Murcha de *fusarium* e podridão radicular em grão-de-bico: taxonomia, sintomatologia e etiologia

Fusarium wilt and rot root in chickpea: taxonomy, symptomatology and etiology

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ABSTRACT

Chickpea (*Cicer arietinum*) wilt and root rot are the most relevant diseases in several production areas. There are some lineage of *Fusarium oxysporum* f. sp. *ciceris*, from the *F. solani* and *F. redolens* complex associated with the wilt symptom. The external disease symptoms are similar; however, both species show different colonization. Traditionally, for many years, the taxonomy of the *Fusarium* genus has been based on the evaluation of morphological markers, being an irrelevant technique to identify species or phylogenetic lineage. Nucleotide sequencing techniques in species from the genus *Fusarium* have broke down species into several different phylogenetic lineages, which are currently called the species complex. Thus, it is believed that many pathogens associated with chickpea that are currently denominated as *formae speciales* may be complex of phylogenetic lineage. *Fusarium* species of different *Fusarium* complex have the ability to induce wilt in different hosts, while other species or phylogenetic lineage, have the ability to induce rot root.

Keywords: Cicer arietinum, disease, Fusarium complex species, identification, phylogenetic lineage

RESUMO

A murcha e a podridão radicular em grão-de-bico (*Cicer arietinum*) são as doenças de maior relevância em diversas áreas de produção. Existem algumas linhagens de *Fusarium oxysporum* f. sp. *ciceris*, do complexo *F. solani* e *F. redolens* associado ao sintoma da murcha. Os sintomas reflexos da doença são semelhantes; no entanto, ambas as espécies mostram colonização diferente. Tradicionalmente, há muitos anos, a taxonomia do gênero *Fusarium* tem sido baseada na avaliação de marcadores morfológicos, sendo uma técnica irrelevante para identificar espécies ou linhagem

filogenética. As técnicas de seqüenciamento de nucleotídeos em espécies do gênero *Fusarium* dividiram as espécies em várias linhagens filogenéticas diferentes, que atualmente são chamadas de complexo de espécies. Assim, acredita-se que muitos patógenos associados ao grão-de-bico atualmente denominados *formae speciales* possam ser complexos de linhagens filogenéticas. Espécies de *Fusarium* de diferentes complexos de *Fusarium* têm a habilidade de induzir murcha em diferentes hospedeiros, enquanto outras espécies ou linhagens filogenéticas têm a capacidade de induzir podridão radicular.

Palavras-chave: *Cicer arietinum*, doença, Complexo de espécies *Fusarium*, identificação, linhagens filogenéticas.

1 INTRODUCTION

Chickpea (*Cicer arietinum* L.) is the second most important vegetable after *Phaseolus vulgaris* L. This botanical species is important in several parts of the world, being cultivated in several tropical, subtropical and temperate regions. The main regions with the highest production are found in countries in South and West Asia, South and North America and Australia (Faostat, 2018). Initially, *C. arietinum* was domesticated in the Mediterranean region, India and Ethiopia and introduced in Mexico, Argentina, Chile, Peru, Australia and Brazil (Van der Maesen, 1987).

However, due to the anthropogenic activity, several pathogens may have been disseminated via propagating material (Jukanti et al., 2012), mainly *Fusarium* species through the seed, which is the form of propagation in chickpea (Cabral et al., 2016). There are several records of *Fusarium* associated with this legume in the literature, causing a reduction in seed production (Haware and Nene, 1980; Navas-Cortés et al., 2000b). The losses caused by *Fusarium* in India, Spain and Tunisia vary between 10 to 70% (Singh and Dahiya, 1973; Haware and Nene, 1980; Trapero-Casas and Jiménez-Díaz, 1985; Halila and Strange, 1996), with the occurrence of late and early wilting causing up to 65 and 94% of yield losses, respectively (Haware and Nene, 1980), reaching 100% depending on agroclimatic conditions, cultivar and the virulence and aggressiveness of *Fusarium*. In Brazil, 10 to 15% reduction in production is reported due to the infection by *F. oxysporum* f. sp. *ciceris* (Stangarlin et al., 2016).

The most important worldwide phytosanitary problems in chickpea are wilting and root rot. Currently, the wilt caused by *F. oxysporum* f. sp. *ciceris* is the main disease of this culture (Jiménez-Gasco et al., 2004). However, *F. redolens* has been reported to be associated with chickpea plantations in Spain, curiously causing symptoms similar to *F. oxysporum* f. sp. *ciceris* (Jiménez-Fernández et al., 2011). In Iran and Brazil, isolates from the *F. solani* f. sp. *pisi* and *F. solani* were associated with symptoms of root rot in chickpea (Hasanzade et al., 2008; Cabral et al., 2016; Azevedo et al., 2017). Recently, *Fusarium proliferatum*, a member of the *Fusarium fujikuroi* complex, was reported causing wilted chickpeas in Cuba (Duarte-Leal et al., 2020).

This review will address the results of research performed mainly in Brazil, involving the *Fusarium* pathosystem versus chickpea, highlighting relevant aspects such as taxonomy, symptomatology and etiology of the disease.

2 FUSARIUM TAXONOMY

From the description of F. roseum (https://www.gbif.org/en/species/5251675), this genus has undergone several changes according to the evolution of science, mainly after molecular identification. To identify Fusarium three species concepts are currently applied. The morphological concept, where identification is based on the evaluation of the morphological markers of the anamorph and teleomorph, which are often not enough to confirm the identity of the species; the biological concept defines that individuals or intercrossing populations that are able to reproduce sexually and leave viable and fertile descendants are of the same species; although there are signs that the sexual reproduction of Fusarium oxysporum f.sp. cubense may occur due to the formation of protoperithecial structures at the crossings (Fourie et al., 2009), for lineages of Fusarium oxysporum complex it is not possible to apply the concept of biological species due to difficulty to obtain the sexual phase of this group. For some species of *Fusarium solani* complex, it is possible to apply this technique. Currently, Fusarium solani complex has eight biological species formally described (Costa et al., 2016; Matuo and Snyder, 1973). The sexual reproduction process is coordinated by gene regions called *Mating type*. Under laboratory conditions, to perform sexual induction, these regions are previously identified through the PCR process, using specific primers, both determining the Mating types it is possible to select possible fertile isolates through in vitro crossings (Leslie and Summerell, 2006; Kerényi et al., 2004). The concept of phylogenetic species, which is based on the evaluation of gene sequences. The application of the concept of phylogenetic species to the genus Fusarium is a new methodology that can clarify taxonomic difficulties, such as the separation of genetically similar species (O'Donnell et al., 1998; Leslie and Summerell, 2006).

With the advent of molecular identification techniques, the taxonomy of the *Fusarium* genus has undergone several changes, specifically, in relation to genetic identity. Thus, many genetic entities called species are being represented by species complexes. Regarding the terminology formae speciales, applied to *F. oxysporum and F. solani*, some time ago it was proved that isolates of a particular *formae speciales* are composed of several distinct phylogenetic lineages, showing that this term should not be applied as a taxonomic category (O 'Donnell et al., 1998).

In recent years, research with the objective of revealing the etiology of *Fusarium* species has shown that identification only through morphological markers are flawed. As an example, the causal agent of wilt in sugar beet (*Beta vulgaris* L.), previously using only the concept of morphological

species, was called *F. oxysporum* f. sp. *betae*, currently we know that the pathogen does not belong to the *Fusarium oxysporum* complex, but to the *Fusarium fujikuroi* complex. Morphologically, this fungus share similar markers such as the production of chlamydospores and microconidia in short monophyalides, generating confusion and uncertainty when applying the concept of morphological species (Secor et al., 2014). Interestingly, in addition to the different formae speciales of *F. oxysporum*, there are other species of *Fusarium* belonging to the *F. fujikuroi* complex that induce wilt. As an example, *F. udum*, a member of the *F. fujikuroi* complex that induces wilt in *Crotalaria* spp. and pigeon pea [Cajanus cajan (L.) Mills.] (Pfenning et al., 2019).

3 SYMPTOMATOLOGY

Symptoms of *F. oxysporum* f. sp. *ciceris* in chickpea can occur at all stages during plant growth (Trapero-Casas and Jiménez-Díaz, 1985; Nene and Reddy, 1987; Haware, 1990). Susceptible cultivars show symptoms such as: chlorosis of the lower leaves, yellowing of leaves, leaf necrosis (dry leaves), drop of petioles, stems and leaflets, discoloration of vascular tissues, partial wilting of branches and in the final stage, total wilting of the plant (Prasad and Padwick, 1939; Singh et al., 2007; Gaur et al., 2010). Histological distortions in the vascular tissue of the affected roots and stems are due to the formation of dense gels and occlusions, contributing to delay the flow of water and nutrients, as well as the development of the plant (Jiménez-Díaz et al., 2015). Symptoms appear within 25 days after sowing (early wilting), being more evident at the beginning of flowering, between 6-8 weeks after sowing (late wilting) (Jiménez-Díaz et al., 2015).

F. oxysporum f. sp. *ciceris* has the species *C. arietinum* as its specific host, although it can infect other plant species such as lentils (*Lens sculenta* Moench), peas (*Pisum sativum* L.), alfalfa (*Medicago sativa* L.) and fava beans (*Vicia faba* L.), but without causing evident symptoms of fusariosis (Trapero-Casas and Jiménez-Díaz, 1985). In addition, the symptoms in *C. arietinum* and the aggressiveness are dependent on the pathotype, the race of *F. oxysporum* f. sp. *ciceris* and the cultivar present in the area. In the populations of *F. oxysporum* f. sp. *ciceris* there are two pathotypes that exhibit differential symptoms, which are called the yellowing and the withering pathotype (Trapero-Casas and Jiménez-Díaz, 1985). The first induces progressive leaf yellowing, while the second induces wilting, severe chlorosis, sagging and vascular discoloration. *F. oxysporum* f. sp. *ciceris* have eight pathogenic races (0, 1A, 1B/C, 2, 3, 4, 5 and 6), where races 0 and 1B/C cause yellowing syndrome and the other races (1A, 2, 3, 4, 5 and 6) cause wilting syndrome.

Races of *F. oxysporum* f. sp. *ciceris* can be identified by the reaction of disease severity in 10 different chickpea cultivars (Table 1). Symptoms of wilt in this host allows to identify 8 physiological races of *F. oxysporum* f. sp. *ciceris*, divided in two groups, according to the severity of the symptoms

of the infected plants (Trapero-Casas and Jiménez-Díaz, 1985). Based on inoculations in diverse differentiating cultivars, it is observed that different races cause different symptoms, showing these symptoms are related with the center of origin of the different races (Table 2) (Correll, 1991; Jiménez-Gasco et al., 2001). Race 0 is the least virulent among all *F. oxysporum* f. sp. *ciceris*, while race 5 is the most virulent (Jiménez-Díaz et al., 1993; Navas-Cortés et al., 2000a; Jiménez-Gasco et al., 2001). Navas-Cortés et al. (2007) demonstrated that this difference in virulence and induction of symptom between breeds 0 and 5 is also related to the combination of inoculum density and soil temperature in different chickpea cultivars.

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Differential lines	Races							
	0	1A	1B/C	2	3	4	5	6
12-071/10054	S*	М	S	R	R	R	R	Μ
JG-62	R	S	S	S	S	S	S	S
C-104	Μ	Μ	R/M	S	S	S	S	Μ
JG-74	R	R	R	S	R	R	М	R
CPS-1	R	R	R	S	Μ	Μ	Μ	R
BG-212	R	R	R	S	Μ	Μ	R	R
WR-315	R	R	R	R	S	R	R	R
ICCV-2	R	R	R	S	S	S	S	Μ
ICCV-4	R	R	R	S	S	S	S	Μ
P-2245	S	S	S	S	S	S	S	S

Table 1. Differential lines of *Cicer arietinum* to pathogenic races of *Fusarium oxysporum* f. sp. ciceris.

Source: Jiménez-Díaz et al. 1993; Jiménez-Gasco et al. 2004. *S (susceptible), M (moderately susceptible) and R (resistant).

The production of cell wall degradation enzymes is an important virulence factor in *Fusarium* wilt, since the fungus needs to penetrate and grow through the root cortex and colonize the xylem. In chickpea plants inoculated with breeds 0 and 5 of *F. oxysporum* f. sp. *ciceris* the production of cell wall degradation enzymes was positively correlated with the development of the disease (yellowing and wilting), however polygalacturonase and pectate lyase were the only enzymes identified in the roots and stems and showed highest specific activity, being generally higher for race 5 than for race 0 (Jorge et al., 2006).

Although *F. oxysporum* f. sp. *ciceris* have a distinct geographic distribution, there are reports of *Fusarium* species and other soil-dwelling fungi associated with chickpea causing diseases with symptomatic yellowing, root rot and wilt similar to that of *F. oxysporum* f. sp. *ciceris* (Table 2), making it difficult to diagnose the pathogen under field conditions. Therefore, it is evident that it is not possible to precisely determine which species is associated only with reflex symptoms. In addition, this presumptive diagnosis can be problematic, especially when it comes to plant breeding

programs and management of the disease. Different strains of *Fusarium* show different levels of aggressiveness or virulence, evidently, the best solution is the correct identification of the pathogen.

Table 2. Geographic distribution of Fusarium oxysporum f. sp. ciceris and other fungi and Oomycetes associated with Cicer
arietinum.

Race/ Pathogen	Source	References
Race 0	Spain, Israel, Lebanon, Syria,	Halila and Strange, 1996
	Tunisia, Turkey, USA	Jiménez-Díaz et al. 1993
		Jiménez-Gasco et al. 2001
Race 1A	Spain, India, Israel, Morocco,	Haware and Nene, 1982
	USA	ICRISAT, 1989
		Jiménez-Gasco et al., 2001
Race 1B/C	Spain, Israel, Syria, Tunisia,	Jiménez-Díaz et al., 1993
	Turkey, USA	Jiménez-Gasco et al., 2001
Race 2, 3 e 4	India	Haware and Nene, 1982
		Jiménez-Gasco et al., 2001
Race 5	Spain, Morocco, USA	Jiménez-Díaz et al., 1993
	-	Jiménez-Gasco et al., 2001
Race 6	Spain, Israel, Morocco, USA	Jiménez-Díaz et al., 1993
	-	Jiménez-Gasco et al., 2001
Pythium spp.	Brazil, USA, Canada, Spain,	Westerlund et al., 1974
Pythium ultimum	India, Iran, Turkey	Harverson, 2011
2		Nene et al., 2012
Phytophthora megasperma	Argentina, USA, Spain,	Harverson, 2011
P. medicaginis	Australia, India, Pakistan	Nene et al., 2012
Verticillium albo-atrum	Pakistan, Tunisia, Syria	Nene et al., 2012
Rhizoctonia solani	India, USA	Westerlund et al., 1974
R.bataticola	Australia, Bangladesh, Iran,	Harverson, 2011
	Kenya,	Andrabi et al., 2011
	Lebanon, Myanmar, Mexico,	Nene et al., 2012
	Nepal, Pakistan, Spain,	
	Sudan, Turkey and USA	
Macrophomina phaseoli	Spain, USA	Westerlund et al., 1974
_		Trapero-Casas and Jiménez-Díaz
		I

1985

Fusarium oxysporum	Brazil, USA, Spain, Asia, Africa, Southern Europe	Trapero-Casas and Jiménez-Díaz, 1985 Harverson, 2011 Nene et al., 2012 Azevedo et al., 2017	
Fusarium solani	Brazil, Argentina, Chile, Mexico, USA, India, Spain, Syria	Trapero-Casas and Jiménez-Díaz, 1985 Harverson, 2011 Andrabi et al., 2011 Nene et al., 2012 Azevedo et al., 2017 Cabral et al., 2016	
F. falciforme	Brazil	Cabral et al., 2016	
F. solani f. sp. pisi	USA	Westerlund et al., 1974	
F. eumartii	Spain	Trapero-Casas and Jiménez-Díaz, 1985	
F. redolens	Lebanon, Morocco, Pakistan, Spain	Jiménez-Fernández et al., 2011	

The differences observed in the aggressiveness of the isolates may be the result of genetic variability in the population, since the isolates were obtained from different chickpea cultivars. For *F. oxysporum* f. sp. *ciceris*, differences in aggressiveness between isolates can be explained by the existence of different pathotypes and races of the pathogen (Haware and Nene, 1982; Trapero-Casas and Jiménez-Díaz, 1985; Jiménez-Díaz et al., 1993; Navas-Cortés et al., 1998).

The symptoms from the interaction between species of *Fusarium* in chickpea may differ in terms of symptomatology. In Brazil, when evaluating symptoms of wilt and root rot in chickpea plantations, both cases exhibited similar symptoms in the aerial part of the plants, initially, the leaves showed yellowing, evolving to necrosis and drought. Based on the reflex symptoms, it is not possible to differentiate between wilt and root rot, requiring the assessment of the conducting vessels. In *F. oxysporum*, darkening of the stem tissue was observed, while in *F. solani* colonization is restricted to the root tissues. For both *Fusarium* species, the initial symptoms correspond to the period in which the plant begins its reproductive period (Azevedo et al., 2017). However, high disease incidence was observed the second week after inoculation with injury to the root system, when compared to the pathogenicity test done by direct seeding. In addition, it was observed that *F. oxysporum* isolates had a higher disease incidence and aggressiveness compared to *F. solani* isolates (Azevedo et al., 2017).

In the interaction of chickpea and isolates of the *F. solani* complex, it is believed plant wilts occur due to the blockage of water and nutrients in the area, while in colonization by *F. oxysporum*

the fungus migrates into the tissues, colonizing the intercellular spaces, consequently blocking the conducting vessels. In this case, the parasitism mechanisms of these isolates in relation to symptoms have not yet been fully elucidated, and there may also be a fungus-cultivar interaction of chickpea and environmental factors. In several pathosystems that involve the interaction of *F. solani* and its formae speciales, the symptoms are restricted to the root system, causing root rot or red rot in legume species. Eventually, reflex symptoms of root rot are leaf necrosis due to the action of toxin production by the pathogen (Jin et al., 1996; Aoki et al., 2003; Navi and Yang, 2008).

Genetic changes, through mutation processes, seem to occur in strains of *F. oxysporum* and these genetic changes result in greater virulence, which can be favored by the interaction of resistant or susceptible cultivars. As an example, the cotton wilt pathogen seems to have evolved from a local strain of native *F. oxysporum*, associated with wild cotton (*Gossypium* spp.) and slightly virulent to *G. hirsutum* (Davis et al., 2006).

For long time the pathogenic variations of *Fusarium* species have puzzled many phytopathologists since the discovery of physiological races terminology. Most recent research has focused on the molecular mechanisms that allow pathogens to avoid recognition by plant defenses. From an evolutionary perspective, however, mutations and recombination between avirulence (avr) genes in sexually reproducing pathogens have been postulated as the mechanisms responsible for race variation (Hermosa et al., 2000).

3 ETIOLOGY OF WILT AND ROOT ROT IN CHICKPEAS

In Brazil, as previously described, the occurrence of *F. oxysporum* f. sp. *ciceris* in chickpea was not related. Therefore, the pathogenic *Fusarium* species may have been introduced through contaminated seeds and/or be endemic populations present in areas cultivated with legumes, as noted by Cabral et al. (2016) and Azevedo et al. (2017) who obtained isolates of *F. solani* and *F. oxysporum* from the same commercial area of chickpea (Cristalina-GO) in planted succession with soybeans [(Glycine max (L.) Merrill] and beans. In previous studies evaluating seed health with seven chickpea genotypes, an average incidence of 6.5% of *Fusarium* sp. were observed in a total of 700 seeds, but among the genotypes the incidence varied between 0 to 13.75% (unpublished data). In another study, we also observed disease incidence occurring in all genotypes between one and four weeks, after transplanting chickpea seedlings into pots with autoclaved soil, which partially reinforces our scientific hypothesis about the spread and survival of *Fusarium*. In Brazil, as there is no fungicide registered for the control of *Fusarium* in chickpeas, the use of *Trichoderma* with different mechanisms of action constitutes a potential in the biocontrol of fusariosis (Azevedo et al., 2020).

The *Fusarium* genus includes some of the most important fungal species associated with various diseases in plants of economic importance. This genus is represented by several complexes of species or phylogenetic lineages. In recent years with the application of the concept of phylogenetic species, what was called *F. oxysporum* and *F. solani* have been transformed into several phylogenetic species or lineages. As an example, *F. solani* f. sp. *glycinis*, a root rot pathogen in legumes is represented by 9 genetically distinct species (Aoki *et al.* 2003; Costa *et al.* 2016). While for different pathosystems that involve *F. oxysporum* causing symptoms of wilt, what is called formae speciales, in fact, it is a complex of phylogenetic lineages (O'Donnell et al., 1998; Nirmaladevi et al., 2016).

Phylogenetic studies using *F. oxysporum* f. sp. *ciceris* from different locations, confirmed that the etiological agent of chickpea wilt is represented by a phylogenetic group (Jiménez-Gasco et al., 2002). While in Brazil, Azevedo et al. (2017), while evaluating the collection of isolates obtained from plants with symptoms of wilt, concluded that the etiological agent belongs to a phylogenetic lineage distinct from *F. oxysporum* f. sp. *ciceris*. This result shows the importance of using modern identification techniques, since based on the concept of morphological species it is not possible to distinguish phylogenetic lineages, from different formae speciales. Because the causal agent of chickpea wilt in Brazil is genetically different from the pathogen in different regions of the world, it is probably an indigenous phylogenetic lineage, since the host is an exotic species that was introduced in areas cultivated with legumes. Thus, we believe that throughout the evolutionary history, the fungus migrated to *C. arientienum*. Another possible hypothesis is that this new strain may have migrated from cultivated or wild hosts to *C. arientienum*. Factors such as coevolution with the host plant and determinants such as virulence factors acquired through parasexuality, heterokaryosis and recombination processes may be involved in the evolution of these pathogens (O'Donnel et al., 1998).

This review shows the importance of modern techniques for the identification of *Fusarium*. Diagnosis of species from this genus is increasingly difficult, due to different reasons. The number of taxonomic characteristics within formae speciales is limited or absent. This fact is evidenced when the concept of phylogenetic species is applied, showing that different phylogenetic strains share the same morphological markers. This information was proven for the etiologic agent of banana wilt, what was called *F. oxysporum* f. sp. *cubense* has broken down into nine independent phylogenetic strains (Maryani et al., 2019). Meanwhile, other pathogens such as the etiological agent of beet, formerly known as *F. oxysporum* f. sp. *betae*, in fact has proved to belong to a new species of the *Fusarium fujikuroi* complex by multicoat sequencing, showing that the evaluation of morphological markers such as the presence of short phyaloid chlamydospores is inaccurate and flawed for species identification (Secor et al., 2014).

Another issue that needs to be investigated is the identity of the term physiological race applied to isolates of different formae speciales. Perhaps the different symptoms exhibited by the pathogen that causes chickpea wilt are caused by the fact that the fungal isolates belong to different phylogenetic strains. From a collection of *F. oxysporum* f. sp. *vasinfectum*, races of the pathogen were determined, through inoculation in differentiating cultivars, however, when performing phylogenetic studies, the formation of four genetically distinct clades was observed, curiously, isolates from different races grouped in the same clade. The first clade grouped races 3 and 5, the second clade races 1, 2 and 6, the third clade represented only by race 8 and the fourth clade containing races 4 and 7 (Skovgaard et al., 2001).

4 CONCLUSIONS AND FUTURE PERSPECTIVES

In the chickpea-*Fusarium* pathosystem we believe that there are several phylogenetic lineages of the *Fusarium oxysporum* and *F. solani* complexes associated with wilt and root rot. Future research using nucleotide sequencing techniques should be carried out in order to confirm this hypothesis. The elucidation of the diversity of phylogenetic lineages, within the special forms of *F. oxysporum* is extremely important, especially when it comes to the selection of cultivars with sources of resistance.

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