary quality

For the health test on filter paper, part of the seeds from each lot were subjected to asepsis using the following steps: immersion in 70% alcohol for 1 minute, immersion in 0.5% sodium hypochlorite for 1 minute, and three washes in distilled water for 1 minute each, while the other part of the lots was not subjected to any type of asepsis. Subsequently, the seeds (with and without asepsis) were incubated on a paper substrate (Blotter Test), with four replications of 25 seeds for each lot. For this, Gerbox boxes containing two sheets of sterile germination paper each were used. Germination was inhibited by the freezing method (-20 ± 2 °C) for 24 h and, subsequently, the boxes remained in the BOD chamber for seven days with a photoperiod of 24 h of light and temperature of 20 ± 1 °C (Brasil, 2009a). After this period, the incidence of fungal genera present in the seeds was evaluated according to the specialized literature (Barnett and Hunter, 1998). At the same time, seeds with and without asepsis were also subjected to the aforementioned germination test with counts on the 5th and 7th days after setting up the test, to check whether the asepsis method would lead to losses in vigor and germination.

Obtaining Fusarium spp. isolates

To obtain the isolates, parts of symptomatic seedlings (Figure 1) from the paper roll germination test were collected and placed in Petri plate containing potato-dextrose-agar (PDA) medium. The plates were incubated at 25 ± 1 °C with 12 h of photoperiod for seven days. After growth, the colonies developed in the PDA were purified by the monosporic culture technique (Fernandes, 1993). In total, four isolates were obtained, each of which represented a different lot of buckwheat seeds.

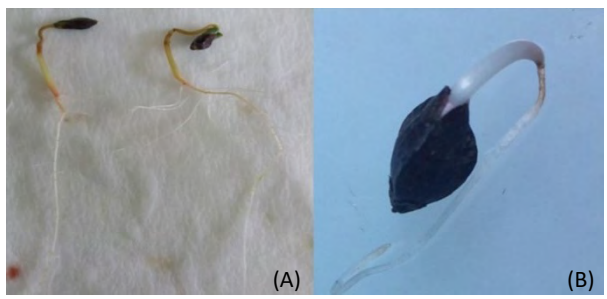

Figure 1. Infected buckwheat seedlings observed in the paper roll germination test. (A) Infected seedlings in the paper roll test. (B) Observation through magnifying glass of the infected collar of the seedling.

Morphological characterization

Culture medium discs containing pathogen structures were transferred to the center of the Petri plate (90 mm) containing PDA culture medium. Subsequently, the plates were incubated at a temperature of 25 ± 1 °C under a 12 h photoperiod. For each isolate found, four replications were used, totaling 16 plates.

For mycelial growth, colony diameter was measured daily using a digital caliper, taking two opposed measurements for seven days, a time in which the colony showed growth on the entire plate. Colony color was determined using the Munsell Soil Color Chart (Munsell Color, Grand Rapidis, MI, USA).

The reproductive structures of the four isolates were characterized through the collection of part of the mycelium, which was transferred to the CLA (carnation leaf-agar) culture medium, where it remained for seven days. After this period, the type of phialides, presence or absence of chlamydospores, microconidia, shape, and size of macroconidia, number of septa, and color of sporodochia were evaluated. For this, slides were prepared to visualize the structures under an optical microscope. Conidia dimensions (length and width) were determined by measuring 30 conidia, with the aid of an OSM eyepiece, coupled to the Olympus BX41® contrast microscope in the 40x lens. Morphological identification was performed according to the classification keys of Nelson et al. (1983) and Leslie and Summerell (2006).

Molecular analysis

For molecular analysis, three of the four isolates presented above were used. After seven days of cultivation in PDA medium, fungal mycelium was sectioned and genomic DNA was extracted with the Quick-DNA™ Fungal/Bacterial Miniprep Kit (Zymo Research®, United States). Genomic DNA samples were subjected to polymerase chain reaction (PCR) in a thermal cycler (2720 Thermal Cycler, Applied Biosystems, United States) for amplification of the elongation factor region (*tef-1*), with the primer pair EF-1 (5'ATGGGTAAGGARGACAAGAC3') and EF-2 (5'GGARGTACCAGTSATCATGTT3') described by O'Donnell et al. (1998).

The fragments were visualized after electrophoresis in a horizontal tank with 1X TBE buffer and agarose gel at 1.0% (m/v) for 40 minutes and at 90V. The amplified samples were purified with EasyPure PCR kit (Transgen Biotech, United States). The sequencing was carried out by the company ACTGene Análises Moleculares Ltda. (Biotechnology Center, UFRGS, Porto Alegre, RS, Brazil) using the automatic sequencer AB 3500 Genetic Analyzer (Applied Biosystems™, Singapore) equipped with 50 cm capillaries and POP7 polymer (Applied Biosystems™, United States). The sequenced fragments were analyzed in the program Staden Package 2.0.0 (Staden et al., 2003) to obtain consensus.

Phylogenetic analysis

The DNA consensus sequences obtained for the three isolates studied were compared with the reference sequences belonging to the *Fusarium incarnatum-equiseti* species complex (FIESC) according to Wang et al. (2019), which are deposited in GenBank (https://www.ncbi.nlm.nih.gov/genbank). For the alignment of sequences, the ClustalW algorithm was used and phylogenetic analysis was conducted by adopting the Maximum Likelihood method, using the Tamura 3-parameter model with distribution I, with 1000 replications, using MEGA software version 10 (Kumar et al., 2018). Finally, the sequences obtained from each isolate were deposited in GenBank.

Pathogenicity Test

To obtain the inoculum used in the test, the four isolates were subcultured in PDA medium and cultivated for seven days at a temperature of 25 ± 1 °C under a 12-hour photoperiod. Health plants (seven days), produced in the sand substrate in a germination chamber with controlled temperature and humidity were used. Inoculation was carried out with the incorporation of a disc of culture medium containing mycelium of the isolate (5 mm) in the collar region of the plants. The control treatment consisted of inoculation of one PDA disc (5 mm).

Statistical analysis

The data of the physiological and sanitary quality tests were checked for normality by the Shapiro-Wilk test, using BioEstat 5.0 software, and then transformed to √(x+0.5). Analysis of variance and comparison of means was performed by the Scott-Knott test at a 5% probability level using SISVAR 5.3 software.

RESULTS AND DISCUSSION

Physical and physiological quality

The moisture content of the seed samples ranged from 12 to 13.35% at the beginning of the test (Table 2). Marcos-Filho (2015) mentions that the limit of difference between samples should be at most 2%, an important factor for the determination and reliability of the other tests, while the thousand-seed weight ranged between 13.09 and 16.75 g. These characteristics are considered technological parameters that characterize seed maturation (Marcos-Filho, 2015). Seed moisture is also an important factor when aiming at the control of fungi associated with them.

In a study with açaí seeds, Nascimento and Moraes (2011) verified a high incidence of *Fusarium* spp. when the seeds showed moisture content of 43 and 37% and were kept at a temperature of 10 °C. For buckwheat seeds, as they are orthodox, that is, they can be dried until reaching low moisture contents with no reduction in germination capacity, moisture contents from 12 to 13.35% can be considered ideal for good storage of the lots, without a high incidence of fungi associated with them. However, the same cannot be observed in recalcitrant seeds, as is the case of those from many forest species, which show various problems with storage fungi, as in species of the genus *Eugenia* (Oliveira et al., 2011).

For the germination on the 5th day (Table 3), it was observed that the sample of lot 1 showed the highest vigor, differing from the others, while for germination on the 7th day, the samples of lot 1 and 4 differed from the others, showing higher germination potential. In a study to evaluate the effect of the treatment of buckwheat seeds, Simonetti et al. (2016) observed that the control (sown only with distilled water) had average germination (7th day) of 85%,

Table 2. Moisture content (MC) and thousand-seed weight (TSW) of four samples of buckwheat seeds.

Sample	MC (%)*	TSW (g)*
1	13.35	16.75
2	13	13.09
3	12.99	13.17
4	12	13.36

*Data not subjected to statistical analysis.

Table 3. Means of germination (5th day), germination (7th day), abnormal seedlings and dead seeds of four buckwheat samples.

	Germination (5th day)	Germination (7th day)	Abnormal	Dead
	.....(%).....			
Sample 1	96 A	99 A	1 A	0 D
Sample 2	71 B	89 B	7 A	4 B
Sample 3	71 B	82 B	8 A	10 A
Sample 4	76 B	95 A	4 A	1 C
CV (%)	7.89	8.58	64.86	33.0

Uppercase letters differ statistically in the column by the Scott-Knott test at 5% probability level. CV (%): coefficient of variation.

corroborating the values found here for buckwheat seed lots, which ranged from 81 to almost 100%, thus indicating a high physiological quality of the lots used.

For root and shoot length (Table 4), it can be seen that lot 2 showed the highest lengths, of both roots and shoots, differing from the other lots. Similar behavior was observed for shoot fresh mass (Table 4). For the variables root fresh mass, root dry mass and shoot dry mass (Table 4), there was no difference between the lots used.

Sanitary quality

Six different genera of fungi were observed in the four seed lots, in seeds with and without asepsis (Table 5). Of these six, five are potentially pathogenic (*Fusarium*, *Alternaria*, *Cladosporium*, *Colletotrichum* and *Curvularia*) and one is saprophytic (*Aspergillus*). In this case, the low moisture content of the seeds did not contribute to reducing the incidence of pathogens.

There was a significant difference between seeds with and without asepsis, and for all genera, a reduction in the percentage of incidence was observed when seeds were subjected to asepsis. Seeds that did not receive asepsis had a high incidence of the genus *Fusarium* in all lots and there was no difference between them. For the other fungal genera, there was a difference between the lots, and for *Aspergillus*, which is considered a storage fungus, the highest percentage of incidence observed was in lot 4. For seeds subjected to asepsis, there was a reduction in the incidence of fungi. However, the genus *Fusarium* had the highest incidence, suggesting that the fungus is associated with the seed

Table 4. Means of root length (RL), shoot length (SL), root fresh mass (RFM), shoot fresh mass (SFM), root dry mass (RDM) and shoot dry mass (SDM) of four buckwheat samples.

	RL	SL	RFM	SFM	RDM	SDM
	.....cm.....		.....g/10 pl^{-1}.....			
Sample 1	9.24 B	2.78 B	0.16 A	0.3 B	0.012 A	0.031 A
Sample 2	10.4 A	3.36 A	0.17 A	0.38 A	0.013 A	0.038 A
Sample 3	9.69 B	2.37 B	0.18 A	0.27 B	0.017 A	0.034 A
Sample 4	9.84 B	2.57 B	0.19 A	0.28 B	0.014 A	0.036 A
CV (%)	4.43	7.75	10.02	10.86	26.97	22.82

Uppercase letters differ statistically in the column by the Scott-Knott test at 5% probability level. CV (%): coefficient of variation.

Table 5. Incidence of *Fusarium* sp. (FUS), *Alternaria* sp. (ALT), *Cladosporium* sp. (CLA), *Colletotrichum* sp. (COL), *Aspergillus* sp. (ASP) and *Curvularia* sp. (CUR) in buckwheat seeds subjected or not to asepsis.

	Incidence (%)							
	Without asepsis				With asepsis			
	Samp. 1	Samp. 2	Samp. 3	Samp. 4	Samp. 1	Samp. 2	Samp. 3	Samp. 4
FUS	89 Aa	92 Aa	80 Aa	82 Aa	65 Ab	37 Bb	37 Bb	15 Cb
ALT	55 Aa	26 Ba	46 Aa	12 Ba	7 Ab	1 Ab	6 Ab	2 Ab
CLA	5 Ab	3 Aa	0 Aa	7 Aa	23 Aa	0 Ba	9 Aa	5 Aa
COL	5 Ba	29 Aa	0 Ba	3 Ba	0 Ba	12 Ab	0 Ba	0 Ba
ASP	3 Ba	0 Bb	0 Ba	41 Aa	3 Ba	20 Aa	3 Ba	2 Bb
CUR	0 Aa	0 Ab	0 Aa	0 Aa	1 Ba	7 Aa	0 Ba	2 Aa

Uppercase letters differentiate lot within each asepsis method and lowercase letters differentiate asepsis method within each lot by the Scott-Knott test at 5% probability level.

not only externally, but also internally, with the potential to cause damage before or after emergence. For seeds with asepsis, the highest percentage of *Fusarium* observed was in lot 1, while lot 4 had the lowest incidence.

In studies with fungal communities from buckwheat seeds, Kovacec et al. (2016) found that buckwheat seeds comprise a variety of typical fungi that colonize buckwheat plants, and their frequency is reduced with seed storage. Thus, one-year storage periods reduced the incidence of fungi to levels lower than 5%, eliminating the need for fungal treatment in seeds during storage.

In a study with *Nicotiana tabacum* L. seeds, Ishizuka et al. (2018) concluded that they can carry several fungi, especially *Alternaria alternata*, *Fusarium verticillioides* and *F. pallidoroseum*. They also showed that the species *A. alternata* causes damage to the physiological quality of the seeds, because it affects germination, resulting in the death of the seeds. Studies with *Pinus taeda* evaluating the transmission of *Fusarium* sp. to seedlings showed no transmission. However, *Fusarium subglutinans* caused seed rot in the germination stage (Silva et al., 2017).

For buckwheat, Morral and McKenzie (1975) and Nyvall (1989) reported pre- and post-emergence damping-off caused by *Fusarium* spp. Thus, due to the high incidence of this fungus and the damage that some of these species may cause, it was necessary to conduct pathogenicity tests to check whether the fungal species associated with the seeds had the capacity to cause damage to the plants.

Table 6 shows the germination (5th day), abnormal seedlings and dead seeds of the four lots subjected to asepsis or not of seeds. It can be observed that, within each lot, seeds with asepsis showed lower germination (5th day) compared to seeds without asepsis. At the same time that germination decreased, there was an increase in the percentage of abnormal seedlings and dead seeds in the method with asepsis. This result can probably be attributed to the concentration and time of exposure of the seeds to the product used, which hampered germination, causing abnormalities and increasing the percentage of dead seeds. Fantinel et al. (2013) also found increase in the number of dead seeds using asepsis of seeds with 70% alcohol and 1% sodium hypochlorite.

Among the four samples of seed lots used, samples of lots 3 and 4 had the highest reduction in germination (5th day). This can be explained by the fact that these samples had large amounts of seeds with visible mechanical damage, probably caused during harvest, resulting in cracks in the seed coat, which may have allowed the entry of the asepsis product into the embryo, hence compromising germination.

For germination (7th day), there was no interaction with treatments with and without asepsis (Table 7). However, seedlings that did not develop in the evaluation on the 5th day were able to reach the parameters established for normal seedlings and could be counted in the final germination count.

Symptoms of the disease in the paper roll germination test

The occurrence of collar rot and root rot (Figure 1) in buckwheat was observed in a controlled environment, during germination tests without asepsis in paper roll, in the four samples of seed lots used, ranging from 8 to 12% between

Table 6. Means of germination (5th day), abnormal seedlings and dead seeds of four seed lots subjected or not to asepsis.

	Without asepsis				With asepsis			
	(%)							
	Samp. 1	Samp. 2	Samp. 3	Samp. 4	Samp. 1	Samp. 2	Samp. 3	Samp. 4
Germ. (5th day)	91 Aa	83 Ba	64 Ca	82 Ba	57 Ab	64 Ab	38 Bb	17 Cb
Abnormal	2 Bb	5 Aa	10 Aa	6 Ab	6 Ba	7 Ba	19 Aa	25 Aa
Dead	2 Ba	6 Ba	13 Ab	3 Bb	2 Ca	11 Ba	24 Aa	7 Ba

Uppercase letters differentiate lot within each asepsis method and lowercase letters differentiate asepsis method within each lot by the Scott-Knott test at 5% probability level.

the lots. Thus, these isolates were identified and their pathogenicity in young buckwheat plants was also evaluated.

Morphological characterization

The four isolates of *Fusarium* spp. obtained from seedlings with symptoms showed distinct characteristics among themselves (Table 8). Daily mycelial growth ranged from 2.54 to 7.72 mm. The color of the colonies ranged from light pink to dark red (Figures 2 A, D, G and J) with cottony aerial mycelium of lighter color at the edges and darker color near the center.

The sporodochia (Figures 2 B, E and H) of the colonies grown in CLA medium ranged from light orange to dark orange color, in abundant amount. However, for isolate 2, there was no sporulation and it remained only in its vegetative stage, so it was not possible to visualize such morphological characteristics. For isolates 1 and 3 in colonies produced in CLA, microconidia formed on monophialides can also be observed (Figure 2 L). For isolate 4, macroconidia were observed on polyphialides (Figure 2 M). The presence of chlamydospores was not observed in any isolate, and macroconidia usually had 4-5 septa.

The size of the macroconidia (Table 8) was similar among the isolates and, according to the keys used for identification, it was confirmed that they were isolates of the genus *Fusarium*.

Table 7. Means of germination (7th day) of four buckwheat seed samples subjected to the methods with and without asepsis.

	Without asepsis	With asepsis	Mean
	Germination (%)		
Sample 1	97	93	95 a
Sample 2	89	82	86 b
Sample 3	74	56	65 c
Sample 4	91	68	80 b
Mean	88 a	75 b	

Lowercase letters differ statistically in column and row by the Scott-Knott test at 5% probability level.

Table 8. Morphological characteristics of *Fusarium* spp. isolated from parts of buckwheat seedlings after seven days of incubation in PDA (potato-dextrose-agar) medium, with 24-hour photoperiod, at 25 °C.

Isolate	Diameter (mm)	ADG* (mm.day^{-1})	AGR*	Sporulation (x10^{5}ml^{-1})	Conidium		Septum	Colony color
					Length	Width		
					.....mm.....			
1	54.07	7.72	75.96	8.9	42.8 (33.6 - 51.7)	3.6 (3 - 4.5)	4 - 5	Light pink (5YR 8/2)
2**	37.9	5.41	49.93	-	-	-	-	Dark red (2.5 YR 3/6)
3	74.54	7.02	74.54	4.31	(34.6 - 56.9)	(2.9 - 4.5)	4 - 5	Pink (5YR 7/3)
4	17.8	2.54	16.44	4.76	30.9 (24.6 - 39.9)	3.1 (2.5 - 4.1)	3 - 4	Light red (2.5 YR 7/8)

*ADG = Average daily growth; AGR = Average growth rate.
** Isolate showed no sporulation.

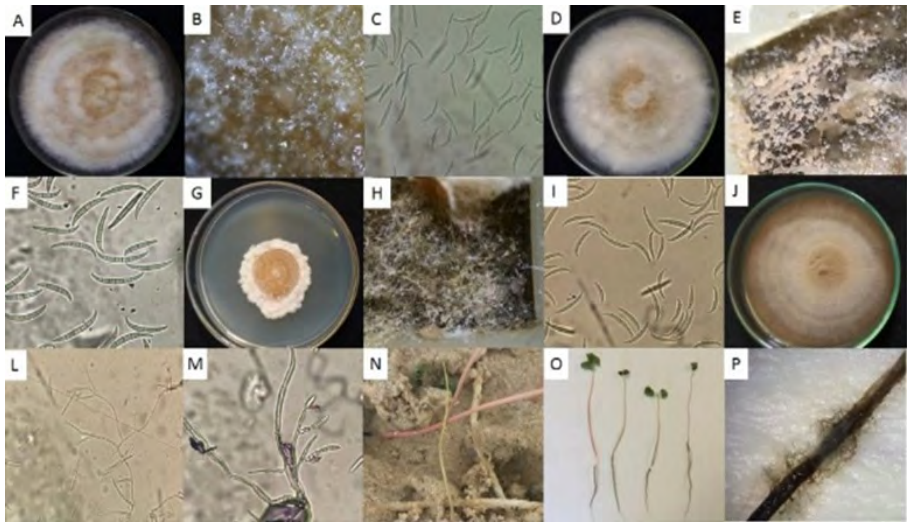

Figure 2. Morphological characteristics of *Fusarium* spp. associated with buckwheat seeds. (A - C) Culture in PDA medium; sporodochium in CLA medium and macroconidia of isolate 1, respectively; (D - F) culture in PDA medium; sporodochium in CLA medium and macroconidia of isolate 3, respectively; (G - I) culture in PDA medium; sporodochium in CLA medium and macroconidia of isolate 4, respectively; (J) culture in PDA medium of isolate 2; (L) monophialides; (M) polyphialides; (N) seedling damping-off in the pathogenicity test in sand; (O) seedlings after pathogenicity test (control on the left, inoculated on the right); (P) re-isolation after pathogenicity test.

Molecular analysis

The three *Fusarium* isolates were identified as *Fusarium incarnatum-equiseti* species complex (Figure 3). Based on the partial gene of the elongation factor 1-alfa (*tef1*-α), the statistical bootstrap support was 87% for the isolate 439TMBR (GenBank: OK135328) and 40% for the isolates 435TMBR (GenBank: OK086070) and 436TMBR (GenBank: OK135327) for the respective clades. These bootstrap supports are insufficient for the discrimination of species within the complex. Wang et al. (2019), in a study on the phylogeny of different species of the FIESC complex, suggested that other genes should be used for analysis, such as CAL, RPB1 and RPB2. A polygenic analysis can be performed in order to obtain the identification at the species level of the isolates used in this study.

The phylogenetic tree (Figure 3) indicates that, morphologically, the isolates 435TMBR and 436TMBR may belong to the species FIESC 23, while the isolate 439TMBR may belong to the species group *F. hainanense* FIESC 26. They are classified as different, because within the complex they present variability. Many species of the FIESC complex have not yet been described in the literature, which makes it important to study them for the identification of species with pathogenic potential in agricultural crops.

Pathogenicity test

Plants inoculated with the four isolates of *Fusarium* sp. showed symptoms 15 days after inoculation, and the symptomatology was similar to the initial results found in the germination test on paper roll. The symptoms observed in the leaves were wrinkling and beginning of yellowing, with subsequent wilting and damping-off of the plants. The roots showed signs of rot. No change was observed for plants in the control treatment (Figures 2 N - O). Plants that showed symptoms were incubated in a humid chamber at 25 ± 1 °C and photoperiod of 12 h for four days, where it was possible to observe the fungal structures characteristic of *Fusarium* sp., demonstrating that there was colonization of the tissues of buckwheat plants (Figure 2 P). Subsequently, the fungi were reisolated, completing Koch's postulates.

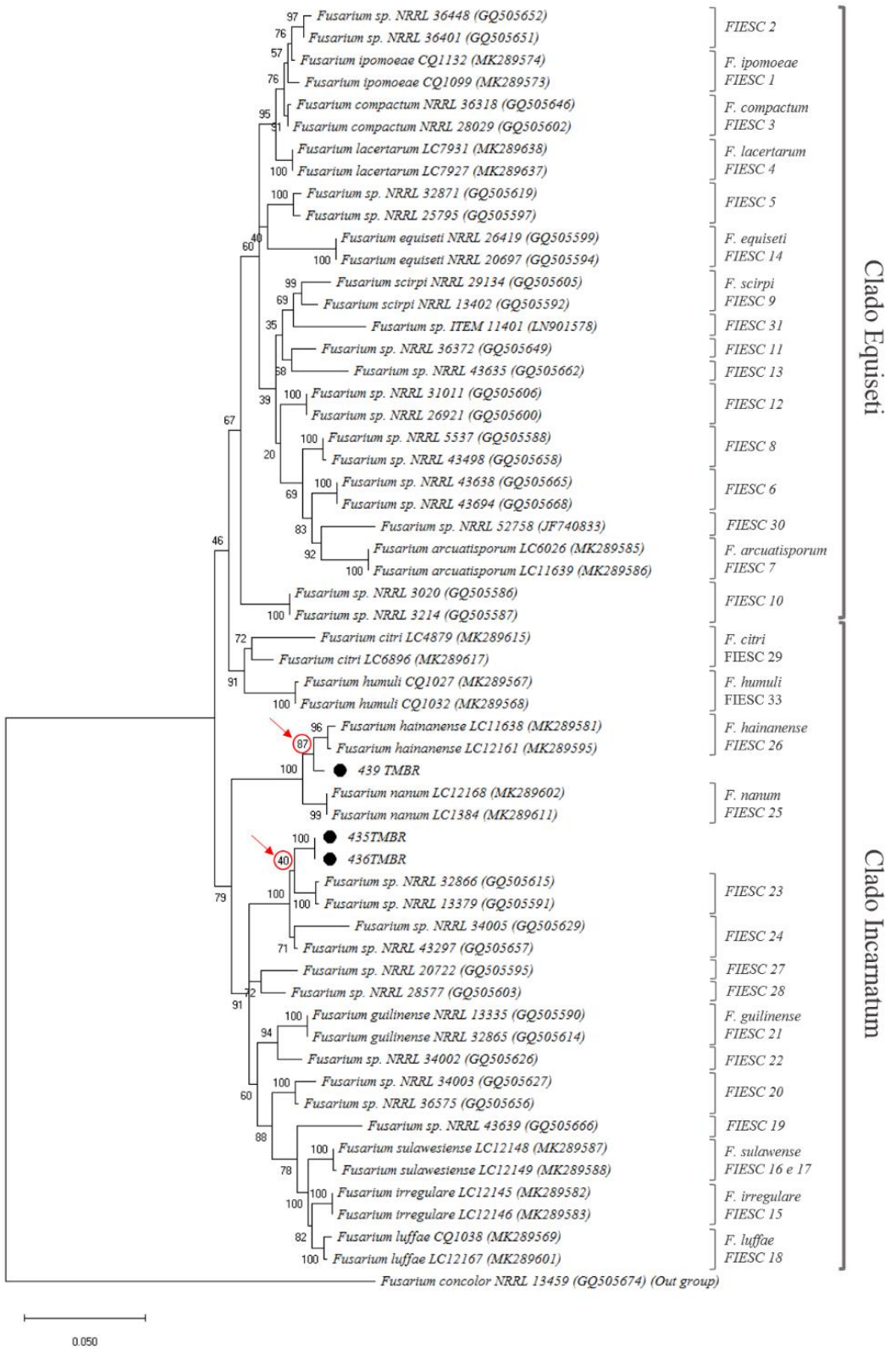

Figure 3. Phylogenetic tree obtained from the sequences of the partial gene elongation factor 1-alpha (*tef1-α*), of three isolates of *Fusarium* spp., associated with buckwheat (*Fagopyrum esculentum* Moench) seeds, showing the phylogenetic relationships of species in the *Fusarium incarnatum-equiseti* species complex (FIESC) according to the Maximum Likelihood statistical method. Numbers on the branches indicate the percentage of repetitions of bootstrap analysis in which the repetitions were observed (1000 repetitions).

The damping-off of young plants, most severe damage, was observed in approximately 8-10% of the evaluated plants. The aggressiveness of the pathogen varies according to the host species and plant. For *Pinus taeda*, symptoms of root rot were observed, and of the 12 isolates of *Fusarium* spp. evaluated, only *F. subglutinans* showed greater aggressiveness to *P. taeda* seedlings (Silva et al., 2017). Maciel et al. (2017), in a study with *P. taeda* and *P. elliottii*, verified seedling damping-off in pre- and post-emergence caused by the species identified as *F. verticillioides* and *F. oxysporum*.

In yerba mate (*Ilex paraguariensis* A. St.-Hil.), two species (*F. solani* and *F. oxysporum*) were observed, which were potentially pathogenic, and the main symptom observed was root rot (Poletto et al., 2012).

However, the presence of a pathogen does not always indicate loss in seed viability. In a study with two species (*Fusarium graminearum* and *Fusarium verticillioides*) and evaluating their effect on the germination of maize seeds, Ramos et al. (2014) concluded that the 16-hour period was sufficient for the inocula of the pathogens to infect the seeds, but nevertheless, none of the species interfered in their germination. However, the cold test (vigor test) showed that the species *Fusarium graminearum* reduced the performance of the lots.

Therefore, it can be observed that there is high variability of *Fusarium* species as well as specificity in relation to the host, while the aggressiveness of the pathogen varies according to the species and the host plant.

CONCLUSIONS

The evaluated samples of buckwheat seeds have high physiological quality, in the germination evaluation (5th and 7th day).

Six different fungal genera (*Fusarium*, *Alternaria*, *Cladosporium*, *Colletotrichum*, *Curvularia* and *Aspergillus*) were found when evaluating the sanitary quality of the seeds.

There is a high incidence of the genus *Fusarium* in all seed samples, which is reduced with the use of asepsis in the seeds. The isolates are identified as *Fusarium incarnatum-equiseti* species complex and are pathogenic to buckwheat plants.

Seed asepsis by the method used reduces germination (5th day) and increases the number of abnormal seedlings and dead seeds.

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