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Response of chickpea genotypes (*Cicer arietinum* L.) to the fungi complex that causes wilt

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ABSTRACT
Objective: The objective of the present study was to evaluate the response of 10 chickpea genotypes from INIFAP to the damage caused by the fungal complex.
Design/methodology/approach: Seedlings (15-day-old) of 10 genotypes (Blanco Sinaloa '92, Blanoro, Combo 743, CUGA2054, HOGA067, CUGA3168, CUGA08-1210, CUGA09-3160, R-12-1509 and R-12-1507) were inoculated by root immersion in a suspension of mycelial fragments of two isolates (high and low virulence) of each fungus: *Fusarium languescens*, *M. phaseolina*, *S. rolfsii* and *S. sclerotiorum*. Disease severity evaluation was performed 30 days after inoculation. The entire experiment was performed twice.
Results: The genotypes showed greater susceptibility to *S. sclerotiorum* and *S. rolfsii* compared to *F. languescens* and *M. phaseolina*. The highly virulent isolates caused a significant difference in the severity of the disease in the genotypes evaluated.
Findings/conclusions: All chickpea genotypes showed susceptibility to the fungal complex that causes wilt.

Keywords: *Fusarium*, *Rhizoctonia*, *Sclerotinia*, *Sclerotium*, genotypes.

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INTRODUCTION

Chickpea (*Cicer arietinum* L.) is the third most important legume in the world, after bean and pea (Vishruta and Nath, 2021), and it is sown in more than 50 countries (Sunkad *et al.*, 2019). It is the only species of the *Cicer* genus that is apt for cultivation and has the capacity of increasing soil fertility, particularly dry soil through atmospheric nitrogen fixation (Jendoubi *et al.*, 2017). Nutritionally, chickpea is high in protein,

dietary fiber and essential minerals, and therefore, it plays a critical role in the fight to reduce hunger and malnutrition in developing countries (Jha *et al.*, 2020; Mwape *et al.*, 2021a). Different biotic and abiotic factors negatively affect the global production of chickpea. Among the main limitations in the production of this crop, there is root rotting and wilting caused by a fungi complex which originates in the soil, including *Fusarium oxysporum*, *Macrophomina phaseolina*, *Sclerotium rolfsii* and *Sclerotinia sclerotiorum*, and these depend on the moment of infection and the amount of inoculum in the soil, reducing production in up to 100% (Rai *et al.*, 2022; Kamthe *et al.*, 2023). Wilting caused by *Fusarium* is one of the most severe diseases in this crop, causing losses in yield of up to 100% under favorable conditions for the infection (Rana *et al.*, 2023). The disease has been associated primarily with *Fusarium oxysporum*, although the phylogenetic evidence points to *Fusarium oxysporum* being a compound of cryptic species (Laurence *et al.*, 2014). It should be mentioned that the *Fusarium* genus includes at least 300 phylogenetically different species (Dongzhen *et al.*, 2020). On the other hand, *M. phaseolina* and *S. rolfsii* are present in the entire world, affecting more than 500 species of plants in more than 100 families (Marquez *et al.*, 2021; Napte *et al.*, 2021). Meanwhile, *S. sclerotiorum* is a necrotrophic fungus with a range of hosts of approximately 600 species of plants and can cause up to 100% of losses in chickpea (Mwape *et al.*, 2021b).

The management of root rotting and wilting in chickpea is difficult, since there is not an effective control measure and it should be done through an integrated management program, including the use of varieties that are tolerant to the disease (Khalifa *et al.*, 2022). Because of this, the objective of this study was to evaluate the response from 10 chickpea genotypes from the improvement program of the National Institute for Forestry, Agriculture and Livestock Research (Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias, INIFAP), to the damage caused by the phytopathogenic fungi complex.

MATERIALS AND METHODS

To prepare the inoculum, two fungal isolates of *Fusarium languescens* (belonging to the complex of the species *F. oxysporum*), *M. phaseolina*, *S. rolfsii* and *S. sclerotiorum* were grown in PDA medium at 25 °C for 10 days. The isolates from each species were distinguished previously according to their level of virulence. In this study, one with high virulence was used and another with low virulence, from each fungus species. The inoculate suspension was adjusted to a concentration of 1×10^5 mycelium fragments mL^{-1} and Tween 20® was added. The roots of the seedlings from the different chickpea genotypes at 15 days of age (Blanco Sinaloa '92, Blanoro, Combo 743, CUGA 2054, HOGA 067, CUGA 3168, CUGA-1210, CUGA09-3160, R-12-1509 and R-12-1507), were inoculated by immersion in the mycelium fragment suspension for 15 minutes. Once the time had passed, the seedlings of the different genotypes were transplanted into pots with sterilized substrate and kept in a greenhouse at temperature of 15 to 35 °C for 30 days. Assessment of the severity of the disease was conducted with a visual scale of 5 categories, where 0=healthy plant, 1≤25%, 2=26-50%, 3=51-75%, 4≥75%. The experiment had a completely random block design with arrangement in divided plots (4 pathogens × 10 genotypes) with 12

replicas. The data obtained were analyzed through analysis of variance and the means comparison was conducted with Tukey's test ($P \leq 0.05$) using the statistical package SAS (version 9.3). The varieties were classified through the statistical method of means cluster analysis, for which the fungi isolates and their level of virulence were used as clustering variables.

RESULTS AND DISCUSSION

All the chickpea genotypes evaluated showed susceptibility to the four species of phytopathogenic fungi, and they presented symptoms of yellowing and wilting. These symptoms have been reported in various studies with artificial inoculations of *Fusarium oxysporum* sensu lato, *M. phaseolina*, *S. rolfsii* and *S. sclerotiorum* in the chickpea crop (Manjunatha and Saifulla, 2018; Lamont and Bennett, 2019; Hale *et al.*, 2020; Babariya and Nath, 2021). A significant difference was found between the chickpea genotypes that were inoculated with highly virulent isolates, and no significant difference was observed between the genotypes evaluated with low virulence isolates (Table 1. Severity of the disease caused by isolates from four phytopathogenic fungi with different level of virulence in 10 chickpea genotypes). In addition, difference was found between the isolates of high and low virulence from each of the species of fungi inoculated (Table 2. Means comparison of severity of the disease caused by isolates of four highly virulent phytopathogenic fungi in 10 chickpea genotypes; Table 3. Means comparison of severity of the disease caused by isolates of four pathogenic fungi with low virulence in 10 chickpea genotypes). The difference between the two levels of virulence and between the fungi species evaluated can be because of the different toxins produced by the phytopathogens to invade their host in the different stages of the crop (Rampersad, 2020; Singh *et al.*, 2021).

Table 1. Severity of the disease caused by isolates from four phytopathogenic fungi with different level of virulence in 10 chickpea genotypes.

Genotype	Disease severity	
	Isolates with high virulence	Isolates with low virulence
Blanco Sinaloa'92	3.63 ab*	3.56 a
Blanoro	3.52 b	3.56 a
HOGA067	3.63 ab	3.54 a
Combo 743	3.65 ab	3.42 a
CUGA2054	3.66 ab	3.54 a
CUGA09-3160	3.88 a	3.68 a
CUGA-3168	3.81 a	3.68 a
CUGA08-1210	3.63 ab	3.54 a
R-12-1507	3.65 ab	3.68 a
R-12-1509	3.65 b	3.60 a

*=Means with the same letter in the same column are not significantly different according to Tukey's test ($P > 0.05$).

Table 2. Means comparison of severity of the disease caused by isolates of four highly virulent phytopathogenic fungi in 10 chickpea genotypes.

Genotype	Disease severity			
	F. languescens	***M. phaseolina***	***S. rolfsii***	***S. sclerotiorum***
Blanoro	3.42 abcd*	2.83d	3.83 abc	4.00 a
Blanco Sinaloa'92	3.50 abcd	3.33 abcd	3.75 abc	3.92 ab
Combo 743	3.25 abcd	3.33 abcd	4.00 a	4.00 a
CUGA2054	3.33 abcd	3.50 abcd	4.00 a	3.83 abc
CUGA-3168	3.83 abc	3.58 abcd	3.92 ab	3.92 ab
CUGA09-3160	3.83 abc	3.67 abc	4.00 a	4.00 a
HOGA067	3.42 abcd	3.33 abcd	3.92ab	3.83 abc
CUGA08-1210	3.58 abcd	3.08 cd	3.92 ab	3.92 ab
R-12-1507	3.42 abcd	3.17 bcd	4.00 a	4.00 a
R-12-1509	3.42 abcd	3.17 bcd	4.00 a	4.00 a

*=Means with the same letter in the same column are not significantly different according to Tukey's test (P>0.05).

Table 3. Means comparison of severity of the disease caused by isolates of four pathogenic fungi with low virulence in 10 chickpea genotypes.

Genotype	Disease severity			
	F. languescens	***M. phaseolina***	***S. rolfsii***	***S. sclerotiorum***
Blanoro	3.42ab*	3.00 b	3.83 ab	4.00 a
Blanco Sinaloa'92	3.25 ab	3.75 ab	3.75 ab	3.50 ab
Combo 743	3.08 ab	3.00 b	3.67 ab	3.92 ab
CUGA2054	3.17 ab	3.67 ab	3.50 ab	3.83 ab
CUGA-3168	3.92 ab	3.17 ab	4.00 a	3.67 ab
CUGA09-3160	3.25 ab	3.58 ab	3.92 ab	4.00 a
HOGA067	3.58 ab	3.17 ab	3.67 ab	3.75 ab
CUGA08-1210	3.33 ab	3.50 ab	3.42 ab	3.92 ab
R-12-1507	3.33 ab	3.58 ab	4.00 a	3.83 ab
R-12-1509	3.00 b	3.42 ab	4.00 a	4.00 a

*=Means with the same letter in the same column are not significantly different according to Tukey's test (P>0.05).

The development of withering caused by *F. oxysporum* s. l., in chickpea can be influenced by the virulence, density of inoculate and environmental conditions. The high temperatures are critical for the development of wilting and the severity of the disease is higher at temperatures between 25-30 °C than 15-20 °C (Chen *et al.*, 2016). Regarding *M. phaseolina*, this fungus can cause losses in yields at high temperatures (30-35 °C) and low soil humidity (Rai *et al.*, 2022). Similarly, the disease caused by *S. rolfsii* is important in areas where the seedling is exposed by high temperatures and high soil humidity (Singh *et al.*, 2022). In the case of *S. sclerotiorum*, the disease is favored by temperatures <28 °C and conditions of high humidity (Willbur *et al.*, 2019).

Various studies mention the production of cell wall degrading enzymes by the fungi complex associated to chickpea wilting. In the case of *Fusarium* (FOSC), this production of enzymes allows the formation of a gelatinous mass that blocks the plant's nutrient and water transport system, causing the discoloration of the vascular system of the root (Sunkad *et al*., 2019). Similarly, *M. phaseolina* releases different toxins and enzymes inside the host, interrupting the plant's defense and resulting in dead cells and the establishment of the disease (Rai *et al*., 2022). In the case of *S. rolfsii*, this fungus produces oxalic acid, reacting synergistically with enzymes that cause maceration in the plant's tissue, in addition to inducing the production of oxygen reactive species including the radical superoxide, hydrogen peroxide, and hydroxyl radical, which damage the membrane and destroy cell organelles (Kumari *et al*., 2020b). Meanwhile, *S. sclerotiorum* secretes cell wall degrading enzymes that facilitate the penetration, maceration of the tissue, and degradation of components of the cell wall (Bolton *et al*., 2006).

According to the results, the isolate of *S. rolfsii* with high virulence caused the highest severity of the disease in the chickpea genotypes Combo 743, CUGA-2054, CUGA09-3160, R-12-1507 and R-12-1509, which is because of its prolific mycelium growth and its capacity to produce sclerotia (Shirsole *et al*., 2019). A similar behavior was observed with the isolates of *S. sclerotiorum* toward the genotypes Blanoro, Combo 743, CUGA09-3160, R-12-1507 and R-12-1509, which is due to its known aggressiveness and ability to attack, colonize and cause damage (Mwape *et al*., 2021a). In contrast, the isolate of *M. phaseolina* with high virulence caused lower level of severity of the disease in the genotype Blanoro; however, Sharma *et al*. (2016) mentioned that the pathogenic variability of *Macrophomina* is due to mutations, hyphae fusions, and mitotic recombination (Manjunatha and Saifulla, 2018). Various studies have reported the production of sclerotia by *S. rolfsii* (Shirsole *et al*., 2019), *S. sclerotiorum* (Bolton *et al*., 2006) and *M. phaseolina* (Mirchandani *et al*., 2023), which are resistance structures, as well as primary source of inoculum for the development of the disease. Sharma *et al*. (2016) reported for *M. phaseolina*, a significant relationship between the formation of sclerotia, the density and the severity of the disease. In the case of *F. oxysporum* s. l., it produces chlamydospores, and the soil infested by these structures is a source of primary inoculum for the development of withering (Jiménez-Díaz *et al*., 2015).

Based on the cluster analysis integrated by genotypes in function of each species of phytopathogenic fungus evaluated and their degree of virulence, three groups were observed: cluster 1 which grouped 2 genotypes (Blanoro and Combo 743), cluster 2 which grouped 7 genotypes (BS'92, CUGA-2054, HOGA-067, CUGA08-1210, R-12-1507, R-12-1509 and CUGA09-3160), and cluster 3 which grouped only the genotype CUGA-3168. In this analysis, Blanoro and Combo 742 were the genotypes that presented the lowest degree of susceptibility to phytopathogens, while the genotype CUGA-3168 presented the highest degree of susceptibility (Figure 1).

The management of wilting caused by *Fusarium* (FOSC) is difficult despite crop rotation or application of fungicides, because of the nature of this pathogen which originates in the soil. In the case of *M. phaseolina*, *S. rolfsii*, *S. sclerotiorum*, something similar happens to what has been described for *Fusarium*, because this group of fungi cannot be controlled efficiently through the use of chemical, physical and cultural control due to its wide range

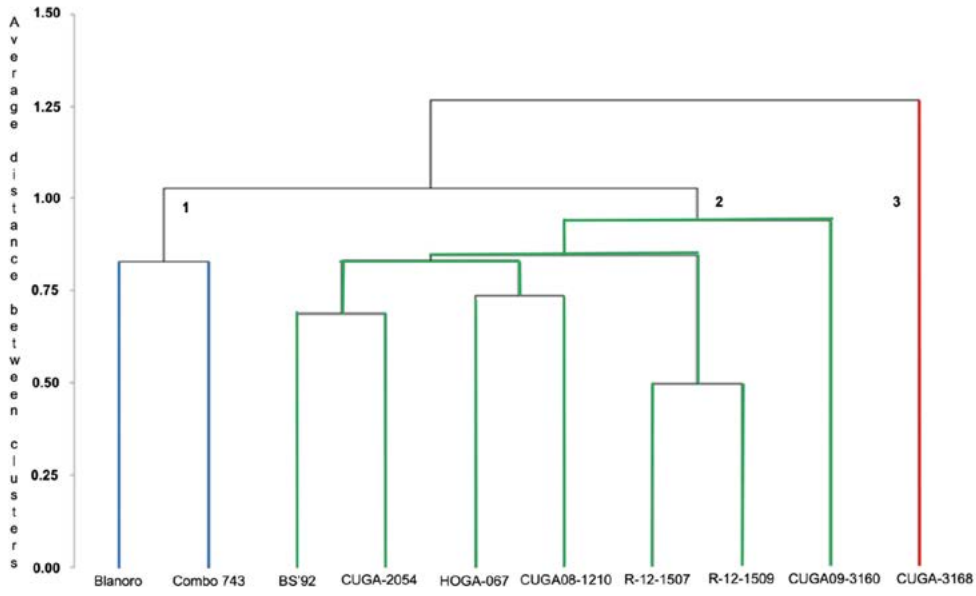

Figure 1. Number of groups integrated between varieties in function of the species of fungi and isolates evaluated.

of hosts and its survival in the soil for long periods of time in form of sclerotia. Therefore, the method with greatest feasible effectiveness and economically viability is the use of tolerant chickpea genotypes (Khalifa *et al.*, 2022; Sheshma *et al.*, 2022).

Diverse studies have conducted the search for sources of resistance to *F. oxysporum* s. l. (Rana *et al.*, 2023), *M. phaseolina* (Mirchandani *et al.*, 2023), *S. rolfsii* (Vishruta and Nath, 2021) and *S. sclerotiorum* (Mwape *et al.*, 2021a) in genotypes of *Cicer* spp.; however, only some genotypes of chickpea resistant to *F. oxysporum* s. l. (Rana *et al.*, 2023) have been found. Therefore, there is a need to continue with the search for sources of resistance to the diverse fungi which originate in the soil that affect the chickpea crop, with the aim of including this resistance to the future chickpea varieties to develop in Mexico.

CONCLUSIONS

The highly virulent isolates of *F. languescens*, *M. phaseolina*, *S. rolfsii* and *S. sclerotiorum* caused higher degree of severity of the disease in the different chickpea genotypes, compared to the damage caused by the isolates with low virulence.

The isolates of *S. rolfsii* and *S. sclerotiorum* caused greater severity of the disease in the different genotypes of chickpea compared to the isolates of *F. languescens* and *M. phaseolina*.

The genotypes of Blanoro and Combo 743 were the ones that presented lower level of susceptibility in response to the infection caused by the four species of phytopathogenic fungi, while the genotype CUGA-3168 was the most susceptible among the 10 genotypes evaluated.

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