FUNGAL DIVERSITY IN LIMA BEAN SEEDS

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ABSTRACT

There is no comprehensive survey of the presence of fungi associated with lima bean seeds. The objective of this study was to evaluate the fungal diversity of 34 samples of lima bean seeds, acquired with farmers and markets during the years 2014 and 2015 in the states of Ceará, Maranhão, Paraíba and Piauí. Subsamples of 400 seeds were sterilized by soaking in 1% NaOCl solution for 3 minutes, followed by two washes in sterile distilled water, and dried at room temperature. Seeds were placed in Petri plates containing Potato Dextrose Agar, and incubated at 25 °C for seven days. Fungal identification was based on morphological markers, and its incidences were quantified. Isolates from the main phytopathogens were also identified by the amplification and sequencing of housekeeping genes. Samples presented variations in diversity and incidence, with the presence of 22 fungal genera. *Aspergillus* spp., *Penicillium* spp., *Curvularia* sp. and *Monilinia* sp. corresponded to 63.76% of the colonies observed. Among the phytopathogens, isolates of *Macrophomina phaseolina*, *Colletotrichum truncatum*, *Rhizoctonia solani*, *Fusarium udum* and *Fusarium oxysporum* were identified by BLAST*n* analysis (99 to 100% DNA similarity) and phylogenetic analysis. *C. truncatum* and *M. phaseolina* presented the highest incidences (0.95% and 1.58%, respectively) among phytopathogens.

Keywords: *Colletotrichum truncatum*, *Macrophomina phaseolina*, *Phaseolus lunatus*, seed pathology

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RESUMO

Não existe levantamento abrangente da presença de fungos associados a sementes de feijão-fava. O objetivo deste trabalho foi avaliar a diversidade fúngica de 34 amostras de sementes de feijão-fava, adquiridas com produtores rurais e em feiras livres, durante os anos de 2014 e 2015, nos estados do Ceará, Maranhão, Paraíba e Piauí. Subamostras de 400 sementes foram desinfestadas, e incubadas a 25 °C durante sete dias em placas de Petri contendo meio de cultura Batata-Dextrose-Ágar. A identificação dos fungos foi realizada com base nos marcadores morfológicos, e suas incidências foram quantificadas. Isolados dos principais fitopatogógenos também foram identificados pela amplificação e sequenciamento de genes "housekeeping". As amostras apresentaram variações na diversidade e incidência, com a presença de 22 gêneros fúngicos. *Aspergillus* spp., *Penicillium* spp., *Curvularia* sp. e *Monilinia* sp. corresponderam a 63,76% das colônias observadas. Dentre os fitopatógenos, os isolados de *Macrophomina phaseolina*, *Colletotrichum truncatum*, *Rhizoctonia solani*,

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Fusarium udum e *Fusarium oxysporum* foram identificados por análise BLAST*n* (99 a 100% de similaridade de DNA) e análise filogenética. *C. truncatum* e *M. phaseolina* apresentaram as maiores incidências (0,95% e 1,58%, respectivamente) dentre os fitopatógenos.

Palavras-chave: *Colletotrichum truncatum*, *Macrophomina phaseolina*, patologia de sementes, *Phaseolus lunatus*

INTRODUCTION

Plant seeds host a broad diversity of microorganisms that may be transmitted to seedlings, locally or systemically, as endophytes and/or pathogens; these microorganisms may also spoil seeds during storage (Kaga et al., 2009, Embaby et al., 2013). Occurrence of microorganisms associated with seeds can lead to reduced vigor and the emergence of seedlings that yield poorly (Parsa et al., 2016). Furthermore, infected seeds may introduce and/or disseminate plant pathogens to new areas of cultivation, which this may result in epidemics (Oliveira et al., 2013).

Lima bean (*Phaseolus lunatus* L.) is a leguminous annual plant with a climbing habit, producing pods with edible grains that have a high protein content (Seidu et al., 2015). In Brazil, it is generally planted in an unirrigated regime in a crop consortium, especially by smallholders (Oliveira et al., 2014). Brazilian Northeastern states are prominent in the production of this legume, and they have traditionally been responsible for more than 85% of national production (IBGE, 2014). Although it is the second most consumed species of the *Phaseolus* genus in Brazil, and despite being susceptible to many diseases of fungal etiology, there is almost no knowledge about the microflora found in the seeds of this legume. In Brazil, the only study of this type was carried out with four samples collected in the state of Paraíba (Araújo et al., 1983).

Phytosanitary quality of seeds is determined by the incidence of microorganisms that cause damage to the seed during storage, or which are transmitted by the seed, causing diseases and reductions in yield (Costa et al., 2013). It is vital to know more about the fungi naturally present in lima bean seeds, a prerequisite to supporting the implementation of more appropriate measures for disease management in the crop.

The objective of this study was to evaluate the fungal diversity of 34 samples of lima bean seeds, acquired with farmers and markets during the years 2014 and 2015 in the states of Ceará, Maranhão, Paraíba and Piauí.

MATERIAL AND METHODS

In counties from the states of Ceará, Maranhão, Paraíba and Piauí, 34 samples of lima beans were acquired from rural producers and open fairs in 2014 and 2015. These seeds were then deposited in the Active Bank of Lima Bean Germplasm at the Federal University of Piauí/UFPI (Table 1).

Accessions were submitted to the seed health test in accordance with the recommendations of Brasil (2009). Subsamples of 400 seeds were disinfected by immersing them in a sodium hypochlorite (NaOCl) 1% solution, for three minutes, followed by two washes with sterilized distilled water (BRASIL, 2009). Seeds were deposited in Petri dishes with a 150 mm diameter, containing Potato-Dextrose-Agar (PDA) medium. Six seeds were distributed equidistantly per plate, where they remained for between five and seven days in an incubator, at a temperature of 25 ± 1 ºC and a photoperiod of 12 hours.

Table 1. Mean incidence and percentage of fungi in seeds of 34 lima bean (*Phaseolus lunatus* L.) accessions

Ascess	Origin	*Aspergilus* sp.	*Colletotrichum truncatum*	*Curvularia* sp.	*Macrophomina phaseolina*	*Penicilium* sp.
UFPI 880	Puxinanã, PB	3.75	0.00	1.00	0.00	1.00
UFPI 881	Matões, MA	2.00	0.00	3.00	0.00	3.75
UFPI 883	Esperantina, PI	9.00	4.00	6.25	3.50	12.00
UFPI 884	Salitre, CE	3.75	0.00	0.75	18.25	6.50
UFPI 885	Presidente Dutra, MA	3.75	0.00	2.00	0.25	4.75
UFPI 886	Novo Oriente, CE	4.50	2.50	2.50	0.00	7.00
UFPI 888	Tianguá, CE	14.25	0.00	9.00	0.00	4.50
UFPI 889	Paraibano, MA	6.00	2.25	4.00	0.00	3.00
UFPI 890	Bocaina, PI	3.00	1.25	1.50	0.00	3.00
UFPI 891	Uruçuí, PI	2.50	0.50	3.00	0.00	7.00
UFPI 892	São Gonçalo, PI	5.75	0.75	4.00	0.00	3.00
UFPI 893	São Gonçalo, PI	3.00	0.00	11.25	0.00	0.00
UFPI 894	Esperantina, PI	3.50	2.75	6.50	8.50	2.75
UFPI 895	José de Freitas, PI	2.25	0.00	1.00	0.00	4.50
UFPI 896	Crateús, CE	1.25	0.00	1.00	0.00	2.25
UFPI 897	Fagundes, PB	3.00	1.50	1.75	0.00	1.25
UFPI 898	Pocinhos, PB	2.00	0.00	1.00	0.00	5.00
UFPI 899	Lagoa Seca, PB	6.00	0.00	0.50	0.00	2.50
UFPI 900	Areias, PB	1.75	0.00	3.00	0.00	3.00
UFPI 902	São Benedito, CE	3.50	0.25	2.00	0.00	0.75
UFPI 903	São Benedito, CE	2.25	0.00	2.25	0.00	0.25
UFPI 904	Parambú, CE	4.50	0.00	3.25	1.00	1.50
UFPI 905	Pedra Branca, CE	3.25	0.00	1.25	1.25	0.50
UFPI 906	Crateús, CE	1.75	0.00	2.75	1.25	2.25
UFPI 907	Tauá, CE	5.25	0.00	2.00	0.00	0.75
UFPI 908	Pedra Branca, CE	6.25	0.75	0.50	0.00	0.00
UFPI 910	Pedro II, PI	16.00	0.25	3.50	1.00	0.00
UFPI 911	Teresina, PI	14.25	0.50	2.25	4.50	4.25
UFPI 912	Passagem Franca, PI	2.50	0.75	2.00	0.75	3.75
UFPI 913	Amarante, PI	16.25	1.75	3.75	1.75	17.00
UFPI 914	Chapada Grande, PI	5.00	0.00	3.75	1.00	3.75
UFPI 915	Miguel Alves, PI	3.00	0.25	8.00	0.00	10.00
UFPI 916	Colinas, MA	19.25	2.25	2.50	2.00	8.00
UFPI 923	Tanque, PI	9.25	10.00	1.50	8.75	3.75
Mean (%)		5.68	0.95	3.07	1.58	3.92

Fragments of hyphae and spores of the fungi were transferred to the PDA medium for later identification using morphological markers. Colonies were quantified for the mean incidence among the 34 accessions, and the percentage of fungal frequency was evaluated by genus or species within the general total colonies formed. Because some fungi presented difficulties in sporulation, they were placed on different culture media, such as Malt Extract, Spezieller nährstoffarmer agar (SNA) and oat agar, and then submitted to different light regimes, such as continuous darkness and a photoperiod of 12 h at 25 ± 1°C. With the purpose of stimulating the production of sclerotia of *Sclerotium* sp., sterilized toothpicks were added to the colonies so that slides could be mounted later and morphological identification carried out. After visual identification, isolates considered to be plant-pathogenic were put on to Petri dishes with PDA medium, incubated at 25 ± 1 °C, under a photoperiod of 12 h, for up to seven days.

After growing, the fungi were preserved in tubes containing mineral oil and maintained at room temperature.

Samples of the isolates from the genera *Colletotrichum*, *Macrophomina*, *Fusarium* and *Rhizoctonia* were identified by gene sequencing. Sequenced genes were: GAPDH (glyceraldehyde 3-phosphate dehydrogenase, primers GDF1 and GDR1) to *Colletotrichum* sp., EF-1α (Elongation factor 1-α, primers EF1-728F) to *Macrophomina* sp. and *Fusarium* sp., and ITS (Internal Transcribed Spacer, primers ITS1 and ITS4) to *Rhizoctonia* sp. DNA was extracted using a Wizard Genomic DNA Purification Kit® (Promega, Madison, WI, USA). Reactions were carried out using a Go*Taq*® Colorless Master Mix kit (Promega, Madison, WI, USA). Fragments amplified were purified using the Wizard® SV Gel kit and PCR Clean-Up System (Promega, Madison, WI, USA) and were sequenced in both sense and anti-sense directions by Macrogen, USA. Sequences edited were compared with other sequences deposited in GenBank, using the program BLAST*n* (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Multiple sequence alignments were generated using the program ClustalW implemented by the program MEGA 6.0 (Tamura et al., 2013). Alignments were corrected manually, and the analysis was carried out with MEGA 6.0 using the maximum parsimony method.

RESULTS AND DISCUSSION

Species of fungus from 22 genera were detected and identified. Fungi that occurred with frequency above 10% were *Aspergillus* spp., *Penicillium* spp., *Curvularia* sp. and *Monilinia* sp., which together corresponded to 63.76% of all the colonies obtained, reaching incidences of 5.68, 3.90, 3.07 and 2.51%, respectively (Table 2). Similar mean incidences had been found in four samples of lima bean seeds obtained in Paraíba State (Araújo et al., 1983). These fungi are commonly associated with seeds, and they do not generally cause diseases in most cultivated species under field conditions. However, they may compromise seed quality, reducing germinative power and embryo death, especially when storage is inappropriate (Embaby et al., 2013).

Penicillium spp. were detected in 31 of the 34 samples analyzed, while *Aspergillus* spp. and *Curvularia* sp. were found in all samples (Table 2). Reports exist of *Aspergillus* spp. and *Penicillium* spp. affecting germination, provoking necrosis of the cotyledons, radicle and initial leaves of cowpea, resulting in abnormal seedlings (Rodrigues & Menezes, 2002). When high incidences of these species are found they may make germination inviable and cause fungal structures to grow on soybean seeds (Costa et al., 2013). Some species of *Penicillium* and *Aspergillus* not only spoil grains and seeds, but may also infect botanical species, producing micotoxins (Riverberi et al., 2010).

Table 2. Fungal diversity in seeds of 34 lima bean (*Phaseolus lunatus* L.) accessions.

Genus/Species	Colony	Frequency[1]	Mean incidence[2] (maximum)
Aspergillus spp.	773	23.89	5.68 (19.25)
Bipolaris sp.	1	0.03	0.01 (0.25)
Chaetomium sp.	1	0.03	0.01 (0.25)
Cladosporium sp.	23	0.71	0.17 (5.75)
Colletotrichum truncatum	129	3.99	0.95 (10.00)
Curvularia sp.	417	12.89	3.07 (11.25)
Eurotium sp.	18	0.55	0.14 (1.50)
Fusarium camptoceras	117	3.62	0.86 (5.00)
F. clamydosporum	13	0.40	0.10 (0.75)
Fusarium spp.	41	1.27	0.75 (4.75)
F. verticilioides	4	0.12	0.03 (0.75)
Fusiccocum sp.	59	1.82	0.43 (3.50)
Geotrichum sp.	2	0.06	0.01 (0.50)
Lasiodiplodia sp.	6	0.19	0.04 (1.25)
Macrophomina phaseolina	215	6.65	1.58 (18.25)
Monilinia sp.	342	10.57	2.51 (9.75)
Nigrospora sp.	14	0.43	0.10 (1.00)
Penicillium spp.	531	16.41	3.90 (17.00)
Pestalotia sp.	7	0.22	0.05 (0.50)
Phytium sp.	49	1.51	0.36 (11.75)
Phomopsis sp.	7	0.22	0.05 (1.50)
Rhizoctonia solani AG2-2WB	1	0.03	0.01 (0.25)
Rhizopus sp.	114	3.52	0.84 (5.00)
Sclerotium sp.	9	0.28	0.07 (0.75)
Talaromyces sp.	21	0.65	0.15 (1.50)
Unidentified	330	10.20	2.43 (14.75)
Total colonies	3244		

[1]Frequency of fungal species expressed as a percentage, considering the total of the colonies obtained.
[2]Mean incidence of fungal species expressed as a percentage, considering the 34 samples, and maximum incidence obtained considering the 34 samples evaluated.

Among the fungi considered important pathogens of leguminous crops, *Colletotrichum truncatum* (incidence of 0.95%) and *Macrophomina phaseolina* (incidence of 1.58%) were found (Table 1). Despite their low incidence, they were prevalent in 50 and 41,17% of the samples, respectively (Table 1). *C. truncatum* is the causal agent of anthracnose in lima bean, leading to seedling death and severe infections in adult plants, causing yield losses (Carvalho et al., 2015). This is the first time the pathogen has been found associated with lima bean seeds, since Araújo et al. (1983) did not register its occurrence. In soybean, *C. truncatum* has already been found in association with seeds, with an incidence of 23.64%, and it is one of the main pathogens transmitted by seeds in this crop (Sousa et al., 2011).

Macrophomina phaseolina is the causal agent of charcoal rot and occurs in more than 500 botanical species, affecting both legumes and grasses (Gupta et al., 2012). Presence of this pathogen in crops such as cowpea and soybean can result in epidemics (Gupta et al., 2012). This study reports the presence of *M. phaseolina* in lima bean seeds for the first time.

Rhizoctonia solani AG2-2WB (incidence of 0.01%) and *Sclerotium* sp. (incidence of 0.07%) were also found (Table 2), evidencing the importance of seeds as a vehicle to transport plant-pathogenic fungi that may compromise crop health. *R. solani* is widely distributed and causes diseases in a variety of crops (Gonzalez et al., 2006). Furthermore, *R. solani* is responsible for causing collapse and rot of lima bean roots, while *Sclerotium* sp. cause stalk rot (Assunção et al., 2011; Silva et al., 2014). Even presenting low incidence, both pathogens can be introduced into new areas of cultivation by infected seeds. Araújo et al. (1983) found *Rhizoctonia* sp. and *Sclerotium rolfsii* with incidences of 3.5% and 0.05% in lima bean seeds. Lima bean presents high susceptibility to the action of *S. rolfsii* (Silva et al., 2014).

Fusarium genus was found in 22 accessions, totaling 64.7% of the samples. Different species were morphologically identified: *F. camptoceras*, *F. clamydosporium* and *F. verticillioides.* Some isolates not identified by morphology were identified by the sequencing of the EF-1α gene. Araújo et al. (1983) found a frequency of 3.83% of *Fusarium* spp. in lima bean seeds, but without identification of the pathogenic and saprophytic species. In lima bean, there are no reports of *Fusarium* spp. causing damage to the seed and being transmitted to the seedling. In other crops, *Fusarium* spp. can inhibit seed germination and may be efficiently transmitted, as occurs with *Fusarium oxysporum*, which is transmitted from seeds to seedlings of common bean at a rate of 13.4% (Parsa et al., 2016).

By sequencing housekeeping genes from the fungal isolates identified by morphological markers was possible to confirm the identity of these isolates. *Macrophomina* sp., *Colletotrichum* sp., *Rhizoctonia* sp. and some *Fusarium* spp. isolates showed 99-100% similarity with *M. phaseolina* (KF553897), *C. truncatum* (KT696309), *R. solani* AG2-2WB (KF907728), *Fusarium udum* (AM295809) and *F. oxysporum* (KF562122) (Table 3). *C. truncatum* isolates grouped with the ex-holotype isolate (CBS 151.35) with 90% bootstrap, while *M. phaseolina* isolates grouped with *M. phaseolina* isolates from other beans and soybean, with 100% bootstrap (data not shown), on a branch distinct from the second species of the genus, *M. pseudophaseolina* (Sarr et al., 2014).

Approximately 10.2% of the fungal colonies were not identified because sporulation was absent (Table 2), even using nutrient poor media (Water-Agar, Malt Extract Agar) and incubation regimes to induce stress (24 h dark, growth under black light, stiletto injury). *Aerobasidium pullalans* and *Marasmium* spp. are examples of fungi that rarely sporulate in culture media and which were found acting as endophytes in common bean (Parsa et al., 2016). Future studies of sequencing the ITS region of the unidentified fungi will be conducted.

Table 3. Identification by Blast*n* analysis of some fungal isolates obtained from lima beans seeds (*Phaseolus lunatus* L.)

Isolate name	Closest species*	Sequence identity	GenBank access code
JM01	*Colletotrichum truncatum*	100%	KT696309
JM02	*Colletotrichum truncatum*	100%	KT696309
JM12	*Fusarium udum*	99%	AM295809
JM32	*Fusarium oxysporum*	99%	KY379216
JM33	*Macrophomina phaseolina*	99%	KF553897
MPM143	*Macrophomina phaseolina*	99%	KF553897
JM41	*Rhizoctonia solani*	99%	KX397678

* Species identity was confirmed by phylogenetic analysis (data not shown).

In this work, *Monilinia* sp., *Phytium* sp. and *Rhizopus* sp. were found causing accentuated rot in seeds. *Nigrospora* sp. and *Cephalosporium* sp. were also identified in lima bean seeds, but the infection did not seem to affect their germination. Fungi associated with seeds may not always be pathogenic; instead, they may merely use the seeds as means of transport, or may remain as saprophytes until the environment becomes favorable to their development (Oliveira, 2013).

With the expanding plantations of lima bean mainly in the Brazilian Northeast, it has become more important to carry out studies to identify the pathogens associated and transmitted by seeds. Results obtained in this work will support studies on the application of management measures for fungal diseases in lima bean, which will allow appropriate strategies to be established. These include the possibility of chemical treatment of seeds and plants, and the development of resistant genotypes.

CONCLUSIONS

There is a high diversity of fungi associated to the 34 lima bean samples from the accessions of the Germplasm Bank of the UFPI.

The highest incidences of fungi in the evaluated seeds were of species associated to seed deterioration, such as *Aspergillus* spp., *Penicillium* spp., *Curvularia* sp. and *Monilinia* sp. Among the phytopathogens, isolates of *Macrophomina phaseolina*, *Colletotrichum truncatum*, *Rhizoctonia solani*, *Fusarium udum* and *Fusarium oxysporum* were identified on the samples.

Colletotrichum truncatum and *M. phaseolina* presented the highest incidences among phytopathogens.

REFERENCES

ARAÚJO, E.; DORNELAS, G. V., LIMA, A.; BRUNO, R. L. A.; OLIVEIRA FILHO, J. J. Avaliação da qualidade das sementes usadas para o plantio no estado da Paraíba. II – feijão mulatinho (*Phaseolus vulgaris* L.), feijão massacar (*Vigna unguiculata* (L.) walp.) e fava (*Phaseolus lunatus* L.). **Agropecuária Técnica**, Areia, v. 4, p. 34-51, 1983.

ASSUNÇÃO, I. P.; NASCIMENTO, L. D.; FERREIRA, M. F.; OLIVEIRA, F. J.; MICHEREFF, S. J.; LIMA, G. S. A. Reaction of faba bean genotypes to *Rhizoctonia solani* and resistance stability. **Horticultura Brasileira**, Vitória da Conquista, v. 29, p. 492-497, 2011.

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. **Manual de Análise Sanitária de Sementes**. Brasília, DF: MAPA/ACS. 2009.

CARVALHO, E. M. S.; BESERRA JR., J. E. A.; BARGUIL, B. M. Lima Bean Diseases. In: FERREIRA, A. S. A.; LOPES, A. C. A.; GOMES, R. L. F. (Eds.). ***Phaseolus lunatus*: Diversity, Growth and Production**. Nova Science Publishers, New York, p.113-133. 2015.

COSTA, D. S.; BONASSA, N.; NOVEMBRE, A. D. L. C. Incidence of storage fungi and hydropriming on soybean seeds. **Journal of Seed Science**, Londrina, v. 35, p. 35-41, 2013.

EMBABY, E. M.; REDA, M.; ABDEL-WAHHAB, M. A.; OMARA, H.; MOKABEL, A. M. Occurrence of toxigenic fungi and mycotoxins in some legume seeds. **Journal of Agricultural Technology**, Bangkok, v. 9, p. 151-164, 2013.

GONZALEZ, D.; CUBETA, M. A.; VILGALYS, R. Phylogenetic utility of indels within ribosomal DNA and beta-tubulin sequences from fungi in the *Rhizoctonia solani* species complex. **Molecular Phylogenetics and Evolution**, v. 40, p. 459–470, 2006.

GUPTA, G. K.; SHARMA, S. K.; RAMTEKE, R. Biology, epidemiology and management of the pathogenic fungus *Macrophomina phaseolina* (Tassi) Goid with special reference to charcoal rot of soybean (*Glycine max* (L.) Merrill. **Journal of Phytopathology**, Weinheim, v. 160, p. 167-180, 2012.

IBGE. **Produção Agrícola Municipal**: Culturas Temporárias e Permanentes. Rio de Janeiro: IBGE. 2014.

KAGA, H; MANO, H.; TANAKA, F.; WATANABE, A.; KANEKO, S.; MORISAKI, H. Rice seeds as sources of endophytic bacteria. **Microbes and Environments**, Tóquio, v. 24, p. 154-162, 2009.

OLIVEIRA, V. A.; MARTINS, L. P.; GONÇALVES, R. C.; BENÍCIO, L. P. F.; COSTA, D. L.; LUDWIG, J. Use of seed treatment with fungicide in control of *Colletotrichum truncatum* and physiological quality of soybean seeds *Glycine max*. **Journal of Biotechnology and Biodiversity**, Tocantins, v. 4, p. 98-106, 2013.

OLIVEIRA, A. E. S.; SIMEÃO, M.; MOUSINHO, F. E. P.; GOMES, R. L. F. Desenvolvimento do feijão-fava (*Phaseulus lunatus* L.) sob déficit hídrico cultivado em ambiente protegido. **Holos**, Natal, v. 1, p. 143-151, 2014.

PARSA, S.; GARCIA-LEMOS, A. M.; CASTILLO, K.; ORTIZ, V.; LOPEZ-LAVALLE, L. A. B.; JEROME, F. B. V. Fungal endophytes in germinated seeds of the common bean, *Phaseolus vulgaris*. **Fungal Biology**, v. 120, p. 783-790, 2016.

RIVERBERI, M.; RICELLI, A.; ZLALIC, S.; FABBRI, A. A.; FANELLI, C. Natural functions of mycotoxins and control of their biosynthesis in fungi. **Applied Microbiology and Biotechnology**, Berlim, v. 87, p. 899-911, 2010.

RODRIGUES, A. A. C.; MENEZES, M. Detecção de fungos endofíticos em sementes de caupi provenientes de Serra Talhada e de Caruaru, estado de Pernambuco. **Fitopatologia Brasileira**, Brasília, v. 27, p. 532-537, 2002.

SARR, M. P.; NDIAYE, M'BAYE; GROENEWALD, J. Z.; CROUS, P. W. Genetic diversity in *Macrophomina phaseolina*, the causal agent of charcoal rot. **Phytopathologia Mediterranea**, Florença, v. 53, p. 250−268, 2014.

SEIDU, K. T.; OSUNDAHUNSI, O. F.; OLALEYE, M. T.; OLUWALANA, I. B. Amino acid composition, mineral contents and protein solubility of some lima bean (*Phaseolus lunatus* l. Walp) seeds coat. **Food Research International**, v. Ontário, 73, p. 130-134, 2015.

SILVA, J. A.; OLIVEIRA, M. G.; SOUZA, L. T.; ASSUNÇÃO, I. P.; LIMA, G. A.; MICHEREFF, S. J. Reação de

genótipos de feijão-fava a *Sclerotium rolfsii*. **Horticultura Brasileira**, Vitória da Conquista, v. 32, p. 98-101, 2014.

SOUSA, T. P.; NASCIMENTO, I. O.; MAIA, C. B.; MORAIS, J.; BEZERRA, G. A.; BEZERRA, J. W. T. Incidência de fungos associados a sementes de soja transgênica variedade BRA valiosa RR. **Agroecossistemas**, Belém, v. 3, p. 52-56, 2011.

TAMURA, K.; STECHER, G.; PETERSON, D.; FILIPSKI, A.; KUMAR, S. MEGA 6: Molecular Evolutionary Genetics Analysis Version 6.0. **Molecular Biology and Evolution**, Oxford, v. 30, p. 2725-2729, 2013.