INDUCED PROTECTION BY *Rhizophagus intraradices* AGAINST *Fusarium* WILT OF TOMATO

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SUMMARY

Fusarium *wilt of tomato, caused by* Fusarium oxysporum *f. sp.* lycopersici *(Fol) is a common disease in tomato. The arbuscular mycorrhizal fungus (AMF)* Rhizophagus intraradices *(previously called* Glomus intraradices*) was evaluated on its bioprotective ability against Fol in tomato cv. Missouri plants. Pathogenicity assays with Fol isolates from races 1, 2 and 3, showed that races 2 and 3 are the most aggressive for this cultivar, being the isolate R_3C_5 from race 3 the most virulent one. The results using this isolate showed that root rot index (RRI) caused by Fol is lower in tomato mycorrhizal plants grown under a low phosphate (20µM) fertilization regime (Fol +AMF +Pi 20µM) compared to non-colonized plants (Fol +Pi 20µM). Fol infested plants subjected to high phosphate (200µM) fertilization (Fol +Pi 200µM) had an intermediate RRI value that was not significantly different in the two treatments. These findings suggest that* R. intraradices *confers tolerance to Fol in tomato cv. Missouri plants and that this mechanism may partially be influenced by improved phosphate nutrition.*

Introduction

Tomato is an important horticultural species worldwide (Giaconi and Escaff, 1995). This crop is cultivated in more than 100 countries with a yearly production close to 2×10^6 metric tons, and Mexico occupies the tenth place (FAOSTAT, 2010). During the autumn-winter 2009-2010 crop season, a total of 53572ha of tomato were cultivated in Mexico, and Sinaloa State contributed with 28.63% of the grown area in the country (SIAP, 2010).

Tomato is susceptible to a wide variety of plagues and diseases, such as the ones caused by pathogenic fungi (Apodaca-Sánchez *et al*., 2002; Carrillo-Facio *et al*., 2003). The most important fungal disease in tomatoes is *Fusarium* wilt caused by *Fusarium oxysporum* Schlechtend.: Fr. f. sp. *lycopersici* (Sacc.) W.C. Snyder & H.N. Hans (Fol), which can affect yield up to 60%, as well as fruit quality (Agrios, 2004). Three pathogenic races have been described for this fungus: race 1 (Saccardo, 1886), race 2 (Alexander and Tucker, 1945) and race 3 (Grattidge and O´brien, 1982). *Fusarium* wilt of tomato is a hypoplastic disease, which causes reduced development, and is similar in most respects to vascular fusarioses of various other plants. However, in *Fusarium* wilt no cortical necrosis of the root system occurs under any set of environmental conditions that have been tested (Walker and Foster, 1946).

Practices to control Fol disease include the employment of chemical fungicides (Song *et al*., 2004). However, their application is becoming more restricted due to the negative side effects on the environment and human health. Other alternatives are the use of resistant varieties, management practices such as flooding and solarization, and most recently, biological control (Mandal *et al*., 2009). The most commonly used microorganisms for biological control include some fungal and bacterial species, such as *Trichoderma* and *Bacillus* spp. (González *et al*., 2004). Some of the microorganisms exhibiting biological control of Fol are able to develop symbiotic interactions with plants, such as the arbuscular mycorrhiza fungi (Steinkellner *et al*., 2011), a mutualistic interaction that is established between members of the phylum Glomeromycota and vascular plants (reviewed in Harrison, 1997). Arbuscular mycorrhiza fungi (AMF) are an essential component of the rhizosphere and they have been identified as organisms causing beneficial effects in growth and yield of different crops (Cavagnaro *et al*., 2006). AMF improve the uptake of different nutrients such as phosphorus, nitrogen, micronutrients and others (Clark and Zeto, 2000; Javaid, 2009). AMF play an important role in disease tolerance (Akhtar and Siddiqui, 2008) and have been used to decrease soil pathogen incidence from genera such as *Aphanomyces*, *Cylindrocladium*, *Fusarium*, *Macrophomina*, *Phytophthora*, *Pythium*, *Rhizoctonia* and *Verticillium* (Harrier and Watson, 2004).

There is a wide range of plant diseases that AMF are able to affect by diminishing their effects. Pepper plants pre-colonized with *Rhizophagus intraradices* (previously named *Glomus intraradices*) showed lower severity and higher survival (100%) of the

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PROTECCIÓN INDUCIDA POR *Rhizophagus intraradices* CONTRA LA MARCHITEZ DEL TOMATE CAUSADA POR *Fusarium*

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RESUMEN

La marchitez del tomate causada por Fusarium oxysporum *f. sp.* lycopersici *(Fol) es una enfermedad común del tomate. El hongo micorrízico arbuscular (HMA)* Rhizophagus intraradices *(previamente denominado* Glomus intraradices*) fue evaluado en cuanto a su capacidad bioprotectora contra Fol en plantas de tomate cv. Missouri. Los ensayos de patogenicidad con aislados de Fol de las razas 1, 2 y 3, mostraron que las razas 2 y 3 fueron las más agresivas para este cultivar, siendo el aislado R_3C_5 de la raza 3 el más virulento. Los resultados de protección inducida usando R. intrarradices contra el aislado de Fol R_3C_5 mostraron que el índice de pudrición radical causado por Fol es más bajo en plantas de tomate micorrizadas (Fol +HMA +Pi 20µM) creciendo en un régimen de bajo fosfato (20µM) comparado a plantas no colonizadas con HMA (Fol +Pi 20µM). Las plantas infectadas con Fol y sometidas a fertilización con 200µM de fosfato (Fol +Pi 200µM) tuvieron un valor del índice de pudrición radical intermedio que no fue diferente estadísticamente en los dos tratamientos. Esto sugiere que* R. intraradices *confiere tolerancia a Fol en plantas de tomate cv. Missouri y que este mecanismo puede ser parcialmente influido por una mejora en la nutrición fosfatada.*

PROTEÇÃO INDUZIDA PELO *Rhizophagus intraradices* CONTRA O APODRECIMENTO DE TOMATE CAUSADO PELO *Fusarium*

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RESUMO

O apodrecimento causado pelo Fusarium oxysporum *f. sp.* lycopersici *(Fol) é uma doença frequente do tomate. O fungo micorrizico arbuscular (FMA)* Rhyzophagus intraradices*, (antes denominado* Glomus intraradices*) fue avaliado por sua capacidade bio-protetora contra o Fol nas plantas de tomate cv Missouri. Os testes de patogenicidade com as amostras do Fol das cepas 1, 2 y 3 mostraram que as cepas 2 y 3 foram mais agressivas para este tipo de cultivar, sendo as amostras R_3C_5 da cepa 3 as que são mais virulentas. O resultados de proteção induzida usando* R. intrarradices *contra isolado Fol R_3C_5 revela que o índice de podridão radical causado pelo Fol é mais baixo nos cultivos de tomate micorrizadas (FOL +FMA +Pi 20µM) crescendo num meio baixo em fosfato (20µM) comparado a plantas nao enxertadas com FMA (FOL +Pi 20µM). As plantas tocadas com o FOL e submetidas a fertilização com 200µM de fosfato (FOL +Pi 200µM) tiveram um valor de índice de podridão radical intermediario, que nao difere estatisticamente nos dois tratamentos. Isto revela que* R. intraradices *concede tolerancia a Fol nos cultivos de tomate cv. Missouri, e que este mecanismo pode ser parcialmente beneficiado pela melhoria na nutrição fosfatada.*

plants 12 days after infection than non-colonized plants (Espinosa-Victoria *et al*., 2004). *Medicago truncatula* plants associated to *R. intraradices* showed a tolerance increase against *Xanthomonas campestris* pv. *vesicatoria*, which was correlated with the differential regulation of genes involved in defense responses in the aerial part of the plants (Liu *et al*., 2007). A significant decrease in severity and incidence of *F. solani* in common bean has also been documented when using a mixture of AMF that included *R. intraradices*, *Glomus mosseae*, *G. clarum*, *Gigaspora gigantea* and *Gi. margarita* (Askar and Rashad, 2010). Tomato plants infected with Fol showed reduced severity of disease 20 days post inoculation when associated with *Glomus macrocarpum* 75% and *G. fasciculatum* 78% (Kapoor, 2008). In tomato cv. Oogatafukuju, susceptible to Fol race 1, the inoculation with *G. etunicatum* suppressed the *Fusarium* propagule number in the rhizosphere soil of tomato and decreased the *Fusarium* wilt severity causing an increase in the numbers of both actinomycetes and bacteria, compared to the non-inoculated control (Ren *et al*., 2010).

In Sinaloa State, Mexico, *Fusarium* wilt of tomato caused by Fol has been detected over the last years causing over 50% crop loss in some fields (Apodaca-Sánchez, 2006). Fol isolates affecting tomato have been grouped in three races according to their ability for infecting different cultivars (Reis *et al*., 2004). Races 1, 2, and 3 were characterized and reported in Culiacan Valley, Sinaloa (Valenzuela-Ureta *et al*., 1996; Ascencio-Álvarez *et al*., 2008). Recent studies using genotyping of 26 Fol isolates collected in the 2008-2009 agricultural cycle in Sinaloa showed that 23 isolates belonged to race 3 and were widespread throughout Sinaloa, while the three isolates identified as race 2 came from the Culiacan Valley (Sánchez-Peña and Cauich-Pech, 2010). The main goal of this study was to evaluate the protective effect that AMF may exert against Fol. The present work, reports the bioprotective effect of *R. intraradices* against a highly virulent Fol race 3 isolate in tomato cultivar Missouri. In addition, some Fol isolates obtained from different tomato fields affected by *Fusarium* wilt in Culiacan Valley in 2004-2005 were characterized molecularly and their race identity confirmed by genotyping as belonging to races 1, 2 and 3.

Materials and Methods

Fol pathogenicity assays on tomato cv. Missouri

Fusarium isolates were obtained from infected tomato stem tissue collected in Culia-

can Valley, Sinaloa, Mexico, during the crop season 2004-2005. Once isolates were purified, they were maintained in sterile river sand/or crio-preserved in a sterile solution of glycerol 15% at -70°C. Isolates used were previously identified as Fol by ITS rDNA sequencing and races determined by differential tomato genotyping. To confirm susceptibility of the tomato cv. Missouri (Seminis), three isolates per race were selected for races 1 (R_1C_1, R_1C_2, R_1C_3) and race 2 (R_2C_2, R_2C_3, R_2C_5); and for race 3 two isolates were chosen (R_3C_2 and R_3C_5). Pathogenicity assays consisted in inoculating Fol isolates 21 days after germination of tomato seeds in pots. The pots contained 250g of a 3:1 mix of sterile sand and vermiculite. Fol inoculum was obtained from plates grown at 25°C on water-agar medium for two weeks. After conidia formation a suspension in sterile water was obtained and adjusted to 6×10^6 conidia/ml. Fol inoculation consisted on the application of 2ml of the conidia water suspension directly to 21-day seedling roots. The plants were grown under greenhouse conditions (~13h of natural light), with temperatures between 15 and 25°C. Irrigation and fertilization was performed once every three days using 50ml of Hoagland´s solution (Millner and Kitt, 1992). The substrate pH was typically 6.5. Twenty days post-inoculation the *Fusarium* wilt symptoms were evaluated according to a severity scale designed to evaluate symptoms on the aerial part of the plant (Marlatt *et al*., 1996). The pathogenicity assay consisted in a complete randomized design with five replicates per treatment, nine treatments in total (three isolates per race) and a control. Experiments were performed twice with similar results.

Bioprotection of tomato plants with R. intraradices against Fol

The material used in this study was obtained from monoxenic cultures consisting of hairy root cultures of *Daucus carota* clone DC2 colonized with the AM fungal species *R. intraradices* (Bécard and Piché, 1992). Spores produced under these *in vitro* conditions were used for inoculation of tomato roots. *R. intraradices* was established in culture with transformed carrot roots as described by Bécard and Fortin (1988) and Doner and Bécard (1991). Spore suspensions were prepared as previously described by St-Arnaud *et al*. (1996). Six hundred *R. intraradices* spores were applied to each tomato cv. Missouri plant eight days post-germination. Plants not receiving AMF spores were mock inoculated (these received the last spore wash containing residual spore exudates). Plants were watered and fertilized with half ionic strength Hoagland´s solution modified with 20 (Millner and Kitt, 1992) and 200µM (Chrispeels *et al*., 1999) phosphate; this last concentration was used for the high phosphate treatments. The high phosphate treatment normally inhibits AMF colonization, but it becomes an important control in mycorrhizae research since it is used as a control of the nutritional status of the plant, allowing discrimination between physiological responses caused by AMF colonization or affected by phosphate, which includes biotic responses such as pathogen defense. The plants were kept under greenhouse conditions (~13h under natural light) from December to February, with temperatures between 12 and 25°C. Pots were watered three times a week with two fertilizations per week (50ml of Hoagland´s solution with the different phosphate treatments).

Forty days post-inoculation (dpi) of *R. intraradices*, plant roots were stained with trypan blue and used to calculate efficiency of colonization using the intersect method (McGonigle *et al*., 1990). At that time, plants were inoculated with 1ml of a water suspension containing 1.2×10^7 Fol conidia/ml from the R_3C_5 isolate, which showed the highest severity of the disease on the previous bioassay. A complete randomized design was applied using ten replicates per treatment. Six treatments were set up. A treatment with high phosphate (Pi 200µM); a treatment with high phosphate and the fungal pathogen (Fol +Pi 200µM); an absolute control (Pi 20µM); a fungal pathogen control (Fol +Pi 20µM); and two treatments with arbuscular mycorrhiza: AMF +Pi 20µM and Fol +AMF +Pi 20µM. Phosphate was supplemented as potassium phosphate.

Fifty five days post-inoculation, six out of the ten replicates were harvested for each treatment and were used to obtain dry weight of shoots and roots. Measurement of root rot index was based on the severity scale described by Gardezi *et al*. (2001). The rest of the replicates were harvested and their roots obtained and stained with trypan blue, after clarification of root tissue (Phillips and Hayman, 1970), to evaluate colonization efficiency of *R. intraradices* (McGonigle *et al*., 1990).

Statistical analysis of data

The data of experiments with completely randomized designs were analyzed by ANOVA test and the means were separated using the minimum significant differences test (LSD) and considered significant at $P\leq0.05$. SAS version 9.0 was used for statistical analyses.

Results

Fol pathogenicity assays on tomato cv. Missouri

Tomato cv. Missouri was susceptible to R_2C_2, R_2C_3, R_3C_2 and R_3C_5 isolates, which were identified as Fol races 2 and 3 and tolerant to R_1C_1, R_1C_2, R_1C_3 isolates which are Fol race 1 and to the Fol 2 isolate R_2C_5 (Table I). Forty five days after inoculation with R_1C_1, R_1C_2, or R_1C_3 race 1 isolates, plants only showed slight rot symptoms and some shortening with respect to the control (Figures 1a, b). Severity index analysis (Marlatt *et al*., 1996) showed that they were statistically similar between both control and all three Fol race 1 (R_1C_1, R_1C_2, R_1C_3) infected plants (Table I). R_2C_2 and R_2C_3 Fol isolates showed slight unilateral wilting in older leaves (Figure 1c). Fol race 3 isolate R_3C_2 showed *Fusarium* wilt symptoms in tomato cv. Missouri and was different to control non-inoculated plants (Figure 1d). Fol race 3 isolate R_3C_5 showed the most severe symptoms with chlorosis, wilting, dwarfism and death of the plants (Figure 1e).

In terms of severity index of disease (Marlatt *et al*., 1996), the isolate belonging to race 3 R_3C_5 was statistically similar to the R_3C_2, R_2C_2 and R_2C_3 isolates (Table I). Race 3 isolates were different to the control plants, R_2C_5 and race 1 isolates (R_1C_1, R_1C_2, and R_1C_3). Tomato cv. Missouri was tolerant to R_1C_1, R_1C_2, R_1C_3 and R_2C_5 isolates, showing very mild symptoms that were not statistically different from the control non-inoculated plants and a lower tolerance to race 2 isolates (R_2C_2 and R_2C_3). Race 3 Fol isolates were the most

TABLE I
Fusarium WILT OF TOMATO MEASURED AS SEVERITY INDEX (MARLATT *ET AL*., 1996) IN TOMATO SEEDLINGS CV. MISSOURI 45 DPI AFTER INOCULATION WITH FOL ISOLATES

Isolate	Severity index
Control	1.0 c
R1C1	1.8 bc
R1C2	1.8 bc
R1C3	1.8 bc
R2C2	2.4 ab
R2C3	2.6 ab
R2C5	1.8 bc
R3C2	2.8 ab
R3C5	3.4 a

Similar letters indicate that the treatments with those isolates do not differ statistically. The data were analyzed by ANOVA test and the means were separated using LSD, $\alpha= 0.05$.

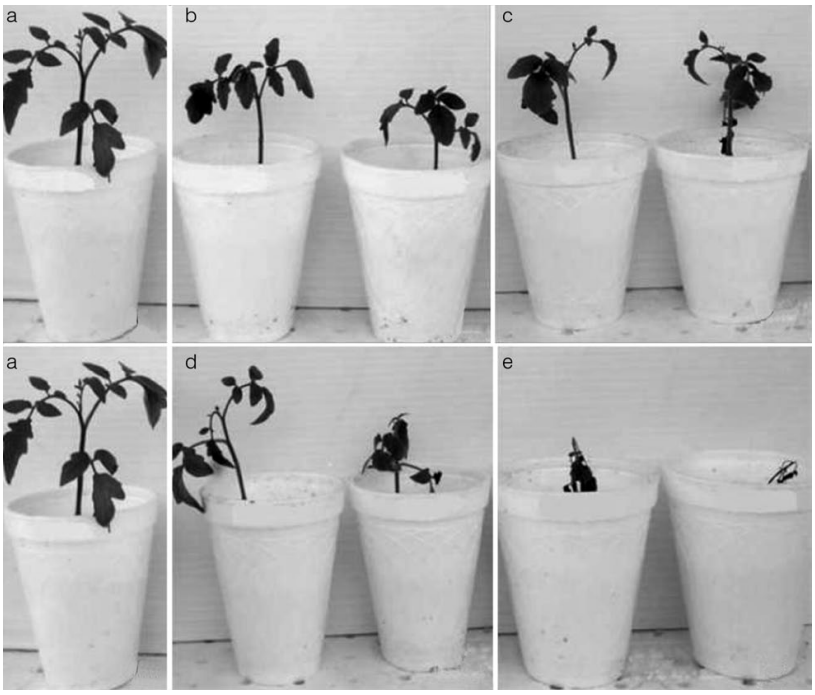

Figure 1. Pathogenicity tests with different Fol races on tomato cv. Missouri plants. a: non-inoculated control, no apparent symptoms were observed (severity class 1); b: Fol isolates R_1C_1 (left) and R_1C_2 (right), only some wilting of the leaves and shortening of the stem is observed; c: Fol isolates R_2C_2 (left) and R_2C_3 (right), unilateral wilting of the foliar tissue is shown in basal leaves; d: Fol isolate race 3 (R_3C_2), show shortening and high levels of wilting and shortening of the plant; e: Fol isolate race 3 (R_3C_5), which showed the highest levels of pathogenicity.

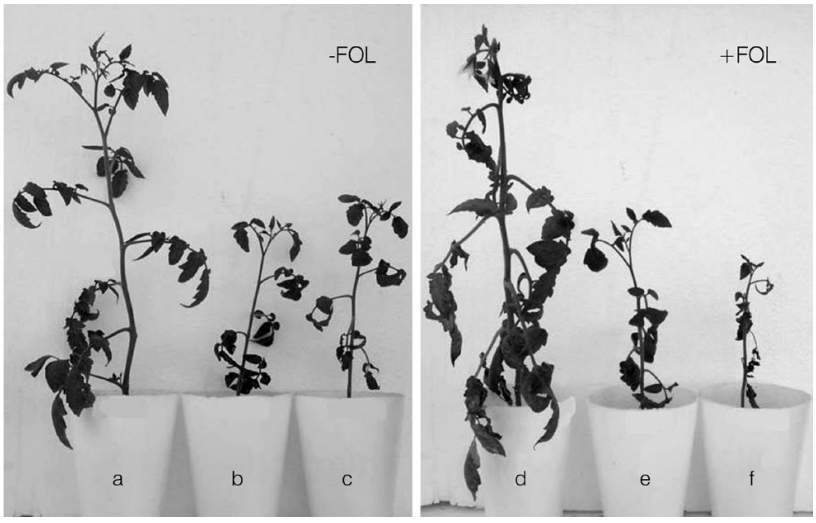

Figure 2. Bioprotection assays in tomato cv. Missouri, 45 days post-inoculation of Fol isolate R_3C_5, average temperature 15-25°C. a: Pi 200µM; b: AMF +Pi 20µM; c: Pi 20µM; d: Fol +Pi 200µM; e: Fol +AMF +Pi 20µM; f: Fol +Pi 20µM.

virulent to this cultivar, particularly isolate R_3C_5.

Disease protection of tomato plants with R. intraradices against Fol

Bioprotection assays were initiated once the fungal colonization with *R. intraradices* had been established for five weeks. Efficiency of colonization at this time averaged 23.33% of the root system. The Fol isolate used for this assay was R_3C_5, since it presented the highest level of damage to the plants when analyzed in the previous assay.

Typical Fol symptoms obtained at the end of this assay were coincident with those observed in the previous pathogenicity assays. Absolute control plants did not show any symptoms of Fol disease (Figure 2a). Even though all treatments without Fol did not present any characteristic symptoms associated to the pathogen, leaf rolling and dark leaf coloration was observed in those treatments were Pi 20µM was used (Figures 2b, c). These symptoms were attributed to nutritional stress for phosphate (Taiz and Sieger, 2010) and the limitations of growth in the root tissue due to the length of time that the plants needed to be maintained in the pots. Control plants inoculated with Fol +Pi 20µM suffered plant death (not shown) and, in some cases, moderate chlorosis, wilting, shortening and defoliation compared to the absolute control plants (Figure 2d).

Uninfested AMF colonized plants (AMF +Pi 20µM) did not show any disease symptoms (Figure 2b) and showed a better foliar appearance with respect to the absolute control plants (Pi 20µM; Figure 2c). In Fol infested mycorrhizal plants (Fol +AMF +Pi 20µM) symptoms were found only in one out of five plants, showing chlorosis, wilting and plant shortening. The other replicates only showed a slight wilting of basal leaves, and chlorosis was absent (Figure 2e).

Non-inoculated plants with 200µM phosphate (Pi 200M) did not show any symptoms and developed more biomass compared to the plants grown on 20µM phosphate either AMF colonized (Figures 2e, f) or non-colonized (Figures 2b, c). Two out of five plants infested with Fol (Fol +Pi 200µM) and fertilized with 200µM phosphate showed severe chlorosis, wilting and plant shortening (not shown) with respect to the Fol uninfested ones with high phosphate control (Pi 200µM; Figure 2a). The other replicates showed wilting in basal leaves and plant shortening (Figure 2d).

Biomass measured as dry weight in control plants and plants infected with Fol, and colonized or not-colonized with AMF, did not differ statistically among all 20µM phosphate treatments (Table II). Variance analysis for biomass in shoots and roots showed that plants grown under a high phosphate regime developed much better and were statistically different when compared to the 20µM plant treatments.

R. intraradices decreased significantly the disease severity measured as root rot index (RRI; Gardezi *et al*., 2001) of AMF colonized plants growing in Fol infested soil. Arbuscular mycorrhiza decreased plant damage under a low phosphate fertilization regime (20µM). The RRI in Fol infested plants grown under high phosphate (Fol +Pi 200µM) was statistically similar to both the Fol +Pi 20µM and the Fol +AMF +Pi 20µM low phosphate treatments, whereas these last two conditions were different among them (Table II).

By the end of the experiments (75 days post inoculation with *R. intraradices*) when differential responses were observed, plants colonized with AMF (Fol +AMF +Pi 20µM; AMF +Pi 20µM) had an efficiency of colonization of 93.3 and 90% respectively (Table II). All types of AMF internal structures and a good external hyphal network

TABLE II
BIOPROTECTION OF TOMATO PLANTS INOCULATED WITH *Rhizophagus intraradices* AGAINST FOL 45 DPI AFTER INOCULATION WITH FOL ISOLATE R_3C_5

Treatments	Shoot dry weight (g)	Root dry weight (g)	RRI*	Myc (%)
Pi 20µM	0.845 b	0.447 b	1.0 c	0.0
Fol +Pi 20µM	0.593 b	0.290 b	3.6 a	0.0
AMF +Pi 20µM	0.863 b	0.472 b	1.0 c	90.0 a
Fol +AMF +Pi 20µM	0.755 b	0.437 b	2.3 b	93.3 a
Pi 200µM	3.223 a	2.907 a	1.0 c	0.0
Fol +Pi 200µM	3.680 a	4.078 a	3.0 ab	0.0

*According to the scale reported by Gardezi *et al.* (2001).
Similar letters indicate that treatments with those isolates do not differ statistically. The data were analyzed by ANOVA test and the means were separated using LSD, α= 0.05. RRI: root rot index, Myc: mycorrhization.

were observed at this time (data not shown).

Discussion

In several regions of the world, tomato plants are attacked by Fol, generating great economical losses (Ascencio-Álvarez *et al*., 2008). This disease is considered one of the most important affecting this crop, and its control can be difficult. This difficulty has stimulated the search for alternatives of biological control (Fravel *et al*., 2003).

We tested the susceptibility of tomato cv. Missouri in pathogenicity assays and found that it was tolerant to race 1, but susceptible to races 2 and 3 of Fol. A race 3 isolate (R_3C_5) was identified as the most pathogenic one to tomato cv. Missouri (Table I) among the tested isolates.

The interaction between plant roots and arbuscular mycorrhiza can increase the tolerance to some biotic and abiotic stress factors. Different mechanisms involved in this tolerance include a higher capability of the arbuscular mycorrhiza to incorporate water, toxic compounds, mineral nutrients and to tolerate diseases (Augé 2001; Elsen *et al.,* 2003; Quoreshi and Khasa, 2008; Hernández-Ortega *et al*., 2012). The bioprotective effect of *R. intraradices* against Fol (isolate R_3C_5) showed typical Fol invasion symptoms in some plants (data not shown). Nevertheless, at the end of the bioassay the symptoms were less severe than in non-mycorrhizal plants infected with Fol (Figure 2). Similar observations are described with other biological control agents, such as *Paenibacillus lentimorbus* and *Trichoderma* sp. used against *F. solani* in tomato (González *et al*., 2004), as well as with arbuscular mycorrhiza used against *F. oxysporum* f. sp. *gladioli* in *Gladiolus grandiflorus* (Gardezi *et al*., 2001).

Previous work using *G. mosseae* (Steinkellner *et al*., 2011) and *R. intraradices* (Akköprü and Demir, 2005) showed a bioprotective effect against Fol. Fol infected plants colonized by *R. intraradices* showed higher phosphate levels either when alone or combined with different rhizobacteria compared to Fol infected plants grown under a low phosphate regime (Akköprü and Demir, 2005). This suggests that the severity of Fol disease might be affected by the phosphate status of the plants. In the present study it was found that arbuscular mycorrhiza caused a protective effect against Fol, but when compared with the plants growing in high phosphate it was observed that phosphate also increased tolerance to Fol (Table II). This suggests that the tolerance mechanism mediated by *R. intraradices* may be at least partially due to an improved phosphate status of the plant. These results agree with those of other authors who also suggest that the increased tolerance by AMF can be associated with improved nutrients, especially phosphate concentration (Davis and Menge, 1980; Graham and Menge, 1982). However, some researchers presumed that the increased tolerance by AMF might not be completely related to this factor (Caron *et al*., 1986; Akköprü and Demir, 2005; Kapoor, 2008). Several hypotheses have been proposed to explain the mechanisms of the increased resistance in mycorrhizal plants: improvement of plant nutrition (Davis and Menge, 1980; Graham and Menge, 1982), competition for space (Azcón-Aguilar and Barea, 1996), modified microbial flora in the rhizosphere (Filion *et al*., 1999; Ren *et al*., 2010), and induced systemic resistance in the plant (Whipps, 2004).

The profuse root colonization by *R. intraradices*, as shown in this study, could have helped to protect the plant tissue against the pathogen by competing for the same niche. At the end of the bioprotection assay the root tissue was completely colonized by the AMF (>90%). The AM fungal symbiont probably can compete for space with any other organisms that invade the root system when the percentage of root colonization is high. Nevertheless, in this case, at the time the plants were infected with Fol, the percentage of colonization was only 23%. Thus, explaining the induction of tolerance to Fol in tomato cv. Missouri only by space competition would be insufficient. It has been shown that the pattern of global gene expression is modified when a plant interacts with *R. intraradices* (Paszkowski *et al*., 2006; Liu *et al*., 2007), indicating that there is a systemic response that allows for the protection of the root system, as well as the induction of tolerance to foliar pathogens such as *Xanthomonas campetris* pv. *vesicatoria* in *Medicago truncatula* (Liu *et al*., 2007). This induced protection by AMF colonization is accompanied by an induction on the expression of a great number of phosphate-related and defense-related genes.

In conclusion, the present results suggest that AMF colonization shows a bioprotective effect against Fol in tomato cv. Missouri and that this mechanism may be partially mediated by an improved phosphate nutritional state. These findings suggest that AMF sometimes may act as bioprotective agents especially in soils where phosphate might be low or unavailable by increasing the nutritional status of the plants.

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REFERENCES

Agrios GN (2004) *Plant Pathology*. 4th ed. Academic Press. New York, USA. 635 pp.

Akhtar MS, Siddiqui ZA (2008) Biocontrol of a root-rot disease complex of chickpea by *Glomus intraradices*, *Rhizobium* sp. and *Pseudomonas straita*. *Crop Prot. 27*: 410-417.

Akköprü D, Demir S (2005) Biogical control of *Fusarium* wilt in tomato caused by *Fusarium oxysporum* f. sp. *lycopersici* by the AMF *Glomus intraradices* and some rhizobacteria. *J Phytopathol. 153*: 544-550.

Alexander LJ, Tucker CM (1945) Physiological specialization in the tomato wilts fungus *Fusarium oxysporum* f. sp. *lycopersici*. *J. Agric. Res. 70*: 303-313.

Apodaca-Sánchez MA (2006) Enfermedades causadas por *Fusarium oxysporum* en el tomate (*Lycopersicon esculentum*). In *Memorias del Curso de Enfermedades de Hortalizas*. Fundación Produce Sinaloa. Culiacán, Sinaloa, México 79 pp.

Apodaca-Sánchez MA, Zavaleta-Mejía E, García-Espinoza R, Osada-Kawosoe S, Valenzuela-Ureta JG (2002) Frecuencia de campos infestados con *Fusarium oxysporum* f. sp. *radicis-lycopersici* en Sinaloa, México y su control. *Mex. J. Phytopathol. 20*: 1-7.

Askar AA, Rashad YM (2010) Arbuscular mycorrhizal fungi: biocontrol agent against

common bean *Fusarium* root rot disease. *Plant Pathol. J. 9*: 31-38.

Ascencio-Álvarez A, López-Benítez A, Borrego-Escalante F, Rodríguez-Herrera SA, Flores-Olivas A, Jiménez-Díaz F, Gámez-Vázquez AJ (2008) Marchitez vascular del tomate: I. Presencia de razas de *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) Snyder y Hansen en Culiacán, Sinaloa, México. *Mex. J. Phytopathol. 26*: 114-120.

Augé RM (2001) Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis. *Mycorrhiza 11*: 3-42.

Azcón-Aguilar C, Barea JM (1996) Arbuscular mycorrhizas and biological control of soil-borne plant pathogens. An overview of the mechanisms involved. *Mycorrhiza 6*: 457-464.

Bécard G, Fortin JA (1988) Early events of vesicular-arbuscular mycorrhiza formation on Ri T-DNA transformed roots. *New Phytol. 108*: 211-218

Bécard G, Piché Y (1992) Establishment of vesicular-arbuscular mycorrhizal in root organ culture: review and proposed methodology. In Norris JR, Read DJ, Varma AK (Eds.) *Methods in Microbiology: Techniques for Study of Mycorrhiza*. Academic Press. New York, USA. pp 89-108.

Caron M, Fortin JA, Richard C (1986) Effect of phosphorus concentration and *Glomus intraradices* on *Fusarium* crown and root rot of tomatoes. *Phytopathology 76*: 942-946.

Carrillo-Facio JA, Montoya-Rodríguez TJ, García-Estrada RS, Cruz-Ortega JE, Márquez-Zequera I, Sañudo-Barajas AJ (2003) Razas de *Fusarium oxysporum* f. sp. *lycopersici* Snyder y Hansen, en tomate (*Lycopersicon esculentum* Mill.) en el Valle de Culiacán, Sinaloa, México. *Mex. J. Phytopathol. 21*: 123-127.

Cavagnaro TR, Jackson LE, Six J, Ferris H, Goyal S, Asami D, Scow KM (2006) Arbuscular mycorrhizas, microbial communities, nutrient availability, and soil aggregates in organic tomato production. *Plant Soil 282*: 209-225.

Clark RB, Zeto SK (2000) Mineral acquisition by arbuscular mycorrhizal plants. *J. Plant Nut. 23*: 867-902.

Chrispeels MJ, Crawford NM, Schroeder JI (1999) Proteins for transport of water and mineral nutrients across the membranes of plant cells. *Plant Cell 11*: 661-675.

Davis RM, Menge JA (1980) Influence of *Glomus fasciculatus* and soil phosphorus on *Phytophthora* root rot of citrus. *Phytopathology 70*: 447-452.

Doner LW, Bécard G (1991) Solubilization of gellan gels by chelation of cations. *Biotechnol. Techn. 5*: 25-28.

Elsen A, Baimey H, Swennen R, De Waele D (2003) Relative mycorrhizal dependency and micorhyza-nematode interaction in banana cultivars (*Musa* spp.) differing in nematode susceptibility. *Plant Soil 256*: 303-313.

Espinosa-Victoria D, González-Mendoza D, Placencia de la Parra J, García-Espinosa R (2004) Reducción de la incidencia de *Phytophthora capsici* Leo. en el sistema radical de plántulas de chile pre micorrizadas con *Glomus intrarradices*. *Terra Latinoam. 22*: 317-326.

FAOSTAT (2010) *Food and Agriculture Organization of the United Nations*. www.faostat.fao.org

Filion M, St-Arnaud M, Fortin JA (1999) Direct interaction between the arbuscular mycorrhizal fungus *Glomus intraradices* and different rhizosphere microorganisms. *New Phytol. 141*: 525-533.

Fravel D, Olivain C, Alabouvette C (2003) *Fusarium oxysporum* and its biocontrol. *New Phytol. 157*: 493-502.

Gardezi AK, Cetina-Alcalá VM, Ferrera-Cerrato R, Velásquez-Mendoza J, Pérez-Mercado CA, Larqué-Saavedra M (2001) Hongos micorrízicos arbusculares como componentes de control biológico de la pudrición causada por *Fusarium* sp. en gladiola. *Terra Latinoam. 19*: 259-264.

Giaconi V, Escaff M (1995) Cultivo de Hortalizas. 11th ed. Editorial Universitaria. Santiago, Chile. 337 pp.

González R, Montealegre J, Herrera R (2004) Biological control of *Fusarium solani* in tomato by the bioantagonists *Paenibacillus lentimorbus* and *Trichoderma* sp. *Cienc. Inv. Agr. 31*: 21-28.

Graham JH, Menge JA (1982) Influence of vesicular-arbuscular mycorrhizae and soil phosphorous on take-all disease of wheat. *Phytopathology 72*: 95-98.

Grattidge R, O´Brien RG (1982) Ocurrence of a third race of *Fusarium* wilt of tomatoes in Queensland. *Plant Dis. 66*: 165-166.

Harrier LA, Watson CA (2004) The potential role of arbuscular mycorrhizal (AM) fungi in the bioprotection of plant soil-borne pathogens in organic and/or other sustainable farming systems. *Pest Manag. Sci. 60*: 149-157.

Harrison MJ (1997) The arbuscular mycorrhizal symbiosis: an underground association. *Trends Plant Sci. 2*: 54-56

Hernández-Ortega HA, Alarcón A, Ferrera-Cerrato R, Zavaleta-Mancera HA, López-Delgado HA, Mendoza-López MR (2012) Arbuscular mycorrhizal fungi on growth, nutrient status, and total antioxidant activity of *Melilotus albus* during phytoremediation of a diesel-contaminate substrate. *J. Env. Manag. 95*: S319-S324.

Javaid A (2009) Mycorrhizal mediated nutrition in plants. *J. Plant Nutr. 32*: 1595-1618.

Kapoor R (2008) Induced resistance in mycorrhizal tomato is correlated to concentration of jasmonic acid. *On line J. Biol. Sc. 8*: 49-56.

Liu J, Maldonado-Mendoza IE, Lopez-Meyer M, Cheung F, Town C, Harrison M (2007) Arbuscular mycorrhizal symbiosis is accompanied by local and systemic alterations in gene expression and an increase in disease resistance in the shoots. *Plant J. 50*: 529-544.

Mandal S, Mallick N, Mitra A (2009) Salicylic acid-induced resistance to *Fusarium oxysporum* f. sp. *lycopersici* in tomato. *Plant Phys. Biochem. 47*: 642-649.

Marlatt M, Correll J, Kaufman P (1996) Two genetically distinct populations of *Fusarium oxysporum* f. sp. *lycopersici* race 3 in the United States. *Plant Dis. 80:* 1336-1342.

McGonigle TP, Miller MH, Evans DG, Fairchild DL, Swan GA (1990) A new method which gives an objective measure of colonization of roots by vesicular–arbuscular mycorrhizal fungi. *New Phytol. 115*: 495-501.

Miller SP, Kitt DG (1992) The Beltsville method of soil less production of vesicular-arbuscular mycorrhizal fungi. *Mycorrhiza 2*: 9-15.

Paszkowski U (2006) A journey through signaling in arbuscular mycorrhizal symbioses. *New Phytol. 172*: 36-46.

Phillips JM, Hayman DS (1970) Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Trans. Brit. Mycol. Soc. 55*: 157-160.

Quoreshi AM, Khasa DP (2008) Effectiveness of mycorrhizal inoculation in the nursery on root colonization, growth, and nutrient uptake of aspen and balsan poplar. *Biomass Bioenerg. 32*: 381-391.

Reis A, Giordano LB, Lopes CA, Boiteux LS (2004) Novel sources of multiple resistance to three races of *Fusarium oxysporum* f. sp. *lycopersici* in *Lycopersicon* germplasm. *Crop Breed. Appl. Biotechnol. 4*: 495-502.

Ren L, Lou Y, Sakamoto K, Inubushi K, Amemiya Y, Shen Q, Xu G (2010) Effects of arbuscular mycorrhizal colonization on microbial community in rhizosphere soil and *Fusarium* wilt disease in tomato. *Commun. Soil Sci. Plan. 41:* 1399-1410.

Saccardo PA (1886) *Sylloge Hyphomycetum*. *Sylloge Fungorum 4*: 705.

Sánchez-Peña P, Cauich-Pech SO (2010) Incidence of *Fusarium oxysporum* f. sp. *lycopersici* races in tomato in Sinaloa, Mexico. *Plant Dis. 94*: 1376.

SIAP (2010) *Servicio de Información Agro Alimentaria y Pesquera*. www.siap.gob.mx

Song WT, Zhou LG, Yang CZ, Cao XD, Zhang LQ, Liu XL (2004) Tomato *Fusarium* wilt and its chemical control strategies in a hydroponic system. *Crop Prot. 23*: 243-247.

St-Arnaud M, Hamel C, Vimard B, Caron M, Fortin JA (1996) Enhanced hyphal growth and spore production of the arbuscular mycorrhizal fungus *Glomus intraradices* in an *in vitro* system in the absence of the host roots. *Mycol. Res. 100*: 328-332.

Steinkellner S, Hage-Ahmed K, García-Garrido JM, Illana A, Ocampo JA, Vierheilig H (2011) A comparison of wild-type, old and modern tomato cultivars in the interaction with the arbuscular mycorrhizal fungus *Glomus mosseae* and the tomato pathogen *Fusarium oxysporum* f. sp. *lycopersici*. *Mycorrhiza* DOI: 10.1007/s00572-011-0393-z.

Taiz L, Zeiger E (2010) *Plant Physiology*. 5th ed. Sinauer. Sunderland, MA, USA. 782 pp.

Valenzuela-Ureta JG, Lawn DA, Heisey RF, Zamudio-Guzman V (1996) First report of *Fusarium* wilt race 3, caused by *Fusarium oxysporum* f. sp. *lycopersici*, of tomato in Mexico. *Plant Dis. 80*: 105.

Walker JC, Foster RE (1946) Plant nutrition in relation to disease development. III. *Fusarium* wilt of tomato. *Amer. J. Bot. 33*: 259-264.

Whipps JM (2004) Prospects and limitations for mycorrhizas in biocontrol of root pathogens. *Can J. Bot. 82*: 1198-1227.