Research, Society and Development, v. 11, n. 12, e221111234369, 2022
(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v11i12.34369

The first report about *Fusarium falciforme that causes root rot in cassava* cv Rosinha, in Alagoas state, Brazil

O primeiro relato de *Fusarium falciforme* que causa podridão radicular em mandioca cv Rosinha, Alagoas, Brasil

El primer reporte de *Fusarium falciforme* causando pudrición de raíz en yuca cv Rosinha, Alagoas, Brasil

Received: 08/21/2022 | Reviewed: 09/03/2022 | Accept: 09/04/2022 | Published: 09/12/2022

Izael Oliveira Silva
ORCID: https://orcid.org/0000-0002-4430-655X
Universidade Federal de Alagoas, Brazil
Centro Educacional Pesquisa Robótica e Inovação, Brazil
Secretaria Estadual de Educação de Alagoas, Brazil
E-mail:Izaelbiologo@gmail.com
Edna Peixoto da Rocha Amorim
ORCID: https://orcid.org/0000-0002-8668-0216
Universidade Federal de Alagoas, Brazil
E-mail: ednaamorim58@hotmail.com
Nelson Augusto Nascimento Junior
ORCID: https://orcid.org/0000-0002-3608-175X
Instituto Federal de Alagoas, Brazil
E-mail: nelsonanjr@gmail.com
Juliana Paiva Carnauba
ORCID: https://orcid.org/0000-0001-7246-8639
Instituto Federal de Alagoas, Brazil
E-mail:juliana.carnauba@ifal.edu.br
Vicente Ferreira de Araújo Neto
ORCID: https://orcid.org/0000-0002-3042-5670
Universidade Federal de Alagoas, Brazil
E-mail: vicente.nt@outlook.com
Georgia Souza Peixinho
ORCID: https://orcid.org/0000-0002-9688-508X
Universidade Federal de Alagoas, Brazil
E-mail: geopeixinho@gmail.com

Abstract
The aim of this study was identifying the root rot causal agents in cassava, cv Rosinha, in Alagoas state, Brazil. Pathogen species were studied, which are responsible for root rot in cassava, through isolation, morphological characterization and pathogenicity confirmation. *Fusarium* sp was emphasized among other isolated genera, which is pathogenic to cassava roots and plants. The phylogenetic study was based on the ITS region of the constructed DNA, using the ‘‘maximum Likelihood Tree’’ method, with a base of 1000 “bootstrap” replicas, what showed 100% of genetic similarity to *Fusarium falciforme*.
Keywords: *Manihot* sp.; Biological control; Molecular biology; Fungus.

Resumo
O objetivo deste estudo foi identificar os agentes causais da podridão radicular na mandioca, cv Rosinha, no estado de Alagoas, Brasil. Foram estudadas espécies de patógenos responsáveis pela podridão radicular na mandioca, através do isolamento, caracterização morfológica e confirmação de patogenicidade. *Fusarium* sp., foi destacado entre outros gêneros isolados, patogênico às raízes e plantas da mandioca. O estudo filogenético foi baseado na região ITS do DNA construído, usando o método da 'Árvore de máxima verossimilhança', com uma base de 1000 réplicas de 'bootstrap', o que mostrou 100% de semelhança genética com *Fusarium falciforme*.
Palavras-chave: *Manihot* sp.; Controle biológico; Biologia molecular; Fungo.

Resumen
El objetivo de este estudio fue identificar los agentes causales de la pudrición de la raíz en la yuca, cv Rosinha, en el estado de Alagoas, Brasil. Se estudiaron las especies de patógenos responsables de la podredumbre de la raíz de la

géneros aislados, se destacó *Fusarium* sp., patógeno de las raíces y plantas de yuca. El estudio filogenético se basó en la región ITS de la construcción de ADN, utilizando el método de "Árbol de Máxima Verosimilitud", con una base de 1000 réplicas bootstrap, que mostró un 100% de similitud genética con *Fusarium falciforme*.
Palabras clave: Manihot sp.; Control biológico; Biología molecular; Hongo.

1. Introduction

Root rot (Manihot esculenta Crantz) is the disease that most limits cassava production (Azevedo et al., 2021) in the North and Northeast regions of Brazil, notably in Alagoas state, where losses reach 70% approximately. The cassava is mainly used in human and animal feeding, as material for the biofuel, biodegradable plastic, meat processing, pasta production, textile and modified starch industries (Oliveira et al., 2011; Silva, et al., 2020). The losses caused by the root rot are limiting factors in the North and Northeast in the country (Machado et al., 2014; Nascimento Junior, 2015; Paiva et al., 2022). However, records about the main plant pathogens that cause root rot are incipient, even though their importance as a tool to make decisions about the best management strategy to be implemented (Baayen et al., 2000; Nalim et al., 2011; O'Donnell et al., 2008; Oliveira et al., 2011).The aim of this study is identifying the root rot causal agents in cassava, cv Rosinha, in Alagoas state, Brazil.

2. Methodology

Soil and roots were collected in the cities Arapiraca, Campo Alegre, Penedo, São Miguel dos Campos and São Sebastião in Alagoas state, Brazil in 2015, where cassava, cv Rosinha, was produced and presented root rot problems. In order to isolate the microorganisms, the serial dilution technique was used and in accordance to Tuite, (1969) methodology, 1.0 mL of the suspension was transferred to the petri dishes with BDA culture medium. Mycelial discs of isolated *Fusarium* sp. were used for DNA extraction, using CTAB protocol according to Doyle and Doyle, (1987). PCR reactions were prepared subsequently, and after that, the product was sent to purification and sequencing with the same primers that were used in the amplification to Macrogen Inc. (Seoul, South Korea). Multiple alignments of nucleotide sequence were prepared (Ronquist et al., 2012), using MUSCLE algorithm (Edgar, 2004) and manually adjusted in MEGA6 package (Tamura et al., 2013). Os isolados obtidos foram amplificados com o gene que codifica para a região ITS-rDNA (Table 1).

Table 1. Description of the initiator used to identify fungal genera.

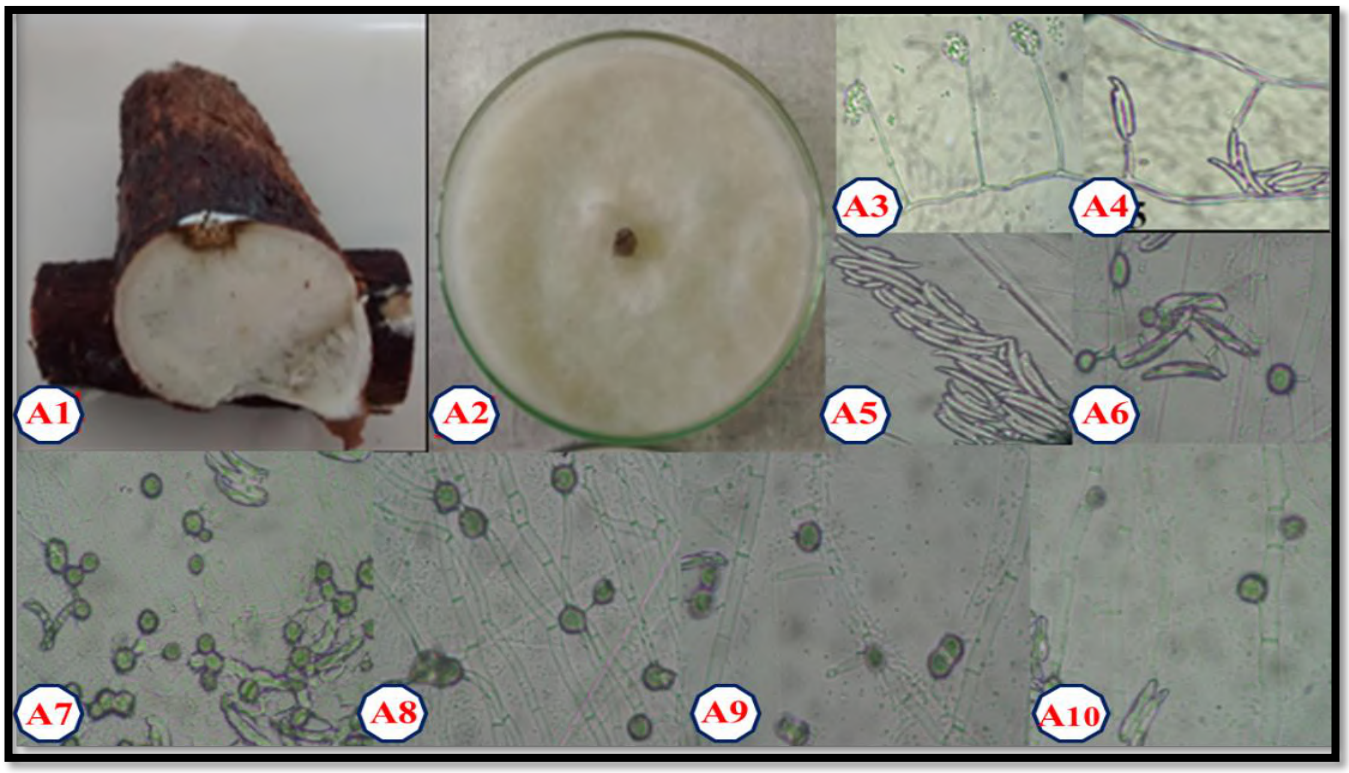
Gene	Primer	Sequence (5'-3')	Reference
ITS	ITS1	TCCGTAGGTGAACCTGCGG	White et al., 1990
	ITS4	TCCTCCGCTTATTGATATGC	

Source: Authors (2022).

3. Results and Discussion

The structures that were produced by the pathogen were observed in the BDA medium, showing macroconidia with 3-7 septa and the size varying from 27-60 µm x 3-5 µm and microconidia with and without septa, measuring 8-16 µm x 2-4 µm, from cylindrical to oval and chlamydospore with an average diameter of 4.7-9.4µm (Figure 1).

Figure 1. Appearance of the dry rot lesion of the cassava root (A1); Aspect of the phytopathogen colony (A2); Conidiophore and microconidium (A3); Conidiophore and macroconidium (A4); Macroconidium (A5); Macroconidium, terminal chlamydospores and interspersed with hyphae (A6; A8; A9; A10); Chlamydospores in chain (A7) Double chlamydospores (A9).

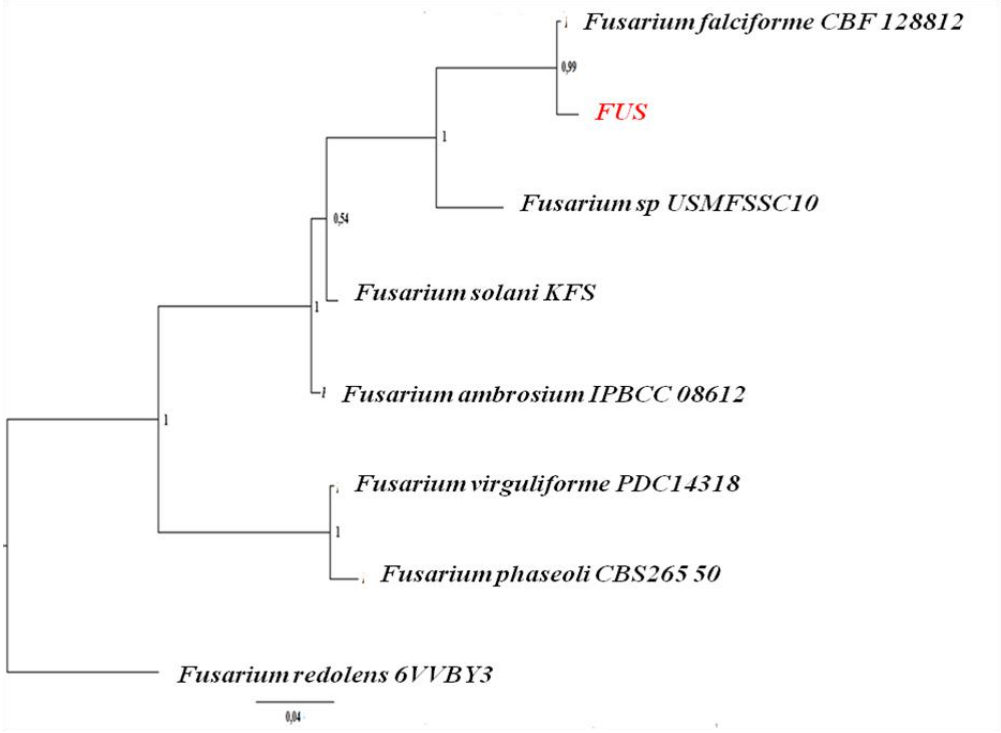


Obtained after 21 days of growth in BDA in the dark at room temperature (28 ± 2 ° C). The images were captured by a digital camera (Olympus IX2-SLP) coupled to an optical microscope with a magnification of 100 to 200x, projected on a computer monitor, using the Cellsenses Standard software (SAMSUNG SDC-415®). 50 measurements were made of each type of structure produced. Source: Authors (2022).

Chehri et al., (2011); O'Donnell and Gray, (1995) and Silva, (2016), state that using only the morphological characters, host pathogenicity and *formae speciales*, may not conclusively reflect the diversity of species and populations in a complex. In this way, the genetic variability and the true phylogenetic relationships, biological species, host range and geographic distribution of these pathogens can be omitted or not defined, leading to misidentification. Thus, the sequencing of genomic regions and molecular studies are strong allies in the identification of phytopathogens

PCR amplification product with ITS1 and ITS4 primers resulted in a fragment of approximately 550 pairs of bases (*pb*). The phylogenetic analysis based on the genic sequences of the ITS (Chehri et al., 2011; Chhetri et al., 2015; Hyde et al., 2014; O'Donnell et al., 2013) region of the DNA of the isolated material, using the ''maximum Likelihood Tree'' method with base on 1000 "bootstrap" replicas, showed 100% reliability that the isolated material belongs to the genus *Fusarium* and 99% similar to the *Fusarium falciforme* sequence that was deposited at *GenBank* (CBS128812). The confirmation is possible from the studied genomic region and the formed phylogenetic tree (Figure 2).

Figure 2. Phylogenetic tree based on the ITS region of *Fusarium sp*. DNA, built using the ‘‘maximum Likelihood Tree’’ method. *Fusarium redolens* 6VVBY3 isolate was used as an “outgroup”. The scale bar represents a change of 0.04 and bootstrap support values of 1000 repetitions are shown in the branches.



Source: Authors (2022).

Thus, it is possible to affirm that from the studied genomic region and the formed phylogenetic tree, the isolated FUS is *F. falciforme* and not *F. solani* as the causal agent of cassava root rot(Alves et al., 2020; Paiva et al., 2022; Silva, et al., 2020a, 2020b; Silva, et al., 2020) . So, this is the first report about this specie as the causer of root rot in cassava.

4. Conclusion

Based on the genomic region (ITS) studied, we can conclude that *Fusarium falciforme* is another species of phytopathogen that causes root rot in cassava.

The morphophysiological characteristics of a phytopathogen together with the molecular tools make the identification more assertive and the decision making in the control of the disease faster.

Other work needs to be carried out to assess the epidemiology and severity of the disease in our state.

References

Alves, R. N. B., Modesto Junior, M. de S., Silva, A. R. B., Nakasone, A. K., & Farias Neto, J. T. de. (2020). *Podridões em raízes de mandioca: Problemas e soluções para o seu controle. 455*(1ª edição), 28f.

Azevedo, P. F. de, Almeida, A. C. de, Marques, R. D., Costa, C. L. da, Benedetti, A. R., Lescano, L. E. A. M., Carvalho, M. C. da C. G., & Matsumoto, L. S. (2021). In vitro inhibition of Fusarium solani by Trichoderma harzianum and biofertilizer. *Research, Society and Development*, *10*(3), e5210312994–e5210312994. https://doi.org/10.33448/rsd-v10i3.12994

Baayen, R. P., O’Donnell, K., Bonants, P. J., Cigelnik, E., Kroon, L. P., Roebroeck, E. J., & Waalwijk, C. (2000). Gene Genealogies and AFLP Analyses in the Fusarium oxysporum Complex Identify Monophyletic and Nonmonophyletic Formae Speciales Causing Wilt and Rot Disease. *Phytopathology*, *90*(8), 891–900. https://doi.org/10.1094/PHYTO.2000.90.8.891

Chehri, K., Salleh, B., Yli-Mattila, T., Reddy, K. R. N., & Abbasi, S. (2011). Molecular characterization of pathogenic Fusarium species in cucurbit plants from Kermanshah province, Iran. *Saudi Journal of Biological Sciences*, *18*(4), 341–351. https://doi.org/10.1016/j.sjbs.2011.01.007

Chhetri, G., Pandey, T., Kumar, B., Akhtar, M. S., & Tripathi, T. (2015). Recombinant expression, purification and preliminary characterization of the mRNA export factor MEX67 of Saccharomyces cerevisiae. *Protein Expression and Purification*, *107*, 56–61. https://doi.org/10.1016/j.pep.2014.11.011

Doyle, J. J., & Doyle, J. L. (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue—World Vegetable Center. *Phytochemical Bulletin*, *19*(1), 11–15. http://www.sciepub.com/reference/60627

Edgar, R. C. (2004). MUSCLE: Um método de alinhamento de sequências múltiplas com menor tempo e complexidade espacial. *BMC Bioinformatics*, *5*(1), 113. https://doi.org/10.1186/1471-2105-5-113

Hyde, K. D., Nilsson, R. H., Alias, S. A., Ariyawansa, H. A., Blair, J. E., Cai, L., de Cock, A. W. A. M., Dissanayake, A. J., Glockling, S. L., Goonasekara, I. D., Gorczak, M., Hahn, M., Jayawardena, R. S., van Kan, J. A. L., Laurence, M. H., Lévesque, C. A., Li, X., Liu, J.-K., Maharachchikumbura, S. S. N., & Zhou, N. (2014). One stop shop: Backbones trees for important phytopathogenic genera: I (2014). *Fungal Diversity*, *67*(1), 21–125. https://doi.org/10.1007/s13225-014-0298-1

Machado, A. R., Pinho, D. B., Oliveira, S. A. S., & Pereira, O. L. (2014). New occurrences of Botryosphaeriaceae causing black root rot of cassava in Brazil. *Tropical Plant Pathology*, *39*(6), 464–470. https://doi.org/10.1590/S1982-56762014000600008

Nalim, F. A., Samuels, G. J., Wijesundera, R. L., & Geiser, D. M. (2011). New species from the Fusarium solani species complex derived from perithecia and soil in the old world tropics. *Mycologia*, *103*(6), 1302–1330. https://doi.org/10.3852/10-307

Nascimento Junior, N. A. (2015). *Efeito da casca de mandioca no controle da podridão radicular causada por Phytophthora sp. Em mandioca de mesa (Manihot esculenta Crantz) var. Rosinha em ambiente irrigado.* [Tese de Doutorado. (Programa de Pós-Graduação em Proteção de Plantas da Universidade Federal de Alagoas., UNIVERSIDADE FEDERAL DE ALAGOAS]. https://ceca.ufal.br/pt-br/pos-graduacao/protecao-de-plantas/documentos/dissertacoes-e-teses/doutorado/2015/efeito-da-casca-de-mandioca-no-controle-da-podridao-radicular-causada-por-phytophthora-sp-em-mandioca-de-mesa-manihot-esculenta-crantz-var-rosinha-em-ambiente-irrigado

O'Donnell, K., & Gray, L. (1995). Phylogenetic Relationships of the Soybean Sudden Death Syndrome Pathogen Fusarium solani f. Sp. Phaseoli Inferred from rDNA Sequence Data and PCR Primers for Its Identification. *Molecular plant-microbe interactions : MPMI*, *8*, 709–716. https://doi.org/10.1094/MPMI-8-0709

O'Donnell, K., Rooney, A. P., Proctor, R. H., Brown, D. W., McCormick, S. P., Ward, T. J., Frandsen, R. J. N., Lysøe, E., Rehner, S. A., Aoki, T., Robert, V. A. R. G., Crous, P. W., Groenewald, J. Z., Kang, S., & Geiser, D. M. (2013). Phylogenetic analyses of RPB1 and RPB2 support a middle Cretaceous origin for a clade comprising all agriculturally and medically important fusaria. *Fungal Genetics and Biology*, *52*, 20–31. https://doi.org/10.1016/j.fgb.2012.12.004

O'Donnell, K., Sutton, D. A., Fothergill, A., McCarthy, D., Rinaldi, M. G., Brandt, M. E., Zhang, N., & Geiser, D. M. (2008). Molecular Phylogenetic Diversity, Multilocus Haplotype Nomenclature, and In Vitro Antifungal Resistance within the *Fusarium solani* Species Complex. *Journal of Clinical Microbiology*, *46*(8), 2477–2490. https://doi.org/10.1128/JCM.02371-07

Oliveira, N. T. de, Alves, J. M. A., Uchôa, S. C. P., Rodrigues, G. S., Melville, C. C., & Albuquerque, J. de A. A. de. (2011). Characterization of cassava clones produced in Roraima for in natura consumption. *Agro@mbiente On-line*, *5*(3), 188–193. https://www.cabdirect.org/cabdirect/abstract/20123022085

Paiva, G. F., Souza, G. H. S. de, Alves, A. R., Silva, B. V. S., Mota, L. H. C., Gonçalves, F. J. T., & Carrasco, N. F. (2022). Avaliação de resistência a Fusarium solani em genótipos de mandioca. *Research, Society and Development*, *11*(9), e23911931734–e23911931734. https://doi.org/10.33448/rsd-v11i9.31734

Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D. L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M. A., & Huelsenbeck, J. P. (2012). MrBayes 3.2: Efficient Bayesian Phylogenetic Inference and Model Choice Across a Large Model Space. *Systematic Biology*, *61*(3), 539–542. https://doi.org/10.1093/sysbio/sys029

Silva, I. O. (2016). *Etiologia da podridão radicular da mandioca de mesa (Manihot esculenta Crantz) var. Rosinha no Estado de Alagoas e Influência de Trichoderma spp. Sobre o desenvolvimento de Fusarium sp* [Tese de Doutorado]. Programa de Pós-Graduação em Proteção de Plantas do Centro de Ciências Agrárias da Universidade Federal de Alagoas.

Silva, I. O., Amorim, E. P. da R., Junior, N. A. N., Peixinho, G. S., Carnaúba, J. P., & Neto, V. F. de A. (2020a). Trichoderma spp antagonism to phytopathogens causing cassava root rot in alagoas. *Revista Científica Multidisciplinar Núcleo do Conhecimento*, *12*(6), 149–177. https://doi.org/10.32749/nucleodoconhecimento.com.br/agronomy-en/trichoderma-antagonism

Silva, I. O., Amorim, E. P. da R., Nascimento Junior, N. A., Assunção, I. P., Carnaúba, J. P., Peixinho, G. S., Santos, M. Q. C. dos, & Araujo Neto, V. F. de. (2020b). *Identificação de patógenos causadores da podridão radicular da mandioca (manihot esculenta crantz) e biocontrole de trichoderma spp sobre fusarium sp*. https://doi.org/10.37423/200702008

Tamura, K., Stecher, G., Peterson, D., Filipski, A., & Kumar, S. (2013). MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. *Molecular Biology and Evolution*, *30*(12), 2725–2729. https://doi.org/10.1093/molbev/mst197

Tuite, J. (1969). Plant pathological methods. Fungi and bacteria. *Plant pathological methods. Fungi and bacteria*. https://www.cabdirect.org/cabdirect/abstract/19701101886