JANAINA MARTINS DE SOUSA

Fusarium solani SPECIES COMPLEX CAUSING ROOT ROT ON BEANS IN BRAZIL

LAVRAS - MG
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Dissertation presented to the Universidade Federal de Lavras, as part of the requirements of the Graduate Program in Agronomy, in concentration area of Phytopathology, to obtain the title of Master.

Dr. Ludwig H. Pfenning
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To myself Janaina Martins de Sousa for accepting this challenge and do not give up in front of several difficulties.

DEDICATE

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ABSTRACT

Bean Root Rot (BRR) causes considerable losses in the production of pulses, such as common beans and cowpea, which are important elements of the alimentary base of Brazilian people. This disease is caused by members of the *Fusarium solani* species complex (FSSC), and the name commonly used for the causal agent is F. solani f. sp. phaseoli. In this study, we identified species of the FSSC associated with BRR in common and cowpea beans in Brazil, based on molecular phylogeny of four loci and morphological markers, and also evaluated its pathogenicity to common bean, cowpea, and soybean. A set of 48 isolates was obtained from symptomatic plants, collected in ten states and Federal District. Based on the phylogenetic analyses of partial DNA sequences of *rpb2*, we identified *F. paranaense* (n = 35), *F. solani* (n = 6), *F. suttonianum* (n = 3) and *F. martii* (n = 2), all members of Clade 3. Two isolates were identified as members of Clade 2, according to analyses of *rpb2, rpb1, tef1,* and ITS regions. Representative isolates of the identified species induced BRR symptoms when inoculated in common bean and cowpea. The main symptoms, similar in both plants, consist of rot starting from the main root extending to the hypocotyl, reducing plant growth. *Fusarium paranaense, F. solani, F. suttonianum,* and *F. martii* induced root lesions on soybean, while *F. martii* induced internerval chlorosis. This study clarifies the etiology of BRR based on modern species concepts and will provide relevant support to disease management and plant breeding studies.

Key words: *Phaseolus vulgaris*, *Vigna unguiculata*, *Fabaceae*, molecular phylogeny, plant disease.

Resumo

A podridão radicular seca (PRS) causa consideráveis perdas na produção de leguminosas, como feijão comum e feijão caupi que são base da alimetação dos brasileiros. Esta doença é causada por espécies do do complexo *Fusarium solani* (FSSC) e comummente se refere a esses agentes causais como *F. solani* f. sp. *phaseoli*. Neste estudo identificamos espécies de FSSC associadas à PRS em feijão comum e feijão caupi no Brasil, com base na filogenia molecular de quatro loci, marcadores morfológicos e avaliamos sua patogenicidade para feijão comum, feijão-caupi e soja. Um conjunto de 48 isolados foram obtidos de plantas sintomáticas de feijão comum e feijão-caupi, coletadas em dez estados e no Distrito Federal. As espécies *F. paranaense* (n= 35), *F. solani* (n= 6), *F. suttonianum* (n= 3) e *F. martii* (n= 2), ambas do Clado 3, foram identificadas por análises filogenéticas da sequência da região gênica *rpb2.* Dois isolados foram identificados como membros do Clado 2, de acordo com as análises das sequencies dos genes *rpb1, tef* and ITS. Os isolados representativos das espécies identificadas induziram sintomas de PRS em feijão comum e feijão-caupi. Os sintomas foram semelhantes, consistindo em podridão a partir da raiz principal estendendo-se até o hipocótilo e reduções do crescimento das plantas. As espécies *F. paranaense, F. solani*, *F. suttonianum, F. martii* induziram lesões nas raízes de soja e *F. martii* induziu clorose internerval em folhas de soja. Este estudo esclarece a etiologia de FSSC e fornece suporte para o manejo de doenças e estudos de melhoramento de plantas.

Palavaras chave: *Phaseolus vulgaris, Vigna unguiculata, Fabaceae*, filogenia molecular, doença de plantas.

Content

PART 1

1 General Introduction

Bean and rice constitute the alimentary base of Brazilian people. For this reason, those food crops are cultivated in a broad range and in whatever place throughout the country, as subsistence farming as well as under the use of high technology and irrigation. In Brazil, cultivars of *Phaseolus vulgaris* L. (common bean), and *Vigna unguiculata* (L.) Walp (cowpea) are most frequent pulses produced in all geographic regions and its production occurs in three different grain harvests during the year (CONAB, 2019).

Bean diseases caused by different pathogens can reduce yield and quality. The main fungal diseases that affect those pulses are white mold (*Sclerotinia sclerotiorum*), angular leaf spot (*Pseudocercospora griseola*), anthracnose (*Colletotrichum lindemuthianum*), and bean root rot (*Fusarium solani* f. sp. *phaseoli*) (Schwartz et al., 2005). Bean Root Rot, also known as *Fusarium* root rot and dry bean Root rot (in Brazil), is reported in many areas, causing a significant reduction in production, and its etiology is not yet clearly defined in Brazil (Schwartz et al., 2005; Chaudhary et al., 2006; Bilgi et al., 2008).

The causal agents of bean root rot are members of the *Fusarium solani* species complex (FSSC). *Fusarium phaseoli*, *F. crassistiptatum* and *F. azukicola* are reported as pathogens of *Phaseolus vulgaris* and *Vigna angularis* in the USA, Canada, and Japan (Aoki et al., 2005, 2012b). *Fusarium falciforme* was reported as a pathogen of *Phaseolus lunatus* in the Brazilian northeast (Sousa et al., 2017), while *F. paranaense*, *F. tucumaniae*, *F. crassistiptatum*, *F. brasiliense*, and *F. virguliforme* are etiologic agents of red root rot and sudden death syndrome of soybean (SDS) (Aoki et al., 2003, 2005, 2012a, b; Costa et al., 2016). In Brazil, those pathogens associated with beans are usually reported as *F. solani* f. sp. *phaseoli* (Macedo et al., 2017; Toledo-Souza et al., 2008).

In this study, we propose to answer the following questions: (i.) Which species of the FSSC are associated with BRR in Brazil? (ii.) Which species of FSSC cause BRR? (iii.) *Fusarium phaseoli*, *F. crassistiptatum* and *F. azukicola* are present in Brazil and associated with common bean and cowpea? (iv.) Different species of FSSC coexist in the same field and in plants with symptoms of BRR? (v.) Can farming technology, such as irrigation or no-till systems, have an influence on species composition or diversity in production fields? (vi.) Pathogenic species to common beans are also pathogens of cowpea and soybean? (vii.) Do any species that cause BRR induce also typical SDS symptoms in beans and soybean?

2 State of The Art

2.1 The main pulses cultivated in Brazil

Phaseolus vulgaris (common bean), and *Vigna unguiculata* (cowpea) are main pulses produced and consumed in Brazil. Other types of beans are grown in crop rotation systems, intercropping, cover crop or green manure, such as *Cajanus cajan* and *Canavalia ensiformis* (Weiler et al., 2019). But the different cultivars of *P. vulgaris* and *V. unguiculata* are pulses used as an alimentary base of Brazilian people, because they are protein sources (Graham & Vence, 2003). Common bean and cowpea have physiological differences, which impact in their geographic distribution and prevalence, but also management. Despite of this, they are cultivated in a broad range throughout the country, as subsistence farming and under use of high technology and irrigation, harvested over all the year (CONAB, 2019; Vale et al., 2017).

Common bean had its origin in Mesoamerica and Andean regions, but is now cultivated around the world (Mamidi et al., 2012). Its domestication occurred 8.000 years ago in Mexico and South America (Bitocchi et al., 2013; Schmutz et al., 2014; Vlasova et al., 2016). Its cycle can complete in 60 to 120 days, varying according to genotype and the environmental

conditions to which plants are submitted (Santos & Gavilaine, 2006). The seeding can occur in three different periods: water beans (1ª crop – august to december), dry bean (2ª crop – january to march), and winter bean (3ª crop – November to April). The first crop season is the main crop in the southern region of Brazil, because it occurs in the rainy season and the harvest happens between November to April. The second crop season occurs in all regions and the harvest is done between March and July. And the third crop season happens mainly with supplemental irrigation, with harvesting between July to October (Posse et al., 2010; Silva, 2015). The main producing states are Paraná, Minas Gerais, Mato Grosso and Santa Catarina (CONAB, 2019).

Cowpea is distributed in tropical and subtropical lands worldwide. The African continent is the center of origin and the crop were brought to Brazil by Portuguese colonizers around the 16th century. Nowadays, it is predominantly cultivated in North and Northeast regions and its genotypes present major tolerance to conditions of drought and high temperature that are present in those regions (Oliveira et al., 2002; Freire Filho, 2011). The cultivation is mostly under subsistence farming, but currently this crop is in expansion to cerrado areas in the Midwest, where it is cultivated as an off-season by producers into large-scale production with high fundamental quality and regularity to feed commercial chain and as cover crop and green adubation used to recover damaged soil (Ramos et al., 2012; Freire Filho, 2011; Vale et al., 2017). Within pathogens that affect those pulses, most important are white mold (*Sclerotinia sclerotiorum*), angular leaf spot (*Pseudocercospora griseola*), anthracnose (*Colletotrichum lindemuthianum*), and bean root rot (*Fusarium solani* f. sp *phaseoli*) (Schwartz et al., 2005).

2.2 Bean Root Rot

Bean root rot (BRR), also known as *Fusarium* root rot or dry bean root rot, is reported in many bean cultivating areas, causing a significant reduction in production. The etiology is still

not clearly defined in Brazil (Schwartz et al., 2005; Chaudhary et al., 2006; Bilgi et al., 2008). Main symptoms of BRR are rot on root tissues, the reason why the disease is called root rot. These symptoms start as a small longitudinal lesion in red or brown colors on the main root, the affected area expands and extends to the hypocotyl. In the flowering stage, plants present yellowing of leaves and reduced growth. The disease results in reduction of pods, grains, and in some cases plant death.

Causal agents of Bean root rot are species of the *Fusarium solani* species complex - FSSC, mostly reported in literature as *F. solani* f. sp. *phaseoli,* according to formae speciales concept (Snyder & Hansen, 1953). Today, identification of species is widely based on molecular phylogeny. Among the soil pathogens present in areas cultivated with beans, *F. solani* f. sp. *phaseoli* is predominant in comparison with others like *Rhizoctonia solani*, expressing higher inoculum density, with 7.10×10^4 propagules/g of soil (Ribeiro Costa & Da Silva Costa, 2004).

Species of the FSSC pathogenic to bean are *F. phaseoli*, *F. crassistiptatum* and *F. azukicola,* reported as pathogens of *Phaseolus vulgaris* and *Vigna angularis* in the USA, Canada, and Japan (Aoki et al., 2005, 2012b). Those species group within Clade 2, together with *F. tucumaniae*, *F. crassistiptatum*, *F. brasiliense*, and *F. virguliforme,* known as the etiologic agents of red root rot and sudden death syndrome of soybean (SDS) (Aoki et al., 2003, 2005, 2012a, b; Costa et al., 2016). *Fusarium falciforme* (Clade 3), was reported as a pathogen of *Phaseolus lunatus,* in Brazilian northeast (Sousa et al., 2017). But most of the studies from Brazil, fungi associated with BRR are usually reported as *F. solani* f. sp. *phaseoli* and thus the etiology of BRR remains unclear (Macedo et al., 2017; Toledo-Souza et al., 2008).

These pathogens are saprophytes, surviving for a long time on crop remains, and produce spores disseminated by water and soil (Casa et al., 2010). Their control is difficult, sterilization of soil that is restricted to small areas, the use of fungicides is not effective to control BRR and

practices such as rotation of cultures is not enough to control fungi with saprophytic ability being applied to reduce inoculum. To obtain resistant cultivars is the main management strategy, so it is necessary to know well the causal agents of disease and the genotype of bean (Michereff et al., 2005).

2. 3 *Fusarium solani* species complex - FSSC

The *Fusarium solani* species complex - FSSC includes significant agricultural important plant pathogens associated with root rots in over 100 crops (Sandoval-Denis et al., 2019). These species were previously considered a single species within section Martiella, based on its morphology (Snyder & Hansen, 1941). According to Leslie & Summerell (2006), the characteristics of *F. solani* cultures in Potato-Dextrose-Agar (BDA) culture medium usually varies in color between white and beige. Aerial mycelium conidia (microconidia) may have a shape oval, ellipsoidal or reniform with 0-1 septa in false heads in long monophialides; macroconidia are relatively large, mainly formed in sporodochia, whose predominant color is beige and less frequently in green and blue and shape ranging from straight to slightly curved; chlamydospores are formed after 2-4 weeks, and can be interspersed between hyphae or at the tip of individual lateral branches or in oval or globose pairs, with or without wall ornamentation. However, the morphological marker of *F. solani* is the long, slender monophialide, which distinguish this species group from other *Fusarium* species.

Another classification concept proposed for *F. solani* was based on sexual compatibility. The use of this concept became known as *Nectria haematococca* mating populations I–VII (Matuo & Snyder, 1973). Now it is known as a complex of species based on molecular phylogenetic analyses, with about 60 defined phylogenetic species (O'Donnell, 2000; O'Donnell et al., 2008; Nalim et al., 2011). Recently phylogenetic studies confirmed FSSC as

part of the monophyletic genus *Fusarium,* which comprises over 300 phylogenetically distinct species distributed among 23 species complexes (Geiser et al., 2020).

The *Fusarium solani* species complex comprises more than 70 species distributed in three distinct subgroups known as clades. In Clade 1 group two species from New Zealand, *F. illudens* and *F. plagianthi*. In Clade 2 group species associated with beans and soybean, while Clade 3 is the most diverse and species rich group with species associated with many different crop plants (O'Donnell et al., 2000; Geiser et al., 2020). Phylogenetic analysis use housekeeping genes like translation elongation factor-1 alpha (*tef*1), second-largest RNA polymerase II B-subunit (*rpb*2) and larger ATP 197 citrate lyase (*acl*1) genes (O'Donnell et al., 2000; Gräfenhan et al., 2011).

Besides studies on phylogeny, molecular tools are used in studies to elucidate the metabolic pathways of FSSC species involved in different types of environments, and to expand the understanding of the impact of gene flow on fungal evolution (Coleman at al., 2009). Species in the FSSC are heterothallic, but a few are homothallic. The biological species concept was already applied in the early seventies, when an infraspecific classification was adopted, separating distinct forms in mating populations (Matuo & Snyder, 1973). Considering that species of FSSC present a diverse set of self fertile (homothallic) and self-sterile (heterothallic) species, which are permitted to be classified into 'mating population'(MP) once those individuals can be fertile with one another, indicating that they are a biological species (Snyder & Hansen, 1941; O'Donnell et al., 2000).

Fusarium solani MP-VI isolates were studied for its ability to live in distinct habitats (Van Etten & Kistler, 1988) and molecular analyses demonstrated these became from genes located on conditionally dispensable supernumerary chromosomes ("CD chromosomes") (Rossman et al., 1999, Miao et al., 1991), which are not required for growth under all conditions and can confer an adaptive advantage in certain habitats (Covert, 1998). Genes of *F. vanettenii*

MP-VI located in these chromosomes are involved in resistance to plant antimicrobials, utilization of specific carbon and nitrogen sources, and in host-specific pathogenicity (Covert, 1998; Van Etten et al., 2001; Rodriguez-Carres et al., 2008).

Virulence factors have been identified in fungi with supernumerary chromosomes, but few studies have focused on identification of virulence factors and plant defense responses at a molecular level in members of FSSC (Coleman, 2016; Hadwiger, 2008). It is known that tolerance mechanisms to phytoalexins represent the best-studied host-specific virulence factors in members of FSSC which possess degradation/modification and efflux mechanisms by which they able to tolerate plant antimicrobial compounds and establish an infection (Coleman & Mylonakis 2009; Van Etten et al., 2001). A few effectors have been identified for example as extracellular DNase h in *F. phaseoli* and *F. vanettenii* (Hadwiger, 2008; Hadwiger et al., 1995). In *F. virguliforme* a small protein, termed FvTox1, is responsible for causing the foliar symptoms of chlorosis and necrosis, ultimately leading to the loss of leaves and pods of infected plants (Brar et al., 2011).

About interaction between *F. phaseoli* and common beans it is known that *F. phaseoli* isolates which produce extracellular kievitone hydratase are highly virulent to common beans and they are less virulent when kievitone hydratase activity is abolished. The kievitone hydratase consists of an acidic glycoprotein which catalyzes a hydration reaction in the dimethylallyl moiety, forming the smallest toxic compound kievitone hydrate (Coleman et al., 2016). The common bean responds to the presence of kievitone with a production of phytoalexins like phaseolidine, phaseolin, and phaseolin isoflavona. However, phytoalexins as phaseolin are detoxified to the less toxic compound 1a-hydroxyphaseolone by a monooxygenase (Kistler and VanEtten, 1981; Turbek et al., 1992).

Many species of Clade 3 are important plant pathogens. As an example, *F. euwallaceae* causing wilt and dieback avocado in Israel and USA (Freeman et al., 2013) and *F. perseae*

causes trunk cankers on avocado in Italy (Guarnaccia et al., 2018). Those species have a global distribution and a wide ecological amplitude, also including strains from soil and agents of plant disease as example *F. solani* associated with cereals in soils in Ethiopia (Bogale et al., 2009) and isolates of *F. solani* causing root and fruit rot of cucurbits in Italy, sweet peppers in United Kingdom and on paprika in Korea (Zaccardelli et al., 2008; Fletcher, 1994; Jee et al., 2005). Species of Clade 3 are also known as opportunistic human pathogens (Zhang et al., 2006; Short et al., 2013).

2.4 Species pathogenic to leguminous plants

Species which cause disease in soybeans are most studied among leguminous plants. In Clade 2 group the causal agents of Sudden death syndrome – SDS, *F. tucumaniae*, *F. crassistiptatum*, *F. brasiliense*, and *F. virguliforme*, reported from USA, Canada, Argentina, and Brazil, while *F. virguliforme* was found only in the USA (Aoki et al., 2003, 2012a). In *Fusarium virguliforme* virulence factors were studied, being elucidated its secreted protein FvTox1, which is responsible for causing the foliar symptoms of chlorosis and necrosis and leading to the loss of leaves of infected plant (Brar et al., 2011). *Phaseolus vulgaris* and *V. angularis* are infected by *F. phaseoli* and *F. azukicola*, respectively in the USA and Japan (Aoki et al., 2005; 2012a). *Fusarium azukicola* showed to be pathogenic to soybean plants inducing symptoms of chlorosis and necrosis and leading to the loss of leaves (Aoki et al., 2012b).

Bean rot root is reported in different regions around the world and in a several growing condition as no till systems (Schwartz et al., 2005; Casa et al., 2011). Common bean is cultivated in succession with soybean in some areas of Brazil (Oliveira et al., 2016) although this practice is not recommended considering improvement of plant pathogens inoculum of both crops. Based on models for predicting distribution of diseases, the strong influence of precipitation and temperature on frequency of this disease was observed. Trends are noted in

relation to increased incidence of the disease in colder regions such as in the state of Minas Gerais, because of environmental conditions (Macedo et al., 2017).

Species of Clade 3 cause root rot in different leguminous plants. In Brazil, *F. paranaense* was reported from distinct regions of the country and cause soybean red root rot (Costa et al., 2015). Regarding beans, *F. falciforme* (FSSC 3+4) was reported as a pathogen of *Phaseolus lunatus* (lima bean) in the northeast region (Sousa et al., 2017). Other FSSC species were reported in leguminous plants used as green fertilization, cover crop or as a strategy to control other plant pathogens like nematodes. A new lineage of FSSC, with morphological characteristics of *F. neocosmoporiellum*, was reported as causal agent of *Crotalaria paulinea* (Sunn Hemp) in Ceara, state of northeastern Brazil (Melo et al., 2016). In Japan, *F. solani* induced root rot on *Lotus japonicus* (Birdsfoot trefoil) (Takeuchi et al., 2007). *Fusarium neocosmoporiellum* was reported to be associated with peanuts in Taiwan (Huang et al., 1998), and with cowpeas, watermelon, and cotton in the United States (Smith, 1899). Isolates of *F. vanettenii* (FSSC 11) were pathogenic on soybean, dry bean, pea and lentil (Chitrampalam & Nelson 2016).

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PART 2

Species of the *Fusarium solani* species complex FSSC causing Bean Root Rot

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Abstract

Bean Root Rot (BRR) causes considerable losses in the production of pulses, such as common beans and cowpea, which are important elements of the alimentary base of Brazilian people. This disease is caused by members of the *Fusarium solani* species complex (FSSC), and the name commonly used for the causal agent is F. solani f. sp. phaseoli. In this study, we identified species of the FSSC associated with BRR in common and cowpea beans in Brazil, based on molecular phylogeny of four loci and morphological markers, and also evaluated its pathogenicity to common bean, cowpea, and soybean. A set of 48 isolates was obtained from symptomatic plants, collected in ten states and Federal District. Based on the phylogenetic analyses of partial DNA sequences of *rpb2*, we identified *F. paranaense* (n = 35), *F. solani* (n = 6), *F. suttonianum* (n = 3) and *F. martii* (n = 2), all members of Clade 3. Two isolates were identified as members of Clade 2, according to analyses of *rpb2*, *rpb1*, *tef1*, and ITS regions. Representative isolates of the identified species induced BRR symptoms when inoculated in

common bean and cowpea. The main symptoms, similar in both plants, consist of rot starting from the main root extending to the hypocotyl, reducing plant growth. *Fusarium paranaense, F. solani*, *F. suttonianum,* and *F. martii* induced root lesions on soybean, while *F. martii* induced internerval chlorosis. This study clarifies the etiology of BRR based on modern species concepts and will provide relevant support to disease management and plant breeding studies.
Key words: *Phaseolus vulgaris, Vigna unguiculata, Fabaceae,* molecular phylogeny, plant disease.

Introduction

Bean and rice constitute the alimentary base of Brazilian people. For this reason, those food crops are cultivated in a broad range and in whatever place throughout the country, as subsistence farming as well as under the use of high technology and irrigation. In Brazil, cultivars of *Phaseolus vulgaris* (L.) (common bean), and *Vigna unguiculata* (L.) Walp (cowpea) are most frequent pulses produced in all geographic regions and its production occurs in three different grain harvests during the year (CONAB, 2019). Bean diseases caused by different pathogens can reduce common bean and cowpea production. The main fungal diseases that affect those pulses are white mold (*Sclerotinia sclerotiorum*), angular leaf spot (*Pseudocercospora griseola*), anthracnose (*Colletotrichum lindemuthianum*), and bean root rot (*Fusarium solani* f. sp *phaseoli*) (Schwartz et al., 2005). Bean Root Rot, known also as *Fusarium* root rot and dry bean Root rot, is reported in many areas, causing a significant reduction in production and whose etiology is still not clearly defined in Brazil (Schwartz et al., 2005; Chaudhary et al., 2006; Bilgi et al., 2008).

The causal agents of bean root rot are members of the *Fusarium solani* species complex (FSSC). *Fusarium phaseoli, F. crassistiptatum* and *F. azukicola* were reported as pathogens of *Phaseolus vulgaris* and *Vigna angularis* in the USA, Canada, and Japan (Aoki et al., 2005,

2012b). *Fusarium falciforme* was reported as pathogen of *Phaseolus lunatus* in the Brazilian northeast (Sousa et al., 2017), while *F. paranaense, F. tucumaniae, F. crassistiptatum, F. brasiliense,* and *F. virguliforme* are etiologic agents of red root rot and sudden death syndrome of soybean (SDS) (Aoki et al., 2003, 2005, 2012a, b; Costa et al., 2016). In Brazil, those pathogens associated with beans are usually reported as *F. solani* f. sp. *phaseoli* (Macedo et al., 2017; Toledo-Souza et al., 2008).

In this study, we propose to answer following questions: i. Which species of FSSC are associated with BRR in Brazil? ii. Which species of FSSC cause BRR in Brazil? iii. *Fusarium phaseoli, F. crassistiptatum* and *F. azukicola* are present in Brazil and associated with common bean and cowpea? iv. Different species of FSSC coexist in the same field and in plants with symptoms of BRR? v. Can farming technology, such as irrigation or no-till systems, interfere with species of FSSC in fields? vi. Pathogenic species to common beans are also pathogens of cowpea and soybean? vii. Do any species that cause BRR induce typical SDS symptoms in beans and soybean?

Material and Methods

Sampling, Isolation and Preservation of isolates

Fungal isolates were obtained from plant material of common bean and cowpea (*Phaseolus vulgaris* and *Vigna unguiculata*) showing BRR symptoms in different growing conditions, such as irrigated and dry farming, no till and conventional farming. Plant samples were collected in different bean production areas from Brazilian states of Bahia, Ceará, Maranhão, Minas Gerais, Mato Grosso, Goiás, Pará, Paraná, Rio Grande do Norte, São Paulo and Distrito Federal (Table 1).

Whenever possible, direct isolations were made by transfer of mycelium or spores observed on the root surface. An indirect isolation method was used to obtain isolates from

symptomatic stems. Fragments of the stem were surface-sterilized with 70% ethanol for 30 s and 2% sodium hypochlorite for 1 min, washed in sterile water and dried on filter paper, and then plated on 2% malt extract agar (20 g of malt extract L^{-1}). From each sample, one isolate was selected as representative for different identified morphotypes. Cultures were incubated at 25 ºC for 4-7 days with a 12 hr light/ 12 hr dark cycle. Single-spore cultures were deposited in the Coleção Micológica de Lavras (CML), Departamento de Fitopatologia, Universidade Federal de Lavras, Minas Gerais, Brazil (http://www.dfp.ufla.br/cml/) (Table 1).

Identification of Morphotypes

A subset of 48 isolates was selected based on morphological markers of members of the FSSC, associated with leguminous disease such as SDS and BRR, and its origin. Colonies grown on synthetic low nutrient agar (SNA) with fragments of carnation leaf for 10 to 14 days at 20 °C in the dark were used for micromorphological characterization. The morphological characteristics such as mycelial growth, microconidia and macroconidia shape and size were analysed and compared with the literature (Aoki et al., 2003, 2005, 2012 a, b, 2013; Costa et al., 2016; Sandoval et al., 2019).

DNA Extraction and PCR

Isolates were grown in malt extract broth medium for three days at room temperature on a rotary shaker (150 rpm). Mycelia were harvested by filtration and total genomic DNA was extracted according to the CTAB protocol (O'Donnell 1992). DNA concentrations were determined using the NanoDrop 2000 apparatus and, visually, on a 1.2% agarose gel by comparing the band intensity with a 1 kb fragment size marker (Invitrogen).

For all selected isolates DNA sequences were generated for the RNA polymerase's second largest subunit (*rpb2*) gene, using primers 5F2 and 7cR (Liu et al., 1999; Sung et al., 2007). Later, a subset of isolates was selected to amplify the genes translation elongation factor 1α (*tef1*), RNA polymerase first largest subunit (*rpb1*), and Nuclear ribosomal rRNA gene

product ITS, using primers EF-1 and EF-2 (O'Donnell et al., 1998), Fa, G2r and R8 (O'Donnell et al., 2013), ITS 5 and NL4, respectively. PCR reactions were performed with the GoTaq® Colorless Master Mix kit (Promega) in a MyCycler Thermal Cycler Thermal Cycler (Bio-Rad, Hercules, CA). Cycling conditions for *rpb2* and *rpb1* following as described by Liu et al., (1999), for *tef1* were those of O'Donnell et al., (2008), and for ITS those of O'Donnell (2000). The amplified fragments were purified with the Wizard® SV Gel and PCR Clean-up System kit (Promega), and then sequenced using the same PCR primers. Bidirectional DNA sequences for each region were generated by Psomagen (Rockville, MD, USA).

Sequencing and phylogenetic analyses

Sequences were assembled using software SeqAssem ver. 07/2008 (Hepperle 2004). Alignments were obtained using ClustalW as implemented in the MEGAX software (Kumar et al., 2018). Sequences of type and reference material of species in the *Fusarium solani* species complex were obtained from GenBank and included in the alignments (Table 3). First, sequences of *rpb2* from fifty isolates were compared with sequences from reference material in GenBank (Table 2). Then, we compared the sequences of *tef1*, *rpb1* and ITS from isolates grouped with Clade 2 with sequences of reference material. Analyses were performed by using maximum parsimony (MP) method for each gene partition and for concatenated dataset in MEGAX software with 1000 bootstrap replications. *Fusarium iludens* and *F. plagianthi* sequences were used as outgroup.

Evaluation of morphological markers

The isolates were characterized morphologically according to Leslie & Summerell (2006) and the characteristics of each isolate were compared with that reported by Aoki et al (2003, 2005, 2012 a, b, 2013) Costa et al (2015). The morphological characteristics as micro- and macroconidia shape and size (30 measurements per isolate), arrangement of conidiogenous cells and presence or absence of false head and chlamydospores were examined after growing

the isolates on synthetic nutrient-poor agar with carnation leaf pieces for 10 to 15 days at 20 ºC. The colony growth radius and mycelium color (surface and reverse) were assessed in isolates grown for four and 14 days, respectively, at 25 ºC on potato dextrose agar (PDA, Merck, Darmstadt, Germany) in the dark.

Pathogenicity test

One isolate representing each identified species was selected for pathogenicity tests, and inoculated in the susceptible cultivars BRS Stylo and Pingo de Ouro of *P. vulgaris* and *V. unguiculata*, respectively. Pathogenicity of species was tested also on *Glycine max*, cultivar Desafio Brasmax, because this crop is often cultivated in succession with common beans and JMS120 and JMAS 169 were obtained in soybean-common bean succession fields.

Isolates were grown in sterilized sorghum grains, during 15 days at 25 ºC with 12h photoperiod. For the inoculation, 6g of grains were added to each pot with soil before being covered with 2 cm soil (Hartman et al., 1997). The negative control consisted of pots with uninfected grain. The test occured along 70 days with four destructive evaluations: vegetative stadium (~30dai), flowering (~45dai), grain filling (~65dai). The evaluation consisted in measurement of main root lesions.

Each plant was removed from the pot (taking care not to lose plant tissue), washed with water and taken to the laboratory where the lessons were the length of the external and internal injuries were measured using a ruler. These experiments were conducted in a greenhouse under a natural photoperiod at 25 – 32 ºC, under daily watering, and three fertilizations with Hoagland solution during plant development. The experiments were repeated twice. Treatments were arranged in a randomized block design.

Koch's postulates were completed by re-isolation of the pathogens from diseased plant tissue, which were then sequenced using the *rpb2* barcode region. Statistical analysis was performed to each test evaluations made, considering the length of the lesions on root, using

ANOVA as implemented in the R studio Software (Ferreira, 2000). Means were compared by the Scott–Knott test at 5% probability.

Results

Molecular phylogeny

Phylogenetic analysis based on the *rpb2* gene showed that 48 isolates represent four distinct species of FSSC Clade 3, and at least one species of FSSC Clade 2. Isolates grouped with the reference sequences of *F. paranaense* (n= 35), *F. suttonianum* (n= 3), *F. martii* (n= 2) and *F. solani* stricto sensu (n= 5) (Fig. 1). Two isolates, JMS120 and JMS169 grouped with reference material of *F. phaseoli*, *F. brasiliense*, *F. crassistptatum*, *F. cuneirostrum*, *F. azukicola* and *F. tucumaniae.* To understand which Clade 2 species these isolates represent we sequenced more three regions, ITS, *tef1* and *rpb1* genes, (Fig 3, Fig S1, Fig S2). But combined dataset (*rpb2*, ITS, *tef1*, and *rpb1*), had not resolution to group JMS120 e JMS169 with a specific Clade 2 species (Fig. 2), however, for the ITS region dataset the isolates JMS169 and JMS120 formed a clade with reference material of *F. brasiliense.*

The alignment of *rpb2* sequences consisted of 820 bp aligned nucleotide positions with 247 variable sites, of which 101 were phylogenetically informative. *Tef* sequences consisted of 708 bp with 195 variable sites, of which 102 were phylogenetically informative. For *rpb1* sequences were 1.625 bp with 507 variable sites, of which 141 were phylogenetically informative. The ITS sequence consisted of 512 bp with 132 variable sites, of which 83 were phylogenetically informative. The combined three sequences consisted of 2.030 bp with 467 variable sites, of which 266 were phylogenetically informative.

Morphological characterization

Morphological characteristics of the assexual stage from species were consistent with those described in the literature (Aoki et al., 2003, 2005, 2012 a, b, 2013; Costa et al., 2015). Species

of Clade 3 shared the same growth pattern, growing faster in comparison with *F. brasiliense* isolates, which shows a slower growth range (Table 1). All species identified formed macroconidia in the aerial mycelium. *Fusarium paranaense* isolates presented variation in colony color mainly white and white with center blue, less frequently color white with center brown, greeen, red, and few isolates presented colony color yellow-brown and brown. (Fig. 4A-F). The isolates showed rapid radial mycelial growth, approximately 2,5 cm after three days at 25 C in the dark on PDA. Microconidia produced in the aerial mycelium only on monophialides presented variable shape, as ellipsoidalis, droplet shape, cylindrical and fusiform, with 0–3-septate, measuring 5–30 × 2,5–5 µm. Macroconidia produced in the sporodochia were mostly 2-5 septate, with 25–57 × 5–10 µm. Macroconidia present a poorly developed foot cell and apical cell briefly rounded, in some isolates, microconidia were straight and cylindrical, and in some cases curved and spindle shaped (Fig. 4G-L).

Fusarium solani isolates present rapid radial mycelial growth of 2,3 cm after 3 d at 25 C and showed colony color from white and white with center varying in blue, green and brown (Fig. 5A-C). Microconidia produced in the aerial mycelium on monophialides showed cylindrical and fusiform shape with 0–3-septate, 3–10 × 1–3 µm. Macroconidia produced in the sporodochia were mostly 3-5 septate, 47–30 × 5–6,5 µm, with foot cell and apical cell symmetric and some are poorly curved (Fig. 5D-H).

Fusarium suttonianum isolates have a rapid mycelial growth, reaching 1,5 cm after 3 d at 25 C. The colony had beige color with brown or purple center. Microconidia produced in the aerial mycelium on monophialides showed cylindrical and fusiform shape, with 0–1-septate, 2,5–27 × 5–2,5 µm (Fig. 6A). Macroconidia produced in the sporodochia were long and wide, cylindrical (sometimes curved), with evident apical and foot cells, mostly 2-5 septate, 47–30 × 5–6,5 µm (Fig. 6B-E).

Fusarium martii isolates have mycelial growth 2,5 cm after 3 d at 25 C. Colonies are white with a blue center. Microconidia produced in the aerial mycelium on monophialides were cylindrical and fusiform with 0–1 septate, 7,5–30 × 5–2,5 µm (Fig. 7A). Macroconidia produced in the sporodochia was long and wide, cylindrical (sometimes curved), with evident apical cells and foot cells, mostly 3-5 septate, 40–57 × 5 µm (Fig. 7C-F).

The isolates JMS120 and JMS169 grows slower than species of clade 3, with mycelial growth of 1 cm after 3 d at 25 C in the dark on PDA (Fig. 8A-B). The colony had a color blue and blue violet. Microconidia with 0–1septate, 7,5–30 × 5–2,5 µm produced in false heads arising from monophialides formed on simple or branched conidiophores. Macroconidia produced in sporodochia presented foot and apical cells symmetrical and rounded with 3-6 septate, 40–57 × 5µm (Fig. 8C-G).

Pathogenicity test

The isolates of *F. paranaense, F. solani, F. suttonianum, F. martii,* and Clade 2 tested induced BRR symptoms in *P. vulgaris* and *V. unguiculata* cultivars (Fig. 9K-V). The symptoms start on vegetative stage (before V4 stage, approximately 25 dag) as a small lesion on the main root which size progresses and extends to the hypocotyl. In the flowering stage, leaves present yellowing and occur in reduced growth. At maturation, there is reduction of pods, grains, and plant death.

On common beans, *F. solani* and Clade 2 isolates tested induced root rot in the vegetative stage different from the other species that only induce symptoms at the flowering stage. *Fusarium paranaense*, *F. solani* and *F. martii* induced miscarriage of flowers of *P. vulgaris* (Fig. 9A-B). In the common bean, mycelium of *F. solani* were observed in the flowers affected area before flowering. *F. suttonianum* induced rot in root and stem (Fig. 9D). On *V. unguiculata* Clade 2 and *F. solani* induce miscarriage of flowers. But, in the repetition of

pathogenicity test any symptoms, such as flowering aborts and mycelium of *F. solani*, were observed on common and cowpea plants inoculated with our Clade 2 isolates.

Significant interaction ($p<0.05$) between factor severity was verified. The severity test grouped the isolates in two groups, letter a highest several and letter b fewer several considering the internal lesion size. Our Clade 2 isolates isolates were the most severe between the species, followed by *F. suttonianum*, *F. solani* in second and third position, respectively (Table 2).

On cowpea, *F. paranaense* and *F. solani* start to induce the disease during vegetative stage (approximately 25 days after germination) and the other species induce symptoms from the flowering (approximately 30 days after germination). Our Clade 2 isolates induced miscarriage of flowers (Fig. 9C). Mycelium was observed in the affected area. The foliar symptoms of yellowing were less severe when compared with common beans. Significant interaction ($p<0.05$) between factor severity was verified. The severity test grouped the isolates in two groups, letter a highest several and letter b fewer several considering the internal lesion size, as observed in common bean Clade 2 isolates were the most severe between the species (Table 3).

On soybean cultivar Desafio Brasmax, all species induced symptoms of root rot (Fig. 9I-J). *Fusarium solani* induced symptoms since the vegetative plant growth and the other species only induced the symptoms during the flowering stage. *Fusarium martii* and JMS120 and JMS169 induce interveinal chlorosis (Fig. 9F-H). To complete Koch's postulates, the morphological characteristics of each tested isolated were compared with preserved material and demonstrated that the same species inoculated in the pathogenicity experiments were re-isolated from diseased roots showing root rot.

Discussion

In this exploratory study we identified *F. paranaense, F. solani, F. suttonianum, F. martii* and Clade 2 species of FSSC as the causal agents of Bean root rot in Brazil. For the first time *F. martii*, *F. paranaense* and *F. suttonianum* were reported as BRR agents on cowpea and common beans, and Clade 2 were reported associated with beans in Brazil fields. The wide distribution and frequency of *F. paranaense* in different environments and fields support its importance as the main causal agent of BRR in Brazil.

In this study, most of the species identified as the causal agents of BRR belong to FSSC Clade 3, which comprises pathogens of important food crops throughout the world (Geiser et al., 2020, Chitrampalam & Nelson 2016, Melo et al., 2016). For example, *F. paranaense,* and *F. solani* have already been reported as pathogens to different plant hosts, such as soybean and *Crotalaria*, and *F. martii* as a pathogen to potato (Costa et al., 2016, Melo et al., 2016, Sandoval et al., 2019). However, in this study, *F. martii*, *F. paranaense* and *F. solani* species were, for the first time, reported as BRR agents on cowpea and common beans.

Around the world, until now, only *F. phaseoli, F. crassistiptatum* and *F. azukicola* had been reported as causal agents of bean root rot, respectively, in the USA, Canada, and Japan (Aoki et al., 2005, 2012b). However, *F. paranaense, F. solani*, *F. suttonianum, F. martii* and Clade 2 species were confirmed as etiological agents of BRR in Brazil based on phylogenetic analysis of *rpb2* sequences (Fig. 1) and pathogenicity test (Table 3).

Furthermore, studies conducted by our team and collaborating researchers have identified *F. suttonianum* as a causal agent of rot in cucurbits and in common beans (Cardoso 2019, personal communication Pfenning and Lobo Junior). This species has a clinical importance worldwide as an opportunistic pathogen of humans and other vertebrates (O'Donnell et al., 2008, Zhang et al., 2006) and now it has been found to affect cowpea, too.

The phylogenetic analysis with concatenate sequences of *rpb2*, *rpb1*, *tef1* and ITS genes was unable to determine which Clade 2 species represent field isolates JMS120 and JMS169 (Fig. 2). Even though both isolates are grouped with *F. brasiliense* on the ITS phylogenetic tree, there is still not enough argument to defend that these isolates represent *F. brasiliense* (Fig.3). Hence, another phylogenetic analysis with other gene regions, such as LSU and IGS (Geiser et al., 2020, Aoki et al., 2005) as well as the application of the biological concept to separate Clade 2 species and identify wich species represent JMS120 and JMS169 isolates, is necessary.

Clade 3 morphology does not provide a precise morphological marker to be used as criteria for species differentiation. However, when we compare Clade 3 and Clade 2 species, the morphology of macroconidia and the colony growth rate can be used as markers to distinguish these groups (Table 1). Isolates JMS120 and JMS169 macroconidia are long and thin (Fig. 8), and the colony growth rate is lower than Clade 3 species. Besides, the color of the Clade 3 colonies, such as *F. paranaense*, varies between blue, green, red, and brown as opposed to Clade 2 colonies, such as *F. tucumaniae*, which varies only within the blue range (Costa et al., 2016, Aoki et al., 2011) (Fig. 4, 5, 6, 7, 8).

Clade 3 present a high morphological plasticity, a wide color variation, and different macroconidia shapes and sizes (Fig. 4, 5, 6, 7). Nevertheless, the species have a fast growth rate, and, even though their macroconidia varies in length, they are always wide macroconidia. (Costa et al., 2016, Sandoval et al., 2019).

All FSSC species in this study produced micro and macroconidia in aerial mycelia, sporodochia, and chlamydospores. Most *F. paranaense* and *F. solani* species had similarities in shape and structure, presenting macroconidia in different lengths (usually short), but similar widths (Figure 4, 5), therefore, it is not possible to distinguish *F. paranaense* from *F. solani* isolates by morphology.

On the other hand, some distinguishing characteristics that can be used as morphological markers were found. *F. martii* presented macroconidia with symmetric, rostrate (pointy) apical and food cells (Fig.6); *F. suttonianum* had a long and wide macroconidia, which confirms the results found by Sandoval et al. (2019) (Fig. 7).

Our Clade 2 isolates were obtained from common beans and cowpea fields planted mainly in succession with soybean, common bean and cowpea cultivated under different environmental and management conditions. In Brazil, common bean and cowpea are cultivated throughout three seasons during a year and in production areas throughout the five regions of the country, which present differences in climate and soil (Da Silva et al., 2019, Rampelotto et al., 2013, Carmello et al., 2016). Management practices change depending on the region, technical support, and culture, regarding the application of a no till, dry cultivation, and irrigation systems.

Besides succession with soybean and pulses, our plant samples were obtained from fields previously cultivated with different crops, such as corn, cassava, banana, rice, sweet potato, black oats, and wheat (Table 1). The diversity of host plants on succession fields where common bean and cowpea are cultivated may explain the number of Clade 3 species observed in this study, considering that these species cause disease in several crops. For example, *F. solani* is a species reported as pathogen to different plants and has now been proved as a pathogen to different bean species in Brazil (Schroers et al., 2016, O'Donnell et al., 2008, Sandoval et al., 2019).

Fusarium paranaense is widely distributed in Brazil, found in the northeastern, midwestern, southeastern and southern regions (Table 1). This species was reported before in the midwest, southeast and south, but, as causing agents for red rot of soybean, and associated with wild beans (Costa et al., 2016; Sousa et al., 2017). Despite *F. paranaense* being considered the major root pathogen of soybean in Brazil, because of its wide distribution in the area, so far

it had not been reported in Brazil's agricultural frontier region, which includes Maranhão, Piauí, Tocantins and Bahia states (MATOPIBA). In this study, we report the presence of *F. paranaense* not only in MATOPIBA causing BRR on cowpea distributed in areas previously cultivated with beans, maize and soybean (Arvor et al., 2017), but also in Ceará and Rio Grande do Norte states (Table 1).

Fusarium solani, F. martii, F. suttonianum, and Clade 2 were found in this study at quite a low frequency. *Fusarium suttonianum,* known as an important opportunistic pathogen of humans and other vertebrates (O'Donnell et al., 2008, Zhang et al., 2006) and identified in association with cucurbits (Cardoso 2019), was the only species obtained from plant samples from the northern region. Our *F. suttonianum* field isolates came from cowpea fields cultivated before with cassava, under dry cultivation and multiple cropping. However, it is not possible to determine the distribution and diversity of FSSC species in northern regions with only a few samples, but now it is known that *F. suttonianum* causes BRR to cowpea crops in that region.

The species *F. solani*, *F. martii*, and Clade 2 were present only in Minas Gerais and Mato Grosso states (southeastern and midwestern regions of the country). All species were obtained from common bean plants in a succession system with soybean. Soybean crops are commonly affected by SDS, a disease caused by FSSC2 species (Aoki et al., 2003, 2005, 2011, Spampinato et al., 2021). The presence of BRR pathogen in soybean and common bean succession fields confirm the hypothesis that these crops are hosts to the same FSSC species and that succession systems promote the survival and multiplication of BRR, SDS and red rot agents between crops.

Fusarium paranaense and Clade 2 were obtained from the same plant found in Bonfinópolis de Minas – Minas Gerais. These species produce, respectively, beige and blue sporodochia on plant tissues (Costa et al., 2016, Aoki et al., 2003, 2005). The sporodochium color, and the macroconidia shape and size are morphological markers used to identify Clade 2

and Clade 3 morphotypes in the same plant. The pathogenicity test verified that *F. paranaense* and Clade 2 are pathogenic to *P. vulgaris*, inducing root rot. It suggests that *F. paranaense* and Clade 2 can co-infect common beans. The co-infection was reported for FSSC isolates associated with dry rot of potato tubers in Poland, for *Fusarium* associated with bacteria on *Triticum,* and for *Fusarium* associated with *Acanthamoeba* on human keratitis infections (Stefańczyk et al., 2016; Dandve et al., 2019; Lin et al., 2009).

The presence of *F. paranaense, F. solani*, *F. suttonianum,* and *F. martii* in fields conducted under no-till and conventional systems, as well as under dry cultivation and irrigated fields support the idea that the management system did not influence or had little influence on the species composition of FSSC on fields, at least the composition of Clade 3. Some studies suggest that management practices affect pathogen populations (Abawi 2000). Also, the lowest and most specific distribution of Clade 2 in fields under no-till systems and under irrigated systems can suggest an interference of field conditions with the kind of Clade 2 clade species in Brazil. New and wider samplings in these areas are necessary to test this hypothesis.

Fusarium paranaense, F. solani, *F. suttonianum, F. martii* and Clade 2 are pathogenic to common bean and cowpea inducing lesions on the main root, yellowing leaves, and reducing growth, which are described as symptoms of BRR (Schwartz et al., 2005; Chaudhary et al., 2006; Bilgi et al., 2008).

Symptoms in flowers, such as abortion and rotting of flowers in common bean and cowpea during the first pathogeniticity test (Fig. 4P, 5L, and 8K) can be a result of temperature conditions. During this essay, the temperature varied between 28 and 31 ºC, and this temperature has already been reported as the cause of abortions in bean flowers before (Portugal et al., 2015). This hypothesis is supported by results on pathogenicity repetitions, which showed a variation of temperature between 26 and 28 ºC, and caused no abortions to the flowers.

F. solani mycelium could have been transported by irrigation water and developed on damaged flowers (Fig. 5L). A presence of mycelium was reported for *F. tupiense,* a member of *Fusarium fujikuroi* species complex, that causes mango malformation (Lima et al., 2012), but in BRR pathosystem, the presence of mycelium and abortion of flowers are not symptoms of the BRR, and the plant infection occurs only on root tissues.

Nevertheless, *F. suttonianum* induced rot in *P. vulgaris* root and stems. This symptom starts on the main root and stretches out along the stem (Fig. 6E). Other FSSC, such as *F. piperis,* induce symptoms on stems, producing perithecium on Black pepper stems (Rocha et al., 2016, Costa et al., 2017).

Regarding the stage of plant in which BRR symptoms start, in our results, the disease can begin in different stages of the plant cycle, but it is during flowering that the symptoms are the most evident, as field technicians and studies about the disease usually report (Schwartz et al., 2005; Toledo-Souza et al., 2008).

Furthermore, the representatives isolated from each species identified in this study presented a difference in virulence level induced on common beans and cowpea (Table 3). Clade 2 isolates were the most virulent pathogens to common bean and cowpea, causing extended lesions and death to the plants. Clade 2 species, such as *F. tucumaniae, F. brasiliense, F. crassistiptatum* and *F. virguliforme* (not reported in Brazil) cause SDS to soybean, and, because our Clade 2 isolates were obtained from areas cultivated in common bean - soybean succession systems, we may conclude that CLADE 2 isolates cause SDS and BRR in Brazil.

Therefore, soybean cultivar Desafio Brasmax, widely cultivated in Brazil and susceptible to SDS, and infected with JMS120 and JMS169, *F. paranaense, F. solani, F. suttonianum,* and *F. martii* presented root rot symptoms (Table 3). Furthermore, Clade 2 and *F. martii* induced leaf symptoms, such as internerval chlorosis (Fig 9 F-J). The internerval chlorosis induced by Clade 2 is the main symptom of SDS of soybean, and is characterized by

a necrosis in the middle of chlorosis (Aoki et al., 2003, 2005, 2012a, b). Besides, *F. azukicola* induces chlorosis and necrosis, and leads to the loss of leaves on soybean, which is also a symptom of the disease induced by *F. tucumaniae, F. crassistiptatum, F. brasiliense,* and *F. virguliforme* (Aoki et al., 2003, 2005, 2012a, b).

However, internerval chlorosis induced by *F. martii* was not the same as the one induced by JMS120 and JMS169, since it only discolored the board of leaves to an orange color. Moreover, the leaf symptom induced by *F. martii* on soybean suggests a different interaction between an Clade 3 species and this plant, which results in leaf symptoms and poses questions about the differences in mechanisms of *F. martii* compared to other Clade 3 species that cause these symptoms. Studies with *F. virguliforme* explained that this leaf symptom is caused by phytotoxin action (Brar et al., 2011). Until now, *F. martii* has been reported as a pathogen to *Solanum tuberosum, Citrus sinensis,* and *Pisum sativum* in Germany, Italy, and the Netherlands, respectively (Sandoval et al., 2019).

All BRR pathogens identified in this study induced root rot on soybean, which confirms the hypothesis that the density of the pathogens, as well as the occurrence of outbreaks and losses caused by SDS and BRR diseases, increases if soybean-common bean succession systems still occur.

Knowing which species FSSC causes BRR is a step to promote sustainable and rational management of beans. Considering that in different climatic conditions different species prevail, and a BRR epidemic depends on the environmental condition, susceptible cultivar and quantity of inoculum to know the causal agents promote support to the right choice about cultivar, fungicide and biocontrol agent.

Moreover, our essays prove that common bean, cowpea and soybean have the same FSSC root rot agents, thus this works provide support to the recommendation of not cultivating those crops in succession systems.

Conclusion

Fusarium paranaense, F. solani, F. suttonianum, F. martii, and Clade 2 cause BRR in *P. vulgaris* and *V. unguiculata*. *F. paranaense, F. solani*, and *F. martii* coexist in the same field. *Fusarium paranaense* and Clade 2 coexist in the same plant. *Fusarium paranaense, F. solani*, *F. suttonianum* and *F. martii* exist in fields under different management (no till and conventional system), irrigation and dry conditions. Our Clade 2 field isolates induced root rot symptoms in both common bean and cowpeas, besides inducing SDS symptoms on soybean. In this study, we prove this species as a nonspecific plant pathogen and causal agent of bean root rot in *V. unguiculata* and *P. vulgaris* and as inducers of rot symptoms on soybean. Clade 2 and *F. martii* induce lesions on roots and leaf chlorosis in soybean.

Perspectives

Crossings are necessary to support the identification of CLADE 2 proposed in this study. To do so, it is necessary to define CLADE 2 tester strains. Further studies are necessary to clarify how *F. martii* induce internerval chlorosis in soybean. Considering the nonspecificity of this species in pulses, the pathogenicity test with other leguminous, produced in the same field as these plants as a cover crops or rotation, can clarify the FSSC pathosystem on those plants in Brazil and promote essential knowledge to improve the management practices in these food crops.

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Figure Captions and Footnotes

Figure 1 Maximum parsimony phylogenetic (MP) tree inferred from *rpb2* gene fragments showing the relatedness of *Fusarium* associated with bean root rot with other species within *Fusarium solani* species complex. The bootstrap values (1000 replicates) from MP ≥ 70% are shown at the internodes. Codes JMS indicate isolates from Brazil. Ex-type and ex-epitype strains are indicated with T and ET, respectively. Strain collection acronyms: CBS: Westerdijk Fungal Biodiversity Institute, Utrecht, Netherlands; CML: Colecão Micológica de Lavras, Universidade Federal de Lavras, Lavras, Minas Gerais, Brazil; NRRL: Northern Regional Research Laboratory, Peoria, Illinois, USA.

Figure 2 Maximum parsimony phylogenetic (MP) tree inferred from combined dataset (*rpb2*, ITS, *tef1*, and *rpb1*), showing the relatedness of *Fusarium* associated with bean root rot with other species within *Fusarium solani* species complex. The bootstrap values (1000 replicates) from MP ≥ 70% are shown at the internodes. Codes JMS indicate isolates from Brazil. Ex-type and ex-epitype strains are indicated with T and ET, respectively. Strain collection acronyms: CBS: Westerdijk Fungal Biodiversity Institute, Utrecht, Netherlands; CML: Colecão Micológica de Lavras, Universidade Federal de Lavras, Lavras, Minas Gerais, Brazil; NRRL: Northern Regional Research Laboratory, Peoria, Illinois, USA.

Figure 3 Maximum parsimony phylogenetic (MP) tree inferred from ITS gene fragments showing the relatedness of *Fusarium* associated with bean root rot with other species within *Fusarium solani* species complex. The bootstrap values (1000 replicates) from MP ≥ 70% are shown at the internodes. Codes JMS indicate isolates from Brazil. Ex-type and ex-epitype strains are indicated with T and ET, respectively. Strain collection acronyms: CBS: Westerdijk Fungal Biodiversity Institute, Utrecht, Netherlands; CML: Colecão Micológica de Lavras,

Universidade Federal de Lavras, Lavras, Minas Gerais, Brazil; NRRL: Northern Regional Research Laboratory, Peoria, Illinois, USA.

Figure 4 Morphological characteristics of *Fusarium paranaense.* A - F. Colony white with center blue, less frequently color white with center brown, greeen, red and white, a few isolates presente colony color brown and yellow brown (isolates jms 112, 65, 173, 116, 118, 30, respectivelly). G. Aerial mycelium. H. Monophialide. I. Macroconidia of sporodochia. J. Mesoconidia. K. Microconidia. L. Chlamydospore. M. Sporodochia formed on carnation leaves on SNA. N. Perithecia. O. Lesions in *P. vulgari*s. P. Necrosis in *P. vulgaris* flowers. Q. Lesions in *V. unguiculata*.

Figure 5 Morphological characteristics of *Fusarium solani.* A - C. white and white with center varying in blue, green and brown. D. Macroconidia in aerea mycelium. E. Monophialide. F. Macroconidia of sporodochia. G. Microconidia. H. Chlamydospore. I. Sporodochia. J. Lesions in *P. vulgari*s. K. Lesions in *V. unguiculata.* L. Necrosis and mycelium in *P. vulgaris* flowers.

Figure 6 Morphological characteristics of *Fusarium suttonianum.* A. colony had a color beige with brown or purple center. B. Macroconidia in aerea mycelium. C. Chlamydospore. D. Macroconidia of sporodochia. E. Microconidia. F. Lesions in *P. vulgari*s. G. Lesions in *V. unguiculata.*

Figure 7. Morphological characteristics of *Fusarium martii.* A. color white with a blue center. B. Perithecia. C. Macroconidia in aerea mycelium. D. Monophialide. E. Macroconidia of sporodochia. F. Microconidia. G. Lesions in *P. vulgari*s. H - I. Lesions in *V. unguiculata*. J. Lesions in soybean root.

Figure 8 Morphological characteristics of Clade 2 isolates. A - B. color white with a blue center. C. Macroconidia in aerea mycelium. D. Chlamydospore. E. Macroconidia of sporodochia. F.

Microconidia. G. Monophialide. H. Sporodochia. I. Lesions in *P. vulgari*s. J. Dead plant of *P. vulgari*s. K. Necrosis n *V. unguiculata*. L. Lesions in root of *V. unguiculata.* N. Lesions in root of *G. max*. N. Internerval chlorosis in *G. max* leaves induced by Clade 2 isolates.

Figure 9 Pathogenicity results. Root in flower: A. *F. paranaense*. B. *F. solani*. C. Clade 2 isolates. D. Dead plant by effecct of Clade 2 isolates. E. root on stem induced by *F. suttonianum*. F. Internerval chlorosis induced by Clade 2 isolates on soybean. G. Initial internerval chlorosis induced by *F. martii* on soybean. H. Internerval chlorosis induced by *F. martii* on soybean. I-J. Rot root caused *F. martii* and Clade 2 isolates. in soybean, respectively. Root of *P. vulgaris*: K. Control. L. *F. paranaense*. M. *F. solani*. N. *F. suttonianum*. O. *F. martii*. P. Clade 2 isolates. Root of *V. unguiculata*: Q. Control. R. *F. paranaense*. S. *F. solani*. T. *F. suttonianum*. U. *F. martii*. V. Clade 2 isolates.

Table 1 FSSC species obtained in this study.

Table 2 List of reference sequence used to molecular phylogeny.

Table 3 Pathogenicity test results

Tables Footnotes

Table 1 Codes JMS indicate isolates from Brazil.

Table 2 *Mean root disease severity ratings of 9 plants for each isolate and two beans cultivars were based on lesion dimensions. Averages followed by the same letter do not differ according to the test at 5% of probability.

Table 3 Codes JMS indicate isolates from Brazil. Ex-type and ex-epitype strains are indicated with T and ET, respectively. Strain collection acronyms: CBS: Westerdijk Fungal Biodiversity Institute, Utrecht, Netherlands; CML: Colecão Micológica de Lavras, Universidade Federal de

Lavras, Lavras, Minas Gerais, Brazil; NRRL: Northern Regional Research Laboratory, Peoria, Illinois, USA; CBS = Westerdijk Fungal Biodiversity Center, Utrecht, The Netherlands.

Legends and footnotes for supplementary material

Figure S1 Maximum parsimony phylogenetic (MP) tree inferred from *tef1* gene fragments showing the relatedness of *Fusarium* associated with bean root rot with other species within *Fusarium solani* species complex. The bootstrap values (1000 replicates) from MP ≥ 70% are shown at the internodes. Codes JMS indicate isolates from Brazil. Ex-type and ex-epitype strains are indicated with T and ET, respectively. Strain collection acronyms: CBS: Westerdijk Fungal Biodiversity Institute, Utrecht, Netherlands; CML: Colecão Micológica de Lavras, Universidade Federal de Lavras, Lavras, Minas Gerais, Brazil; NRRL: Northern Regional Research Laboratory, Peoria, Illinois, USA.

Figure S2 Maximum parsimony phylogenetic (MP) tree inferred from *rpb1* gene fragments showing the relatedness of *Fusarium* associated with bean root rot with other species within *Fusarium solani* species complex. The bootstrap values (1000 replicates) from MP ≥ 70% are shown at the internodes. Codes JMS indicate isolates from Brazil. Ex-type and ex-epitype strains are indicated with T and ET, respectively. Strain collection acronyms: CBS: Westerdijk Fungal Biodiversity Institute, Utrecht, Netherlands; CML: Colecão Micológica de Lavras, Universidade Federal de Lavras, Lavras, Minas Gerais, Brazil; NRRL: Northern Regional Research Laboratory, Peoria, Illinois, USA.

Table S1 Morphotypes morphological makers based on Aoki et al. 2003, 2005, 2011, 2012; Costa et al. 2016.

Figures and tables

Table 1 FSSC species obtained in this study.

Especies	Code	Substrate	Origin	Field history	Cultivation system	Irrigation syste
F. paranaense	JMS 07	*V. unguiculata*	Ibiapina CE	sweet potato, caupi	Conventional tillage	Dryland
	JMS 12	*P. vulgaris*	Ponta grossa MG	oat, commun bean	No-till	Dryland
	JMS 16	*P. vulgaris*	Itutinga MG	soy bean, commun bean (harvest), maize, wheat (off season)	No-till	Irrigated
	JMS 23	*V. unguiculata*	Maracanaú CE	maize	Conventional tillage	Irrigated
	JMS 26	*V. unguiculata*	Maracanaú CE	maize, soy bean	Conventional tillage	Irrigated
	JMS 30	*P. vulgaris*	Ibituruna MG	soy bean	No-till	Dryland
	JMS 32	*P. vulgaris*	Ibituruna MG	soy bean	No-till	Dryland
	JMS 36	*P. vulgaris*	Ponta grossa MG	oat, commun bean	No-till	Dryland
	JMS 39	*P. vulgaris*	Ponta grossa MG	oat, commun bean	No-till	Dryland
	JMS 44	*P. vulgaris*	Taquarivaí SP			Irrigated
	JMS 54	*P. vulgaris*	Nazareno MG	soy bean, wheat, commun bean	No-till	Dryland
	JMS 64	*P. vulgaris*	Arapodi PR	maize, soy bean, black oat, wheat, commun bean		
	JMS 65	*P. vulgaris*	Arapodi PR	maize, soy bean, black oat, wheat, commun bean		
	JMS 69	*P. vulgaris*	Itutinga MG	soy bean, commun bean (harvest), milho, trigo (off season)	No-till	Irrigated
	JMS 70	*P. vulgaris*	Arapodi PR	maize, soy bean, black oat, wheat, commun bean		
	JMS 82	*P. vulgaris*	Arapodi PR	maize, soy bean, black oat, wheat, commun bean		
	JMS 94	*P. vulgaris*	Lavras MG	soy bean	Conventional tillage	Dryland
	JMS 100	*P. vulgaris*	Tangará da Serra MT	maize, commun bean	Conventional tillage	Dryland
	JMS 107	*P. vulgaris*	Tangará da Serra MT	maize, commun bean	Conventional tillage	Dryland

	JMS 112	*P. vulgaris*	Tangará da Serra MT	maize, comum bean	Conventional tillage	Dryland
	JMS 113	*P. vulgaris*	Tangará da Serra MT	maize, commun bean	Conventional tillage	Dryland
	JMS 116	*P. vulgaris*	Rio Verde GO	commun bean, maize, soy bean	No-till	Dryland
	JMS 118	*V. unguiculata*	Balças MA			
	JMS 121	*P. vulgaris*	Tangará da Serra MT	maize, commun bean	Conventional tillage	Dryland
	JMS 134	*V. unguiculata*	Correntina BA	maize, caupi, caupi, banana, rice	Policultive	Flooded crop
	JMS 135	*unguiculata*	Correntina BA	maize, caupi, cassava, banana, rice	Policultive	Flooded crop
	JMS 140	*P. vulgaris*	Tangará da Serra MT	maize, commun bean	Conventional tillage	Dryland
	JMS 143	*P. vulgaris*	Bonfinópolis de Minas MG			Irrigated
	JMS 144	*P. vulgaris*	Rio Verde GO	commun bean, maize, soy bean	No-till	Irrigated
	JMS 145	*P. vulgaris*	Rio Verde GO	commun bean, maize, soy bean	No-till	Irrigated
	JMS 148	*V. unguiculata*	Barauna RN	caupi, cucurbits	Conventional tillage	Dryland
	JMS 149	*V. unguiculata*	Iporangaçu RN	caupi, maize	Conventional tillage	Dryland
	JMS 150	*unguiculata*	Iporangaçu RN	caupi, maize	Conventional tillage	Dryland
	JMS 168	*P. vulgaris*	Lucas do Rio Verde MT	soy bean	No-till	Irrigated
	JMS 173	*P. vulgaris*	Planaltina DF			
F. solani	JMS 02	*P. vulgaris*	Nazareno MG	soy bean, wheat, comum bean	No-till	Irrigated
	JMS 46	*P. vulgaris*	Itapeva SP			Irrigated
	JMS 51	*P. vulgaris*	Arapodi PR	maize, soy bean, black oat, wheat, commun bean		
	JMS 59	*P. vulgaris*	Itapeva SP			Irrigated
	JMS 79	*P. vulgaris*	Itapeva SP			Irrigated
	JMS 130	*P. vulgaris*	Cristalina GO	maize, sorghum, commun bean e soy bean	No-till	Irrigated

F. suttonianum	JMS 160	*V. unguiculata*	Castanhal PA	cassava	Conventional tillage	Dryland
	JMS 162	*V. unguiculata*	Castanhal PA	Cassava	Conventional tillage	Dryland
	JMS 174	*V. unguiculata*	Castanhal PA	Cassava	Conventional tillage	Dryland
F. martii	JMS 55	*P. vulgaris*	Nazareno MG	soy bean, wheat, commun bean	No-till	Dryland
	JMS 61	*P. vulgaris*	Nazareno MG	soy bean, wheat, commun bean	No-till	Dryland
Clade 2	JMS 120	*P. vulgaris*	Bonfinópolis de Minas MG			Irrigated
	JMS 169	*P. vulgaris*	Lucas do Rio Verde MT	soy bean	No-till	Irrigated

Codes JMS indicate isolates from Brazil.

Table 2 List of reference sequence used to molecular phylogeny.

		Genbank accesion			
Species	Code	*rpb2*	*tef*	*rpb1*	ITS
Fusarium acutisporum	CBS 145461 T	LR583814	-	-	-
F. ambrosium	NRRL 22346 T	EU329503	FJ240350.1	-	EU329669
F. azukicola	NRRL 54364	KJ511287	JQ670137	KJ511276	MAEG01000005
	NRRL 54366	KJ511288	JQ670139.1	KJ511277	-
F. bataticola	NRRL 22402 T	FJ240381	-	-	-
F. borneense	NRRL 22579	EU329515	AF178352.1	-	AF178415.1
F. bostrycoides	CBS 144.25 NT	LR583818	-	-	-
F. brasiliense	NRRL 22743	-	AY320145	-	-
	NRRL 22678	-	JQ670133.1	-	-
	NRRL 43350	KJ511285.1	-	KJ511274	-
	NRRL 31757 t	EU329565	AY320148.1	-	EF408514.1
	NRRL 31779	KJ511283	AY320150.1	KJ511272.1	-
F. breve	CBS 144387 T	LR583822	-	-	-
F. breviconum	CBS 204.31 ET	LR583821	-	-	-
F. catenatum	NRRL 54993	KC808355	KC808213.1	-	-
	NRRL 54992	KC808354	-		KC808255.1
F. crassum	CBS 144386 T	LR583823	LR583604	-	LR583709
	Nrrl 36877	-	-		FJ919554.1
F. crassistiptatum	NRRL 31949 T	EU329566	AY320161.1	-	-
	NRRL 31157	FJ240389.1	AY320160.1	KJ511271.1	-
	NRRL 22275	-	AY320158.1	-	-
	NRRL 31104	AY320159.1	-	-	-
F. cuneirostrum	NRRL 31157	KJ511282	-	-	MG967563.1
F. cryptoseptatum	NRRL 22412 T	EU329510	-	-	-
F. cucurbiticola	NRRL 22399	LR583825	-	-	-
F. cyanescens	CBS 518.82 T	LR583826	-	-	-
F. elegans	NRRL 2227	FJ240380	-	-	-
F. euwallaceae	NRRL 54722	JQ038028	JQ038007	-	JQ038014.1
F. falciforme	CBS 475.67 T	LT960558	LT906669	-	MG189935
	NRRL 43529	-	-	JX171541.1	-
	NRRL 32872	-	-	KC808313.1	-
	NRRL 62543	-	-	KC808296.	-
	NRRL 54987	-	-	KC808286.1	-

F. ferrugineum	NRRL 32437	EU329581	DQ246979.1	-	DQ094446.1
F. waltergamsii	NRRL 32323	EU329576	-	-	-
F. haematococcum	CBS 119600 ET	LT960561	-	-	-
F. hypothenemi	NRRL 52782	JF741176	-	-	-
F. illudens	NRRL 22090	JX171601	AF178326.1	-	AF178393.1
F. ipomoeae	NRRL 22657	LR583831	-	-	-
F. kelerajum	CBS 125722 PT	LR583835	-	-	-
F. keratoplasticum	FRC S-2477	JN235897	-	-	-
	CBS 49063 T	LT960562	LT906670	-	LR583721
F. kuroshium	CBS 142642 T	LR583837	KX262216	-	LR583723
F. kurunegalense	CBS 119599 T	LR583838	-	-	-
F. liriodendri	NRRL 22389	EU329506	-	-	-
F. macrospora	CBS 142424 T	LT746331	-	-	-
F. mahasenii	CBS 119594 T	LT960563	-	-	-
F. martii	CBS 115659 ET	JX435256	JX435156	-	JX435206.1
F. metavorans	CBS 135789 T	LR583849	-	-	-
F. mori	NRRL 22230	EU329499	AF178358.1	-	DQ094305.1
F. neocosmosporiellum	CBS 23755	LR583892	LR583663.1		LR583785
	CBS 562.70	-	-	-	-
F. nirenbergiae	NRRL 22387	EU329505	AF178339.	-	AF178403.1
F. noneumartii	CBS 115658 T	LR583852	LR583630	-	LR583745
F. oblongum	CBS 130325 T	LR583853	-	-	-
F. oligoseptatum	CBS 143241 T	LR583854	-	-	KC691566
F. paraeumartii	CBS 487.76 T	LR583855	-	-	LR583747
F. paranaense	CML 1830 T	-	-	-	-
	CML 1993	-	-	-	-
	CML 860	-	-	-	-
	CML 1995	-	-	-	-
F. parceramosum	CBS 115695	JX435249	-	-	-
F. perseae	CBS 144142	LT991909	-	-	-
F. petroliphilum	NRRL 22141	EU329491	-	-	-
F. phaseoli	NRRL 31096	GU170600	-	-	-
	NRRL 31041	JX171643	-	-	-
	NRRL 22411	KJ511278.1	FJ919464.1	KJ511267	-
	NRRL 22277	FJ240380.1	-	-	-

	NRRL 22276 T	JX171608.1	AY220186.1	JX171495	-
	CBS 190.35	-	HE647965.1	-	MH855640
	MIMtc-B4	-	MH541910	-	-
	NRRL 22411	-	-	-	-
	CBS 265.50	KM232375.1	HE647964.1	KM232226	LR583750
	NRRL 31156	FJ240388	AY220187.1	-	-
	CBS 835.85		HE647963.1	-	-
F. piperis	NRRL 22570	EU329513	AF178360.1	-	AF178422.1
F. pisi	CBS 123669 ET	LR583862	-	-	-
F. plagianthi	NRRL 22632	JX171614	-	-	-
F. protoensiforme	NRRL 22178	EU329498	-	-	-
F. pseudensiforme	NRRL 46517	KC691645	-	-	-
F. pseudoradicicola	CBS 145472 T	JF741084	-	-	-
F. pseudensiforme	CBS 143038	LR583867	-	-	-
F. rectiphorum	CBS 125727 T	LR583871	-	-	-
F. riograndense	CM F12570 T	KX534003	KX534002.1	-	KT186366
F. robustum	NRRL 22395	EU329507	AF178341.1	-	AF178405.1
F. silvicola	CBS 123846 T	LR583876	-	-	-
F. solani	CBS 140079 ET	KT313623	-	-	-
	CBS 143241 T	LR58384	-	-	-
	CBS 178.47	LR583860	-	-	LR583751
	NRRL 25388	-	-	-	LR583765
	NRRL 62579	-	KC691538.1	-	-
	NRRL 13952	-	DQ246835.1	-	-
F. solani	CBS 141.90 T	LR583869	-	-	LR583760
F. solani	CBS 114067 T	LR583874	LR583644	-	LR583764
F. solani	CBS 126407 T	LR583846	LR583621	-	LR583731
F. solani	CBS 410.62	LR583824	-	-	LR583710
F. solani	CBS 144390 T	LR583828	LR583607	-	LR583713
	CBS 119601	LR583875	-	-	-
F. spathulatum	NRRL 28541	EU329542	-	-	-
F. stercicola	CBS 142481 T	LR583887	-	-	-
F. suttonianum	NRRL 32858	EU329630	-	-	-
	CBS 146668	LR792611.1	-	-	-
	CML 3972	MK988365.1	-	-	-
	CML 3971	MK988364.1	-	-	-
	Ns263	MN263121.1	-	-	-

	NRRL 54972	-	-	-	-
F. tonkinense	CBS 115.40 T	LT960564	-	-	MG189941
F. tucumaniae	CML 2448	da kedma	-	-	-
	NRRL 31096T	EU329557	AY220181.1	-	AY220231
	NRRL 22744		DQ247651	-	-
	NRRL 31097	FJ240387.1	AY220182.1	-	-
	NRRL 34546	KJ511284.1	AY730886.1	KJ511273	-
	NRRL 31086	KJ511280.1	AY220171.1	KJ511269.1	-
	NRRL 34546	KJ511284	-	-	-
F. vasinfecta	CBS 562.70	LR583901	-	-	-
F. virguliforme	NRRL 22825	EU329533	-	-	-
	NRRL 31041 T	JX171643	AY220193.1	-	AY220239.1
F. yamamotoi	NRRL 22163	EU29496.1	*AF178328.1*	-	AF178394.1
	NRRL 22277	FJ240380	-	-	-

Codes JMS indicate isolates from Brazil. Ex-type and ex-epitype strains are indicated with T and ET, respectively.

Strain collection acronyms: CBS: Westerdijk Fungal Biodiversity Institute, Utrecht, Netherlands; CML: Colecão Micológica de Lavras, Universidade Federal de Lavras, Lavras, Minas Gerais, Brazil; NRRL: Northern Regional Research Laboratory, Peoria, Illinois, USA; CBS = Westerdijk Fungal Biodiversity Center, Utrecht, The Netherlands.

Table 3 Pathogenicity test results

		Lesion on common bean (cm)				Lesion on cowpea (cm)				Lesion on soybean (cm)		
Species	Isolate	Vegetative (~30 dai)	Flowering (~45 dai)	Maturation (~65 dai)		Vegetative (~30 dai)	Flowering (~45 dai)	Maturation (~65 dai)		Vegetative (~30 dai)	Flowering (~45 dai)	Maturation (~65 dai)
F. paranaense	JMS107	0	2,8	0,9	c	1,5	0,6	0,2	b	0	0,7	0,4
F. solani	JMS 130	1,9	1,2	0,7	c	1,5	0,8	0,5	b	1,9	0,5	1,1
F. suttonianum	JMS 174	0	1,0	2,4	c	0	1,0	0,4	b	0	0,2	0,4
F. martii	JMS 61	0,2	0,8	0,8	c	1,5	0,3	0,5	b	1	0,7	0,2
Clade 2	JMS 120	1,5	1,0	4,8	a	0	0,6	2,7	a	0	0,6	0,3
Clade 2	JMS 169	0,1	1,03	3,6	b	0	2,5	1,5	a	0	0,3	0,7

*Mean root disease severity ratings of 9 plants for each isolate on commom bean and cowpea cultivars were based on lesion dimensions. Averages followed by the same letter do not differ according to the test at 5% of probability.

*dai : Days after inoculation.

RPB2
820 bp
1 of 1 tree
565 steps
CI = 0,3435
RI = 0,8308

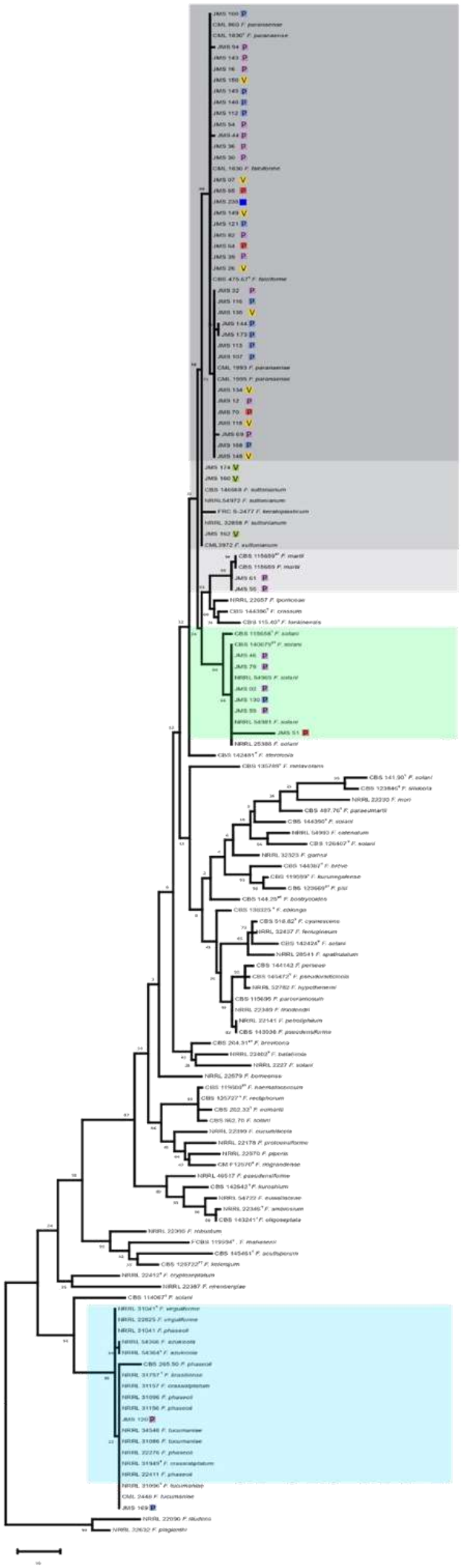

Fig. 1 Maximum parsimony phylogenetic (MP) tree inferred from *rpb2* gene fragments showing the relatedness of *Fusarium* associated with bean root rot with other species within *Fusarium solani* species complex. The bootstrap values (1000 replicates) from MP ≥ 70% are shown at the internodes. Codes JMS indicate isolates from Brazil. Ex-type and ex-epitype strains are indicated with T and ET, respectively. Strain collection acronyms: CBS: Westerdijk Fungal Biodiversity Institute, Utrecht, Netherlands; CML: Colecão Micológica de Lavras, Universidade Federal de Lavras, Lavras, Minas Gerais, Brazil; NRRL: Northern Regional Research Laboratory, Peoria, Illinois, USA.

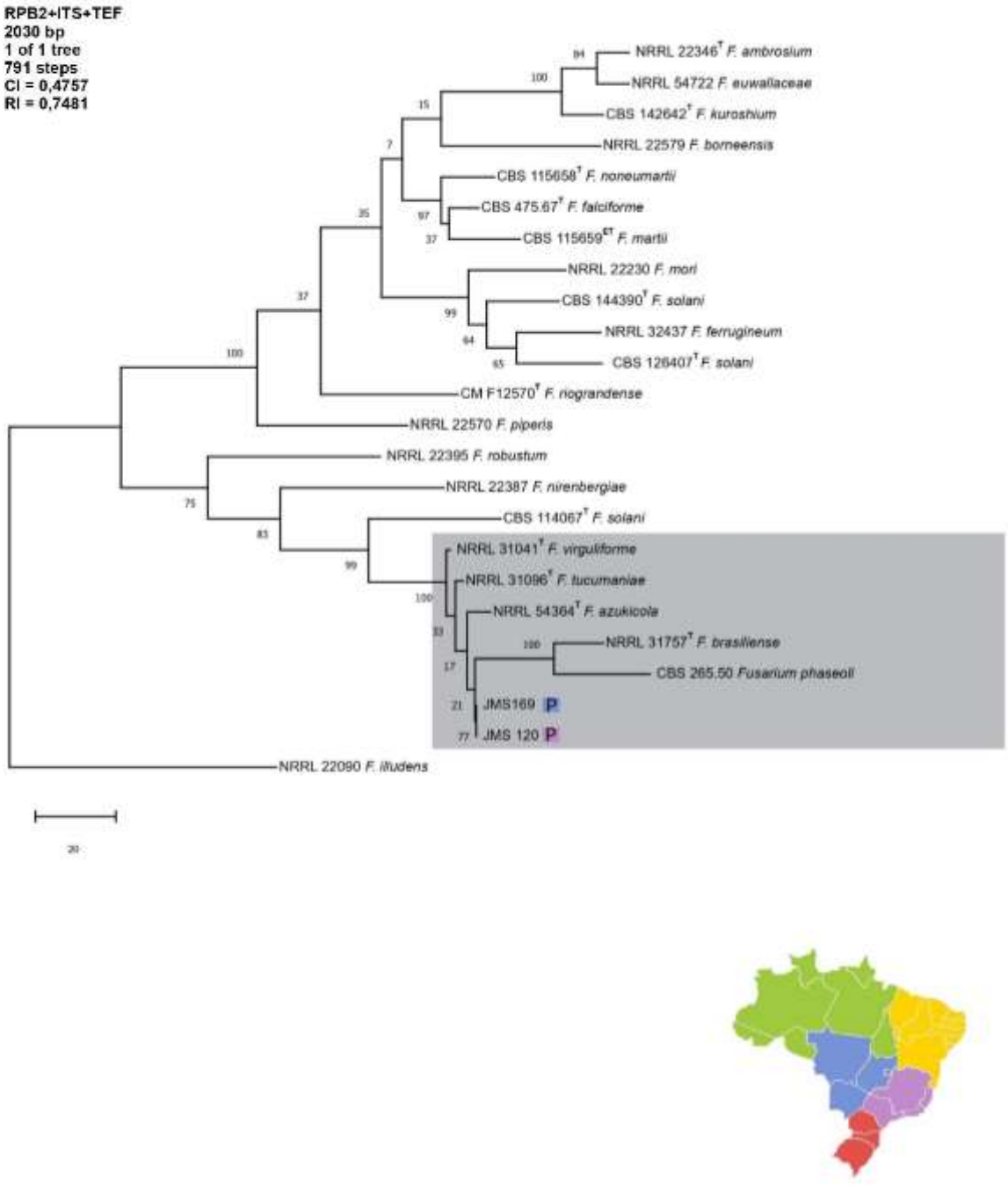

Fig. 2 Maximum parsimony phylogenetic (MP) tree inferred from combined dataset (*rpb2*, ITS, *tef1*, and *rpb1*), showing the relatedness of *Fusarium* associated with bean root rot with other species within *Fusarium solani* species complex. The bootstrap values (1000 replicates) from MP ≥ 70% are shown at the internodes. Codes JMS indicate isolates from Brazil. Ex-type and ex-epitype strains are indicated with T and ET, respectively. Strain collection acronyms: CBS: Westerdijk Fungal Biodiversity Institute, Utrecht, Netherlands; CML: Colecão Micológica de Lavras, Universidade Federal de Lavras, Lavras, Minas Gerais, Brazil; NRRL: Northern Regional Research Laboratory, Peoria, Illinois, USA.

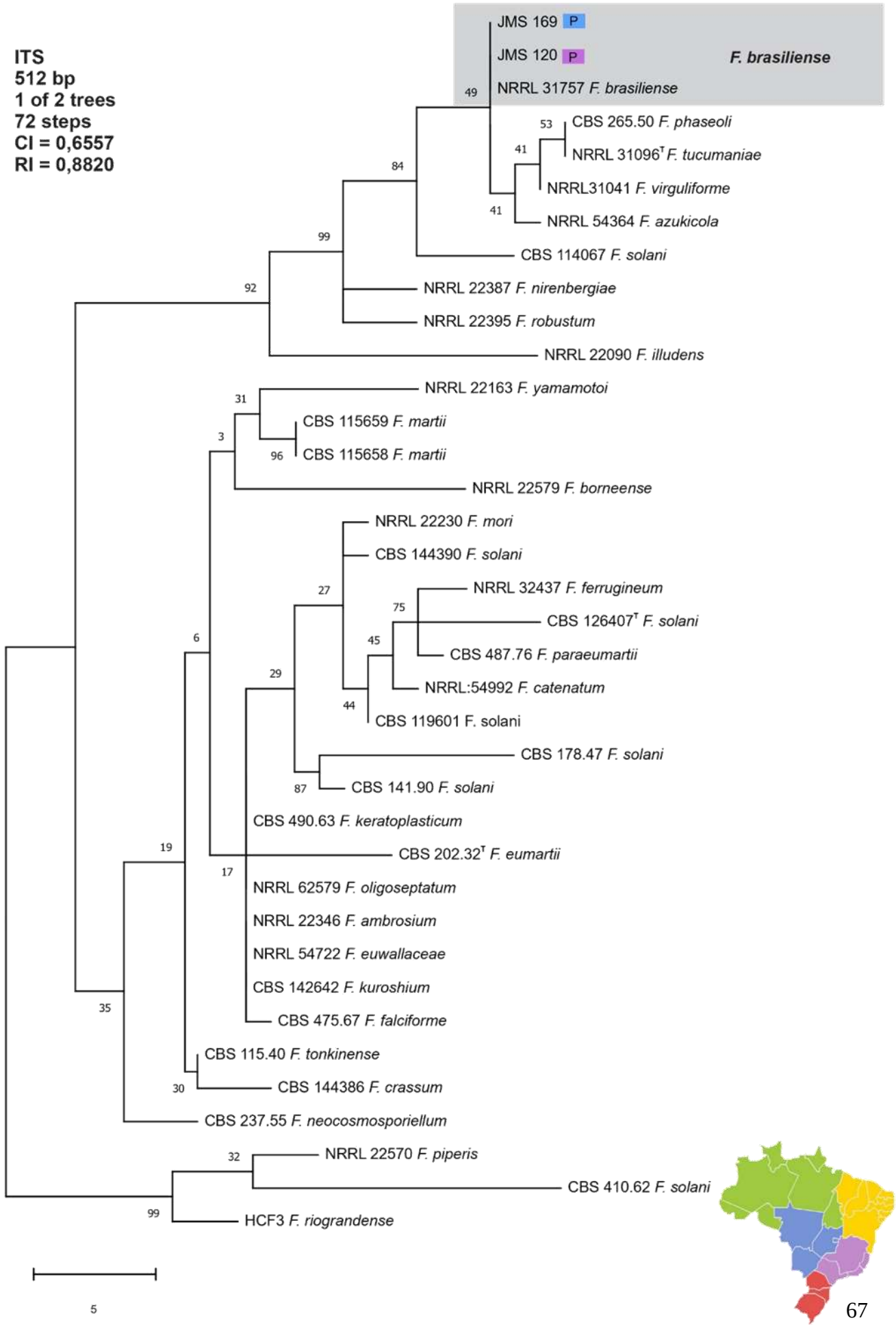
ITS
512 bp
1 of 2 trees
72 steps
CI = 0,6557
RI = 0,8820
JMS 169 P
JMS 120 P
F. brasiliense
NRRL 31757 F. brasiliense
CBS 265.50 F. phaseoli
NRRL 31096T F. tucumaniae
NRRL31041 F. virguliforme
NRRL 54364 F. azukicola
CBS 114067 F. solani
NRRL 22387 F. nirenbergiae
NRRL 22395 F. robustum
NRRL 22090 F. illudens
NRRL 22163 F. yamamotoi
CBS 115659 F. martii
CBS 115658 F. martii
NRRL 22579 F. borneense
NRRL 22230 F. mori
CBS 144390 F. solani
NRRL 32437 F. ferrugineum
CBS 126407T F. solani
CBS 487.76 F. paraeumartii
NRRL:54992 F. catenatum
CBS 119601 F. solani
CBS 178.47 F. solani
CBS 141.90 F. solani
CBS 490.63 F. keratoplasticum
CBS 202.32T F. eumartii
NRRL 62579 F. oligoseptatum
NRRL 22346 F. ambrosium
NRRL 54722 F. euwallaceae
CBS 142642 F. kuroshium
CBS 475.67 F. falciforme
CBS 115.40 F. tonkinense
CBS 144386 F. crassum
CBS 237.55 F. neocosmosporiellum
NRRL 22570 F. piperis
CBS 410.62 F. solani
HCF3 F. riograndense
5

Fig. 3 Maximum parsimony phylogenetic (MP) tree inferred from ITS gene fragments showing the relatedness of *Fusarium* associated with bean root rot with other species within *Fusarium solani* species complex. The bootstrap values (1000 replicates) from MP ≥ 70% are shown at the internodes. Codes JMS indicate isolates from Brazil. Ex-type and ex-epitype strains are indicated with T and ET, respectively. Strain collection acronyms: CBS: Westerdijk Fungal Biodiversity Institute, Utrecht, Netherlands; CML: Colecão Micológica de Lavras, Universidade Federal de Lavras, Lavras, Minas Gerais, Brazil; NRRL: Northern Regional Research Laboratory, Peoria, Illinois, USA.

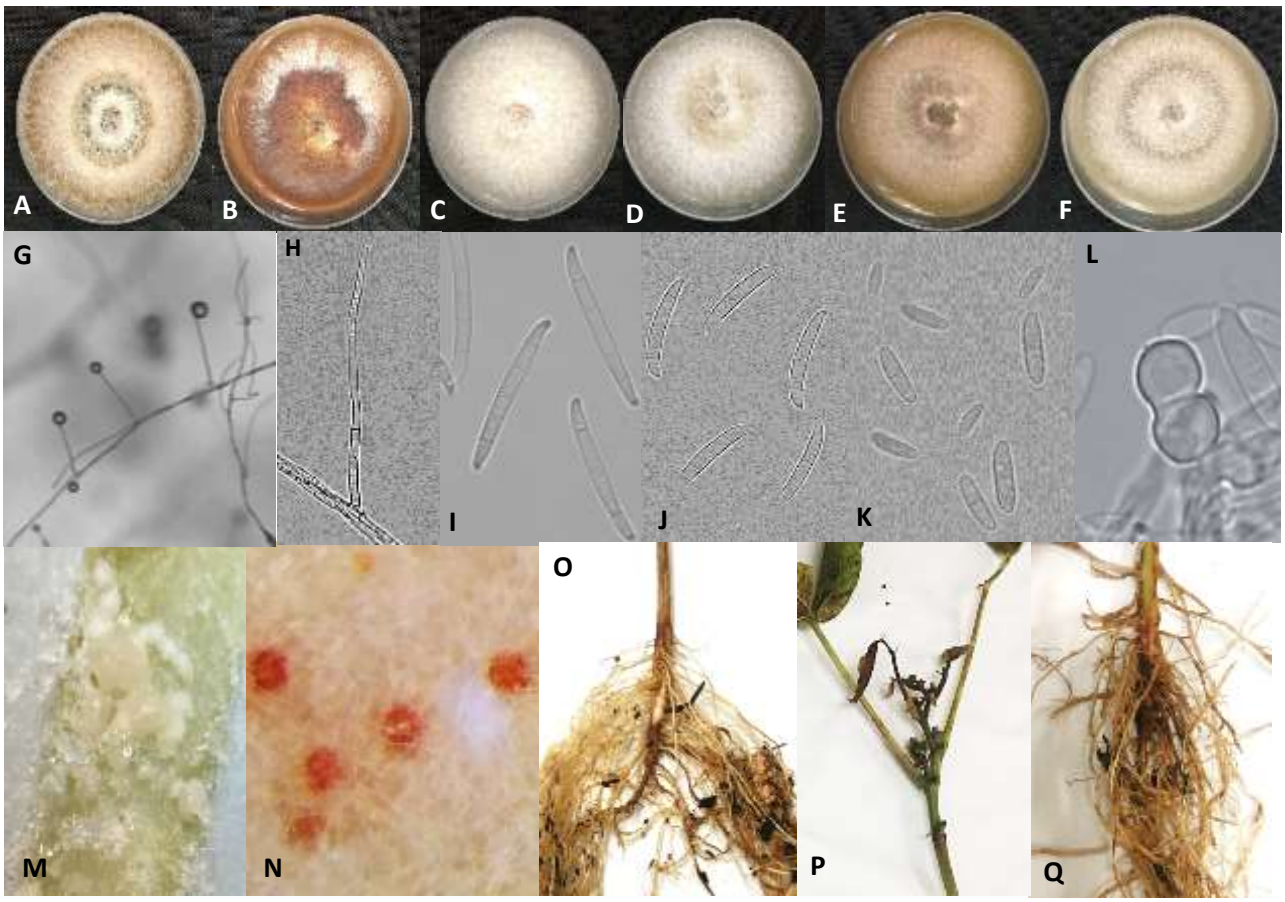

Fig. 4 Morphological characteristics of *Fusarium paranaense.* A - F. Colony white with center blue, less frequently color white with center brown, greeen, red and white, a few isolates presente colony color brown and yellow brown (isolates jms 112, 65, 173, 116, 118, 30, respectivelly). G. Aerial mycelium. H. Monophialide. I. Macroconidia of sporodochia. J. Mesoconidia. K. Microconidia. L. Chlamydospore. M. Sporodochia formed on carnation leaves on SNA. N. Perithecia. O. Lesions in *P. vulgaris*. P. Necrosis in *P. vulgaris* flowers. Q. Lesions in *V. unguiculata*.

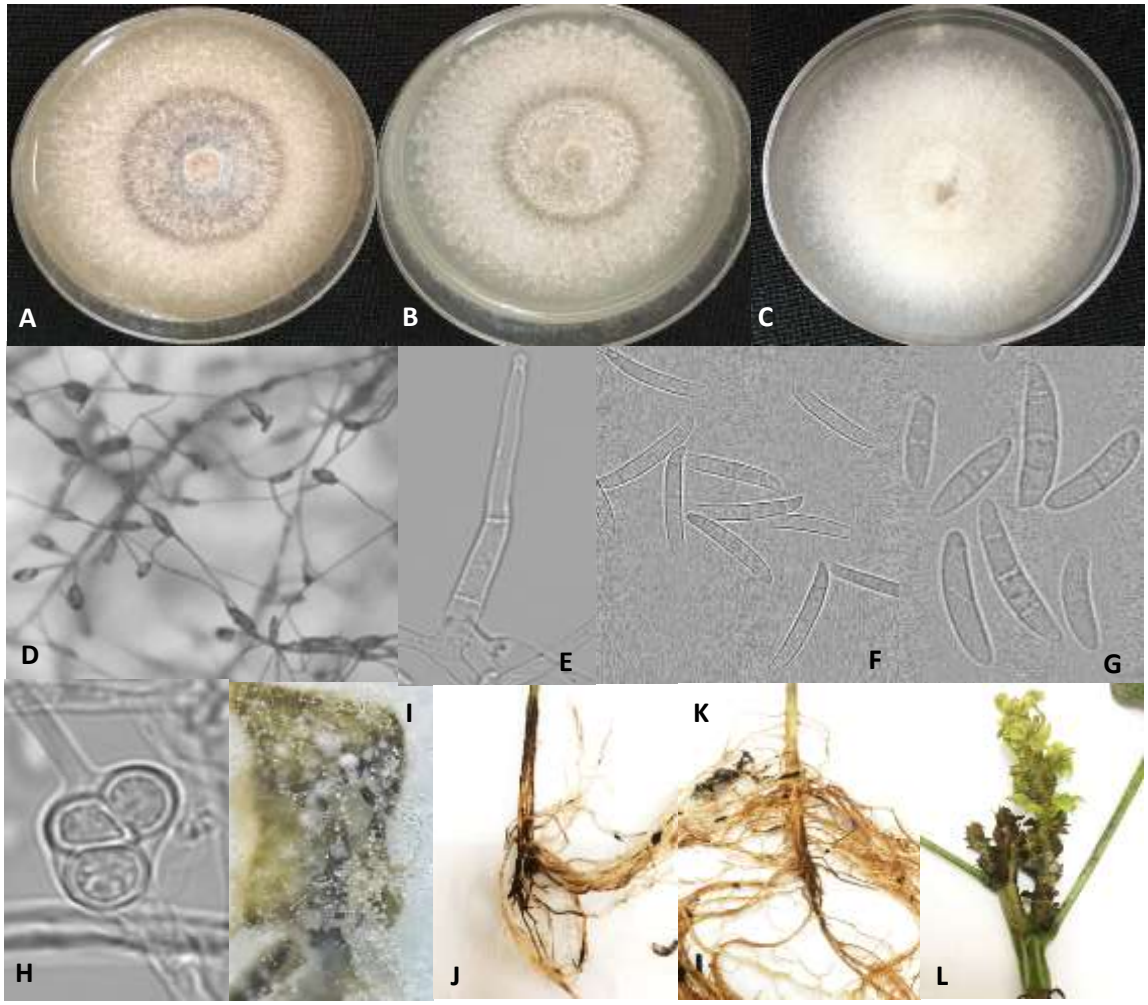

Fig. 5 Morphological characteristics of *Fusarium solani.* A - C. white and white with center varying in blue, green and brown. D. Macroconidia in aerea mycelium. E. Monophialide. F. Macroconidia of sporodochia. G. Microconidia. H. Chlamydospore. I. Sporodochia. J. Lesions in *P. vulgaris*. K. Lesions in *V. unguiculata.* L. Necrosis and mycelium in *P. vulgaris* flowers.

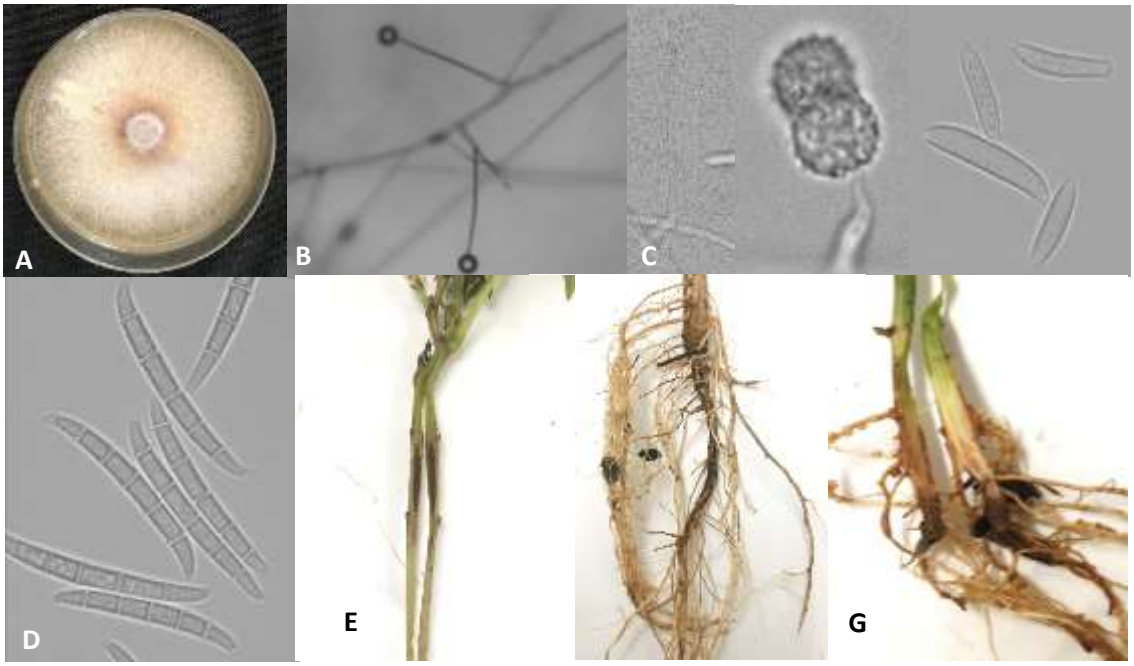

Fig. 6 Morphological characteristics of *Fusarium sutonnianum.* A. colony had a color beige with brown or purple center. B. Macroconidia in aerea mycelium. C. Chlamydospore. D. Macroconidia of sporodochia. E. Microconidia. F. Lesions in *P. vulgaris*. G. Lesions in *V. unguiculata.*

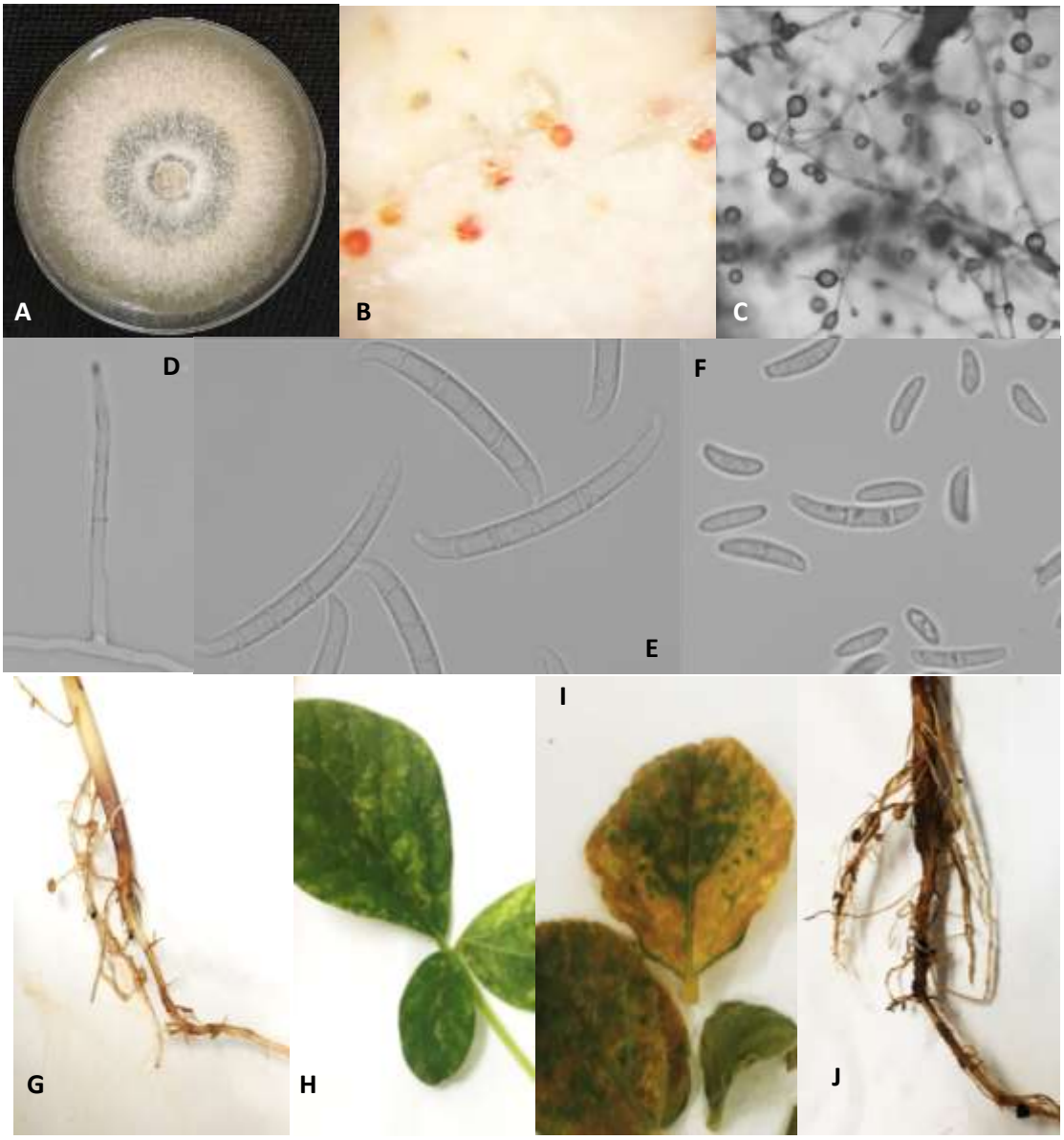

Fig. 7 Morphological characteristics of *Fusarium martii.* A. color white with a blue center. B. Perithecia. C. Macroconidia in aerea mycelium. D. Monophialide. E. Macroconidia of sporodochia. F. Microconidia. G. Lesions in *P. vulgaris*. H - I. Lesions in *V. unguiculata*. J. Lesions in soybean root.

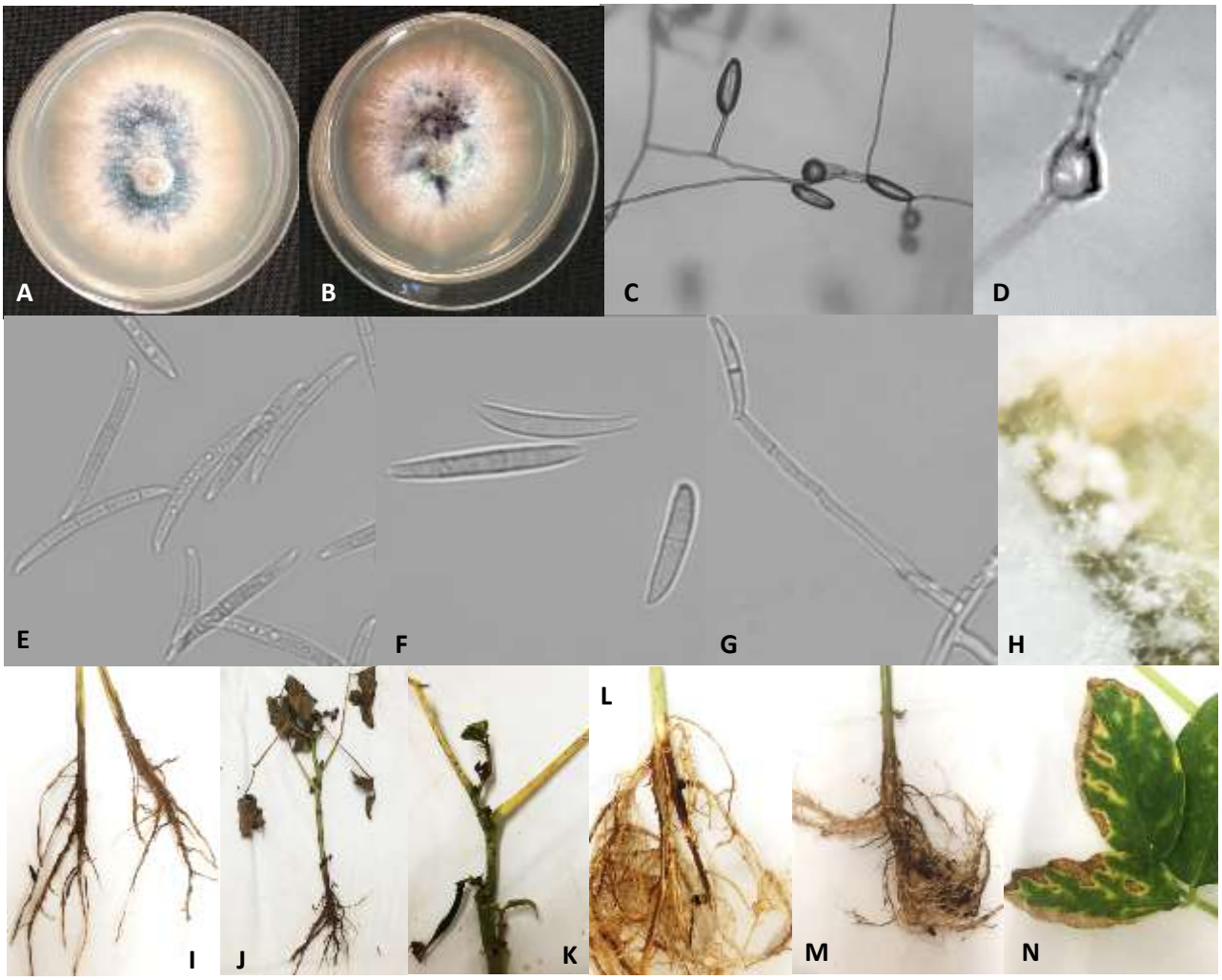

Fig. 8 Morphological characteristics of Clade 2 isolates. A - B. color white with a blue center. C. Macroconidia in aerea mycelium. D. Chlamydospore. E. Macroconidia of sporodochia. F. Microconidia. G. Monophialide. H. Sporodochia. I. Lesions in *P. vulgari*s. J. Dead plant of *P. vulgari*s. K. Necrosis n *V. unguiculata*. L. Lesions in root of *V. unguiculata.* N. Lesions in root of *G. max*. N. Internerval chlorosis in *G. max* leaves induced by Clade 2 isolates.

Fig. 9 Pathogenicity results. Root in flower: A. *F. paranaense*. B. *F. solani*. C. Clade 2 isolates. D. Dead plant by effecct of Clade 2 isolates. E. root on stem induced by *F. suttonianum*. F. Internerval chlorosis induced by Clade 2 isolates on soybean. G. Initial internerval chlorosis induced by *F. martii* on soybean. H. Internerval chlorosis induced by *F. martii* on soybean. I-J. Rot root caused *F. martii* and Clade 2 isolates in soybean, respectively. Root of *P. vulgaris*: K. Control. L. *F. paranaense*. M. *F. solani*. N. *F. suttonianum*. O. *F. martii*. P. Clade 2 isolates. Root of *V. unguiculata*: Q. Control. R. *F. paranaense*. S. *F. solani*. T. *F. suttonianum*. U. *F. martii*. V.

Table 1S Morphotypes morphological makers based on Aoki et al. 2003, 2005, 2011, 2012; Costa et al. 2016.

Especies	Morfological marker
F. phaseoli	Slow mycelial growth, macroconidia with symmetric foot cell and apical cell.
F. azukicola	Slow mycelial growth, macroconidia in the same way as that of *F. tucumaniae*, however wider
F. crassistiptatum	Slow mycelial growth, conidia with rostrate (pointed) apical cell and cell is distinct.
F. tucumaniae	Slow mycelial growth, long and smoothed macroconidia, with foot and apical cell symmetrical.
F. virguliforme	Slow mycelial growth, comma-shaped microconidia.
F. brasiliense	Slow mycelial growth, conidia with foot and apical cell rounded at the ends.
F. paranaense	Rapid mycelial growth and macroconidia of different lengths, but always wide.

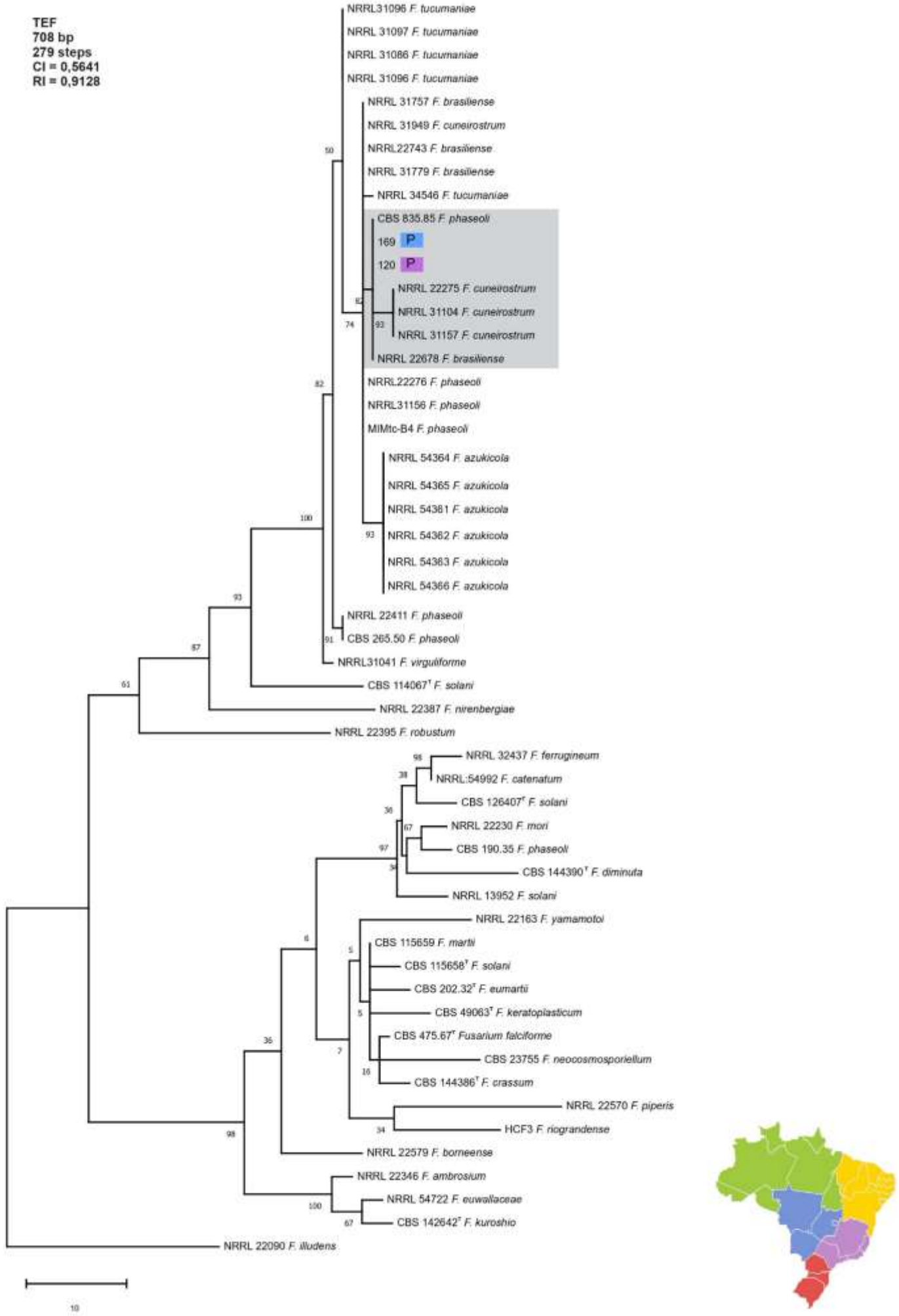
TEF
708 bp
279 steps
CI = 0,5641
RI = 0,9128
NRRL31096 F. tucumaniae
NRRL 31097 F. tucumaniae
NRRL 31086 F. tucumaniae
NRRL 31096 F. tucumaniae
NRRL 31757 F. brasiliense
NRRL 31949 F. cuneirostrum
NRRL22743 F. brasiliense
NRRL 31779 F. brasiliense
NRRL 34546 F. tucumaniae
CBS 835.85 F. phaseoli
169 P
120 P
NRRL 22275 F. cuneirostrum
NRRL 31104 F. cuneirostrum
NRRL 31157 F. cuneirostrum
NRRL 22678 F. brasiliense
NRRL22276 F. phaseoli
NRRL31156 F. phaseoli
MIMtc-B4 F. phaseoli
NRRL 54364 F. azukicola
NRRL 54365 F. azukicola
NRRL 54381 F. azukicola
NRRL 54382 F. azukicola
NRRL 54383 F. azukicola
NRRL 54366 F. azukicola
NRRL 22411 F. phaseoli
CBS 265.50 F. phaseoli
NRRL31041 F. virguliforme
CBS 114067T F. solani
NRRL 22387 F. nirenbergiae
NRRL 22395 F. robustum
NRRL 32437 F. ferrugineum
NRRL:54992 F. catenatum
CBS 126407T F. solani
NRRL 22230 F. mori
CBS 190.35 F. phaseoli
CBS 144390T F. diminuta
NRRL 13952 F. solani
NRRL 22163 F. yamamotoi
CBS 115659 F. martii
CBS 115658T F. solani
CBS 202.32T F. eumartii
CBS 49063T F. keratoplasticum
CBS 475.67T Fusarium falciforme
CBS 23755 F. neocosmosporiellum
CBS 144386T F. crassum
NRRL 22570 F. piperis
HCF3 F. riograndense
NRRL 22579 F. borneense
NRRL 22346 F. ambrosium
NRRL 54722 F. euwallaceae
CBS 142642T F. kuroshio
NRRL 22090 F. illudens
10

Fig. S1 Maximum parsimony phylogenetic (MP) tree inferred from *tef1* gene fragments showing the relatedness of *Fusarium* associated with bean root rot with other species within *Fusarium solani* species complex. The bootstrap values (1000 replicates) from MP ≥ 70% are shown at the internodes. Codes JMS indicate isolates from Brazil. Ex-type and ex-epitype strains are indicated with T and ET, respectively. Strain collection acronyms: CBS: Westerdijk Fungal Biodiversity Institute, Utrecht, Netherlands; CML: Colecão Micológica de Lavras, Universidade Federal de Lavras, Lavras, Minas Gerais, Brazil; NRRL: Northern Regional Research Laboratory, Peoria, Illinois, USA.

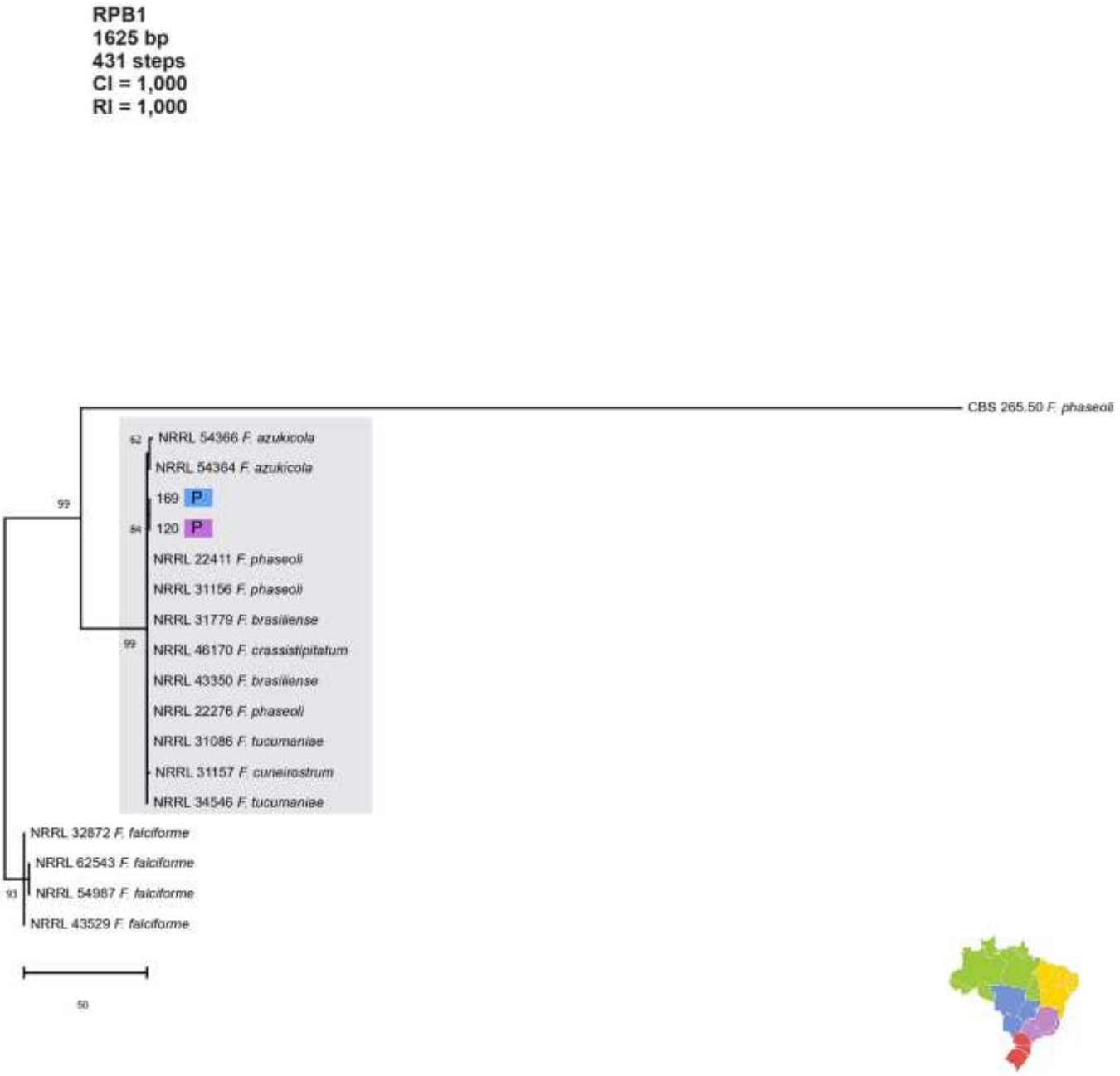

Fig. S2 Maximum parsimony phylogenetic (MP) tree inferred from *rpb1* gene fragments showing the relatedness of *Fusarium* associated with bean root rot with other species within *Fusarium solani* species complex. The bootstrap values (1000 replicates) from MP ≥ 70% are shown at the internodes. Codes JMS indicate isolates from Brazil. Ex-type and ex-epitype strains are indicated with T and ET, respectively. Strain collection acronyms: CBS: Westerdijk Fungal Biodiversity Institute, Utrecht, Netherlands; CML: Colecão Micológica de Lavras, Universidade Federal de Lavras, Lavras, Minas Gerais, Brazil; NRRL: Northern Regional Research Laboratory, Peoria, Illinois, USA.