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Resistance to *Phytophthora capsici* in manzano chili grafted onto CM-334, grown in infested soil, with applications of auxins and *Trichoderma harzianum*

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ABSTRACT

Background/Objective. *Phytophthora capsici* causes losses of up to 100 % in *Capsicum pubescens* and there are no resistant commercial varieties. A viable and sustainable alternative is to use the CM-334 rootstock (*Capsicum annuum*), which is universally resistant to *Phytophthora capsici*.

Materials and Methods. The following was studied: the root biomass of CM-334 when grafting the mazano chili hybrids ‘Maruca’, ‘Jhos’, and ‘Dali’, the resistance of the graft to *P. capsici* in infested soil and its yield (hybrid ‘Dali’), and the root biomass of CM-334 with applications of auxins and *T. harzianum*.

Results. As a rootstock, CM-334 exhibited 50, 53 and 75 % less root volume, fresh weight, and dry weight, respectively, compared to non-grafted hybrids. Using the CM-334 rootstock, there was no incidence of *P. capsici* and the yield decreased by 2 %, and even with *T. harzianum*, alone or in combination with 1200 ppm of IBA, the yield increased by 8 %. The grafted ‘Dali’ hybrid had 32, 50, 50, and 76 % less root length, volume, fresh weight, and dry weight, respectively, compared to the non-grafted hybrid; therefore, it is suggested to apply 1.25 kg ha^{-1} of *T. harzianum* and 1200 ppm of IBA every 20 days to improve root biomass.

Conclusion. Grafting manzano chili onto CM-334 is a viable and sustainable control alternative to reduce *P. capsici* incidence since none of the grafted plants showed wilt symptoms like the non-grafted ones, and the yield was the same as in the first production cycle, with the advantage that grafted plants produce more cycles (4 years), whereas the non-grafted ones die during the first cycle because of the oomycete.

Keywords: *Capsicum pubescens*, *Capsicum annuum*, chili wilt, IBA, root biomass.

INTRODUCTION

Phytophthora capsici is the causal agent of chili wilt, one of the most destructive diseases affecting the *Capsicum* genus (Majid *et al*., 2017). This oomycete can infect the crop at any stage of development, leading to loss of turgor, chlorosis, root rot, leaf drop, and plant death, with damage levels ranging from 10% to 100% (Jiménez-Camargo *et al*., 2018).

Among the alternative methods for controlling chili wilt is the use of resistant rootstocks (Zhao *et al*., 2011). The Serrano Criollo de Morelos 334 (CM-334) stands out for its high resistance to *P. capsici* (Castro-Rocha *et al*., 2016; Naegele and Hausbeck, 2020). However, the use of rootstocks may positively or negatively affect fruit yield and quality (Greathouse *et al*., 2021), possibly due to alterations in root architecture that reduce water and nutrient transport (Ropokis *et al*., 2019). Nevertheless, the exogenous application of auxins and *T. harzianum* can promote and accelerate the formation of adventitious roots in grafted plants (Saini and Deepanshu, 2023; Larios *et al*., 2019).

In manzano chili, Pérez-Grajales *et al*. (2021) confirmed the compatibility between hybrids of this chili type and the CM-334 rootstock under hydroponic conditions. However, it is necessary to evaluate the performance of grafted plants in the presence of *P. capsici*. Therefore, this study assessed the root biomass performance of CM-334 as a rootstock for the commercial manzano chili hybrids ‘Maruca,’ ‘Jhos,’ and ‘Dali’ under hydroponic conditions. Additionally, it evaluated the resistance of grafted ‘Dali’ hybrid plants on CM-334 versus non-grafted plants in soil infested with the oomycete. Furthermore, the effect of auxin and *T. harzianum* applications on fruit yield of the ‘Dali’ hybrid and root biomass of CM-334 was examined.

MATERIALS AND METHODS

Experimental site. The research was conducted under greenhouse conditions at the Universidad Autónoma Chapingo, Estado de México (19° 29’ 22.2” N, 98° 52’ 24.7” W, at an altitude of 2,250 m). The average levels of temperature, relative humidity, photosynthetically active radiation, and CO_2 were 18.7 °C, 68.9%, 525 µmol photons m^{-2} s^{-1}, and 450 ppm, respectively.

Root biomass of grafted and non-grafted hybrids grown in hydroponics. In March 2017, manzano chili plants of the hybrids ‘Maruca,’ ‘Jhos,’ and ‘Dali’ (registered with SNICS 1351, 1298, and 1299) were established in pots and hydroponics under greenhouse conditions, both grafted onto CM-334 and as non-grafted plants. The experiment followed a completely randomized design with six treatments (three grafted hybrids and three non-grafted) and seven replications per treatment, with one plant serving as the experimental and sampling unit.

The plants were cultivated for two years in a greenhouse under hydroponic conditions using a two-stem ‘V’ trellising system, as proposed by Pérez and Castro (2008). In May 2019, root biomass was measured for all six treatments. Roots were extracted from the pots, thoroughly washed with water, and weighed using a precision balance with 0.1 g sensitivity (Ohaus®, Mexico). Root volume was determined by water displacement in a

10 L container graduated in mL. Additionally, dry weight (g) was obtained by drying the samples in an oven (Teknolab®, Mexico) at 70 °C for 72 hours.

Fresh weight, volume, and dry weight data of the root treatments were analyzed using analysis of variance (ANOVA) and Tukey's multiple mean comparison test ($P \leq 0.05$). A Student's *t*-test ($P \leq 0.05$) was applied for comparing two populations (grafted and non-grafted plants). All statistical analyses were performed using SAS 9.1.3.

Resistance to *P. capsici*, fruit yield, and root biomass of grafted and non-grafted plants grown in soil

Morphological characterization of *P. capsici* isolated from soil. To determine the presence of *P. capsici* for use in the experiment, a sporangia recovery technique was developed using a composite sample of silty soil (1 kg) collected from a depth of 30 cm at five different points within a one-hectare plot (Lot X4) of the Fitotecnia experimental field at UACh. This site had a known history of oomycete presence based on symptoms observed in previous crops.

For the isolation of *P. capsici* from soil, the methodology proposed by Aristizábal and Torres (2015) was followed with some modifications. A 10 g soil sample was weighed in seven replicates and mixed with 90 mL of sterile distilled water. A 1 mL aliquot of this solution was then taken and diluted to 10 mL with sterile distilled water, a process repeated twice. A drop of the final dilution was placed in a sterile Petri dish filled with liquid PDA medium at room temperature and left to incubate for seven days to allow mycelial growth. The fungus was purified using the hyphal tip method. For sporangia formation, the isolate was transferred to Petri dishes containing V8-agar medium, where it remained for six days at 26 °C in complete darkness.

To induce oospore release, the oomycete was subjected to thermal stress, alternating two hours at 5 °C with one hour at 26 °C (Ortíz and Camargo, 2005). Sporangia measurements were taken by preparing microscope slides with coverslips, and the length and width of the papillae of 50 sporangia were recorded using the Motic Plus 3.0 software. Morphological identification was carried out using the tabular keys proposed by Martin *et al.* (2012).

Plant material. The plant material used as the scion was the 'Dali' hybrid, selected for its high yield and fruit quality, registered under SNICS 1299. The rootstock was the Serrano Criollo de Morelos 334 (CM-334) cultivar, known for its universal resistance to *P. capsici*. The grafting technique was performed according to the method described by Pérez-Grajales *et al.* (2021).

Management of grafted and non-grafted plants. Seventy days after grafting, both grafted and non-grafted 'Dali' hybrid plants were transplanted into 50 × 50 cm plastic bags (600-gauge) filled with pre-analyzed soil. The plants were cultivated and managed in a greenhouse, as proposed by Pérez and Castro (2008). Three months after transplantation, applications of 4-indole-3-butyric acid (IBA) and *T. harzianum* (Th) were initiated, targeting the roots of both grafted and non-grafted plants for four months, according to the following treatments: 1) 0.18 g of *T. harzianum* per plant every 20 days; 2) 0.18 g of *T. harzianum* combined with 1200 ppm of IBA every 20 days; 3) IBA at 1600 ppm every 15 days; 4) IBA at 1600 ppm every 30 days; 5) IBA at 1600 ppm every 45 days; 6) IBA at 1200 ppm every 15 days; 7) IBA at 1200 ppm every 30 days; 8) IBA at 1200 ppm every 45 days; 9) IBA at 800 ppm every 15 days; 10) IBA at 800 ppm every 30 days; 11) IBA at 800 ppm every 45 days; and 12) the control group with no application of auxins or *T. harzianum*. The experiment followed a completely randomized design with four replications, with one plant serving as the experimental and sampling unit.

Incidence evaluation and AUDPC. The incidence evaluation was carried out by identifying plants showing typical oomycete symptoms and comparing them to the total number of plants in the experiment. To assess damage severity, an arbitrary scale was established based on the percentage of foliar wilting (Figure 1). Using these data, the severity percentage of *P. capsici* infection was calculated, along with the area under the disease progress curve (AUDPC), using the trapezoidal integration method proposed by Shaner and Finney (1977). The data were analyzed using analysis of variance (ANOVA) and Fisher's LSD multiple mean comparison test (P≤0.05) with the SAS 9.1.3 statistical package.

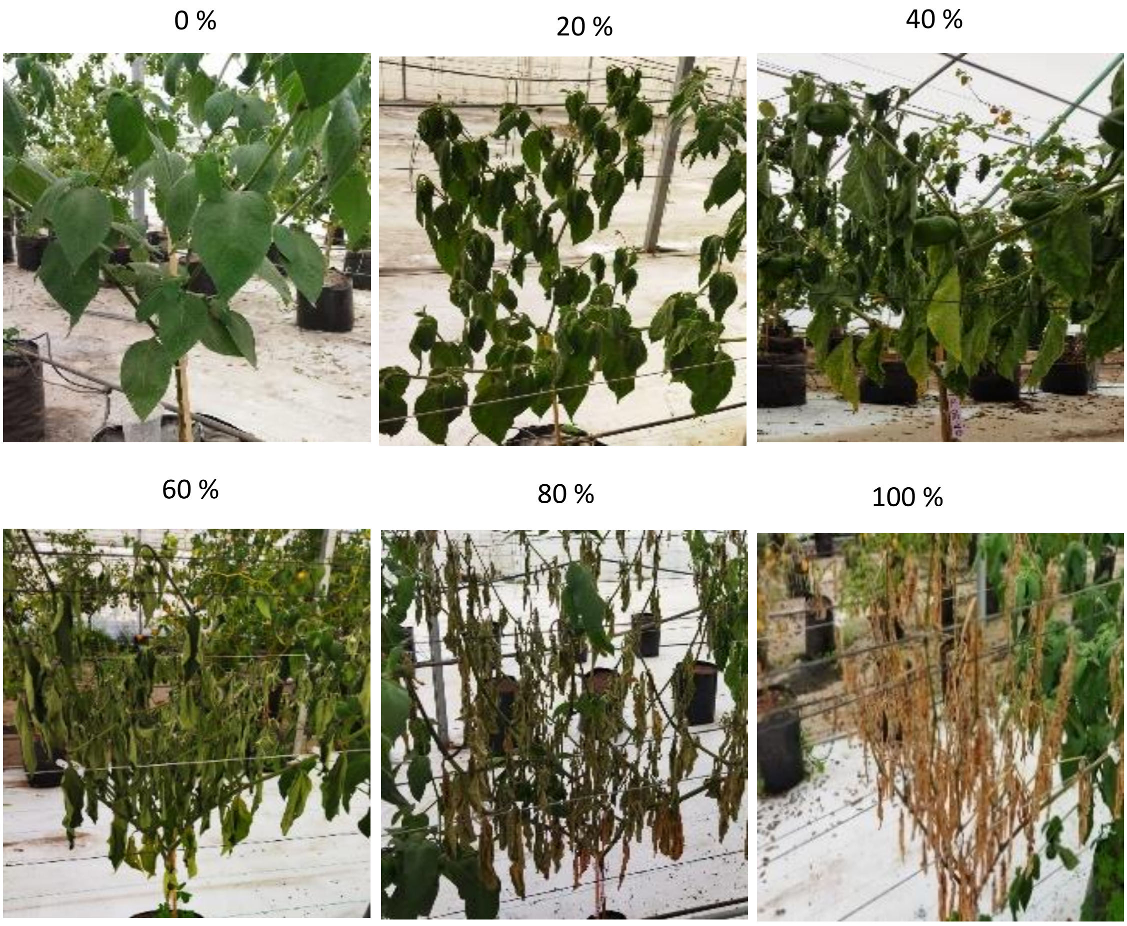

Figure 1. Arbitrary scale for evaluating the incidence and severity caused by *P. capsici* in 'Dali' hybrid manzano chili (*Capsicum pubescens*) plants grown in pots with *P. capsici*-infested soil. Chapingo, Mexico, 2020–2021.

Morphological characterization of *P. capsici* isolated from plant tissue. Once wilting symptoms were observed in non-grafted 'Dali' hybrid plants, three random samplings were conducted at different times, selecting five diseased plants to isolate the causal agent. From each plant, five 1 cm sections of stem and root were taken, disinfected with

3% sodium hypochlorite, and plated on PDA medium (Soto-Plancarte *et al*., 2017). After seven days, the purification process was initiated using the hyphal tip method. Once purified, preparations were made and observed under a compound microscope. Using the Motic Plus 3.0 software, the length and width of the papillae of 50 sporangia were measured. Morphological identification was performed with the aid of the tabular keys proposed by Martin *et al.* (2012).

Yield. Seven fruit harvests were conducted, one every 15 days, recording the total number and weight of fruits per plant. Yield per hectare was calculated by considering a planting density of 0.694 plants/m² multiplied by the fruit weight per plant.

Root biomass. Root biomass determination was carried out 14 months after transplantation, following the completion of the fruit yield evaluation. Roots were thoroughly washed with water to remove as much soil as possible. For each of three plants, root length was measured using a millimeter-graduated measuring tape, and fresh weight was recorded using a precision balance with 0.1 g sensitivity (Ohaus®, Mexico). Root volume was determined by water displacement in a 10 L container graduated in mL (Gamboa-Angulo *et al.*, 2020), and dry weight (g) was obtained by drying the samples in an oven (Teknolab®, USA) at 70 °C for 72 hours.

Statistical analysis. An analysis of variance (ANOVA) was performed, followed by multiple mean comparison using Tukey's test (P≤0.05). A Student's *t*-test (P≤0.05) was applied to compare two populations (grafted and non-grafted plants). All analyses were conducted using the SAS 9.1.3 statistical software.

RESULTS AND DISCUSSION

Root biomass of grafted and non-grafted hybrids grown in hydroponics. The root biomass of non-grafted hybrids was, on average, 52%, 70%, and 50% higher in root volume, fresh weight, and dry weight, respectively, compared to hybrids grafted onto CM-334 (Table 1 and Figure 2). The lower root biomass observed in grafted plants can be explained by the characteristics of the CM-334 rootstock, which is a small-sized, annual plant belonging to *C. annuum*. In contrast, the scion (manzano chili hybrids) is a tall, perennial plant classified as *C. pubescens*. To enhance root formation in the CM-334 rootstock and improve nutrient absorption, the exogenous application of auxins and *Trichoderma* spp. is recommended. In this regard, López-Elías *et al.* (2005) reported increases of up to 42% in root volume, 35% in fresh weight, and 4% in dry weight with applications of indole-3-butyric acid (IBA) in bell pepper and habanero chili. Similarly, Larios *et al.* (2019) observed increases of 41.57% and 55% in root length and volume, respectively, with the use of *Trichoderma* spp.

Table 1 Root volume, fresh weight, and dry weight of manzano chili plants of the hybrids 'Maruca,' 'Jhos,' and 'Dali' grafted onto CM-334 vs. non-grafted plants grown in Chapingo, Mexico. 2017–2019 cycle.

Treatment	Volume (mL)	Fresh weight (g)	Dry weight (g)
'Maruca'	512.86 a*	506.50 a*	133.714 a*
'Maruca', grafted	267.14 b	145.37 b	67.586 b
'Jhos'	468.57 a*	513.57 a*	140.214 a*
'Jhos', grafted	1 97.14 b	164.81 b	70.186 b
'Dali'	515.71 a*	511.69 a*	134.243 a*
'Dali', grafted	204.29 b	148.70 b	67.429 b
Coefficient of variation	13.91	12.82	13.11

Means with the same letters are not statistically different (Tukey, 0.05). Significant according to Student's *t*-test (P≤0.05).

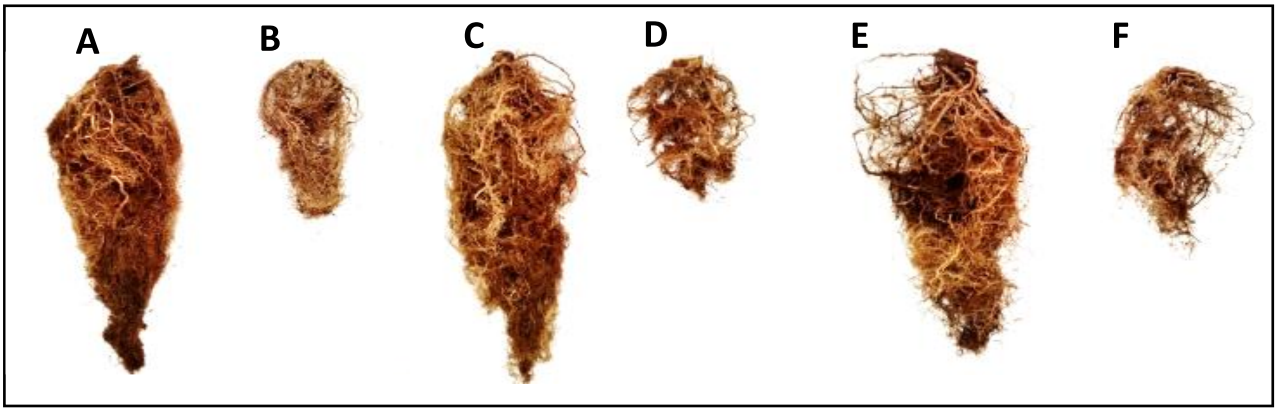

Figure 2. Root of non-grafted vs. CM-334-grafted manzano chili hybrid grown in hydroponics and greenhouse conditions in Chapingo, Mexico (2017–2019). A) Maruca, B) Maruca grafted, C) Jhos, D) Jhos grafted, E) Dali, F) Dali grafted.

Resistance to *P. capsici*, fruit yield, and root biomass of grafted and non-grafted plants

Morphological characterization of *P. capsici* isolated from soil. The isolate obtained from the infested soil exhibited white, cottony, coenocytic mycelial growth with 15 sporangia per mL (aliquot). The sporangia measured 28.24 to 49.61 µm in length, 27.29 to 37.41 µm in width, and 4.8 to 6.5 µm in papilla width (Figure 3). The observed morphological characteristics of the oomycete align with those described by Díaz-Nájera *et al*. (2015) for *P. capsici* in cucurbits and by Silva-Rojas et al. (2009) for *C. annuum*. These findings confirm that the soil used in the experiment was naturally infested with *P. capsici*.

Incidence and AUDPC. In non-grafted plants, the incidence of *P. capsici* reached 70%, with symptoms appearing 100 days after transplantation (DAT). In contrast, no disease incidence was observed in grafted plants, confirming the high resistance level of the Serrano Criollo de Morelos 334 (CM-334) rootstock to *P. capsici* (Lamour *et al*., 2012). These results indicate that grafting manzano chili onto CM-334 is a viable production alternative for soils infested with this pathogen.

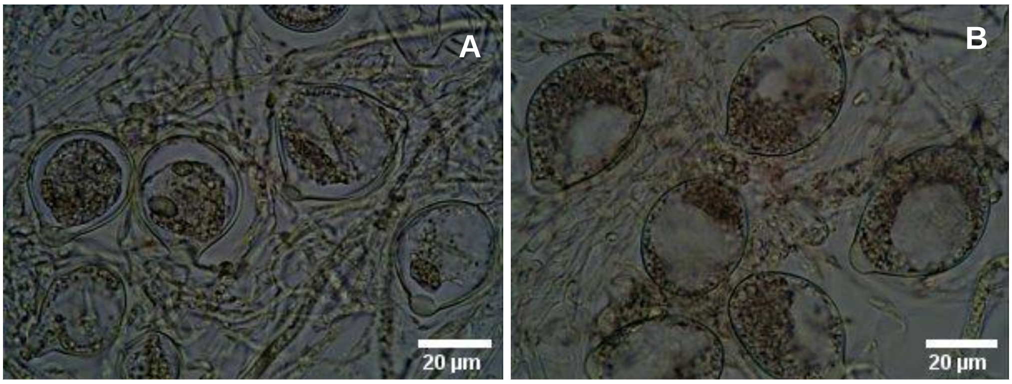

Figure 3. A) Bipapillate sporangium and smooth mycelium; B) Unipapillate sporangium with zoospores of *P. capsici* obtained from infested soil in Chapingo, Mexico.

The symptoms in infested plants began with a loss of foliar turgor, followed by brown lesions on leaf tissues (Figure 1), stem collar, and darkening of the root system. These symptoms are characteristic of chili wilt caused by *P. capsici* and are consistent with those reported by Callaghan *et al*. (2016) and Moreira-Morrillo *et al.* (2022) for the *Capsicum* genus. Symptom severity in affected plants progressively increased until complete wilting of the aerial part and root system necrosis, which was considered 100% damage. Plant death occurred, on average, 14 days after the onset of wilting symptoms, aligning with the findings of Barchenger *et al*. (2018) for mature *Capsicum* plants.

The area under the disease progress curve (AUDPC) confirmed that all treatments involving grafted plants showed no disease symptoms at any phenological stage. Fisher's LSD multiple mean comparison test revealed significant differences among treatments with auxin and *T. harzianum* applications (Figure 4). The highest disease severity was observed in 'Dali' hybrid plants treated with 1200 ppm of IBA every 15 days. In contrast, treatments that included *T. harzianum* showed no significant differences in AUDPC compared to grafted plant treatments, despite a disease incidence of 50%.

Gallegos-Morales *et al.* (2022) reported that *T. harzianum* increased the yield of Serrano chili (*Capsicum annuum* var. Tampiqueño) by 30% and reduced *P. capsici* incidence by 71%. Similarly, Romero-Arenas *et al.* (2017) found that *P. capsici* incidence ranged from 60% to 70% in tomato plants previously treated with *T. harzianum*, attributing this to the antagonistic interaction between the involved microorganisms. Ita *et al*. (2021) indicated that *T. harzianum* exhibits a higher growth rate than *P. capsici*, inhibiting its development and functioning as a biocontrol agent (Mousumi *et al*., 2018). Additionally, La Spada *et al.* (2020) stated that *Trichoderma* can induce root defense responses when phytopathogens penetrate the rhizodermal region, increasing the activity of peroxidase and chitinase enzymes and promoting callose deposition in cell walls.

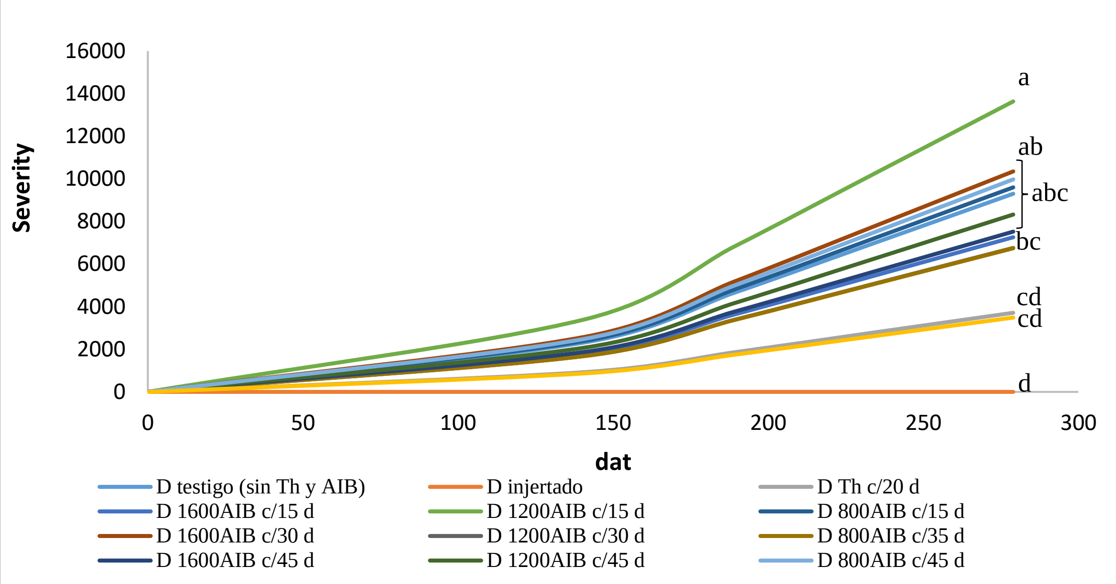

Figure 4. Area under the disease progress curve (AUDPC) of *P. capsici* in manzano chili plants grown in Chapingo, Mexico, from 2020 to 2021. dat: days after transplantation. D: non-grafted 'Dali' hybrid, d: days (application frequency). [z]Same letters indicate no statistically significant differences (Fisher's LSD, *P*≤0.05).

Morphological characterization of *P. capsici* isolated from plant tissue. The isolate obtained from wilted plants exhibited white, cottony, coenocytic mycelial growth, with sporangia measuring 24.01 to 44.45 µm in length and 17.4 to 35.17 µm in width, and bipapillae ranging from 3.3 to 6.8 µm in width (Figure 5). The observed morphological characteristics are typical of *P. capsici* and align with those described by Callaghan *et al*. (2016) and Moreira-Morillo *et al*. (2022) for *C. annuum*. These findings confirm the presence of this oomycete in 'Dali' (*C. pubescens*) plants grown in naturally *P. capsici*-infested soil.

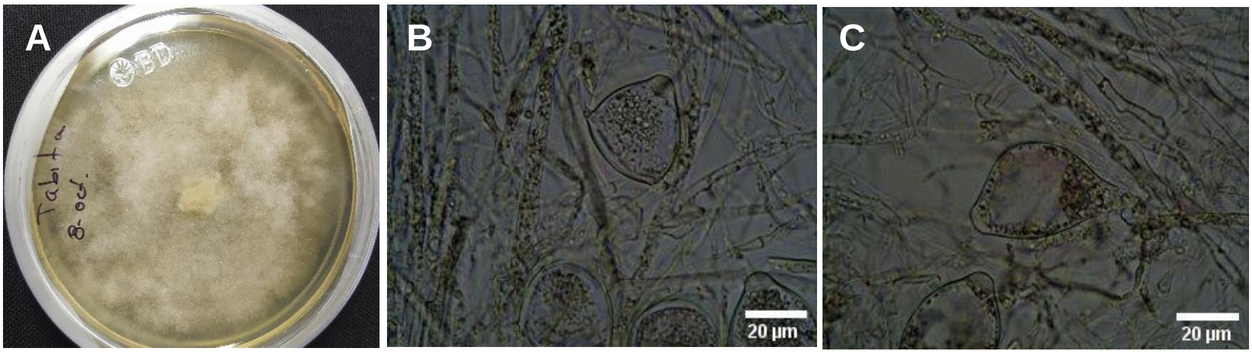

Figure 5. Morphological characterization of *P. capsici* in diseased plants. A) cottony growth, B y C) bipapillate sporangium of *P. capsici* in manzano chili in Chapingo, Mexico.

Yield. The treatments with the highest number of fruits were non-grafted 'Dali' with applications of 800 ppm of IBA every 15 days and grafted 'Dali' with auxin applications combined with *T. harzianum*, averaging 86 and 88 fruits per plant, respectively (Table 2). In contrast, the control treatment (non-grafted 'Dali' hybrid) without auxin or *Trichoderma* applications had the lowest fruit count, with 70 fruits per plant. However, the treatments with the lowest fruit numbers among those receiving auxin applications were 'Dali' at 1200 ppm and non-grafted 'Dali' at 800 ppm every 45 days, both with 66 fruits (Table 2). Therefore, no clear trend was observed in the number of fruits in response to auxin and *T. harzianum* applications, meaning these treatments did not consistently result in a higher fruit count.

Table 2. Means of fruit number, fruit weight per plant, and yield per hectare of manzano chili plants grafted onto CM-334 vs. non-grafted plants, with and without the application of IBA and *T. harzianum*, grown in Chapingo, Mexico. 2020–2021 cycle.

Treatments	Total number of fruits per plant	Total weight of fruits per plant (kg)	Yield (t ha^{-1})
D control without Th and AIB	69.66 h^{z} *	6.00 jk^{ns}	41.64 jk^{ns}
D^{i} control without Th and AIB	74.66 g	5.99 jk	41.55 jk
D Th+AIB 1200 ppm every 20 days	66.66 ij*	6.32 efg*	43.88 efg*
D^{i} Th+AIB 1200 ppm every 20 days	87.66 a	6.85 abc	46.91 abc
D Th 1200 ppm every 20 days	69.66 h*	6.19 ef*	42.98 g*
D^{i} Th 1200 ppm every 20 days	77.66 e	6.85 a	47.54 a
D AIB 1600 ppm every 15 days	83.33 bc*	6.82 ab*	47.35 ab*
D^{i} AIB 1600 ppm every 15 days	70.33 h	6.66 c	46.20 c
D AIB 1200 ppm every 15 days	76.66 ef *	6.69 bc^{ns}	46.43 bc*
D^{i} AIB 1200 ppm every 15 days	74.66 g	6.46 de	44.81 de
D AIB 800 ppm every 15 days	86.00 a*	6.49 d*	45.02 d*
D^{i} AIB 800 ppm every 15 days	81.66 cd	6.40 def	44.39 def
D AIB 1600 ppm every 30 days	80.33 d*	6.29 fgh*	43.68 fgh*
D^{i} AIB 1600 ppm every 30 days	74.33 g	6.17 hi	42.82 hi
D AIB 1200 ppm every 30 days	67.66 i*	6.49 d*	45.02 d*
D^{i} AIB 1200 ppm every 30 days	74.66 g	6.32 efg	43.86 efg
D AIB 800 ppm every 30 days	67.66 i*	6.85 a*	47.54 a*
D^{i} AIB 800 ppm every 30 days	83.66 b	6.67 c	46.29 c
D AIB 1600 ppm every 45 days	74.66 g NS	6.09 ij*	42.26 ij*
D^{i} AIB 1600 ppm every 45 days	73.66 g	5.91 kl	41.04 kl
D AIB 1200 ppm every 45 days	75.33 fg*	5.97 jkl*	41.43 jkl*
D^{i} AIB 1200 ppm every 45 days	65.66j	5.82 l	40.41 l
D AIB 800 ppm every 45 days	65.66 j *	6.19 ghi*	41.70 jk*
D^{i} AIB 800 ppm every 45 days	67.66 i	5.8 kl	40.81 kl
Coefficient of variation	2.80	3.74	3.74

D: non-grafted 'Dali' hybrid, Di: 'Dali' hybrid grafted onto the CM-334 rootstock, Th: *T. harzianum*, AIB: 4-indole-3-butyric acid, ^{z}Same letters indicate no statistically significant differences (Tukey, 0.05), Significant and ns: Not significant according to Student's *t*-test (P≤0.05).

In contrast, when fruit weight per plant was extrapolated to yield per hectare, two treatments showed the highest fruit yield: grafted 'Dali' with *T. harzianum* applications every 20 days and non-grafted 'Dali' with 800 ppm of IBA every 30 days, both yielding 47.54 t ha^{-1}. These were followed by non-grafted 'Dali' with auxin applications at 1600 ppm every 15 days (47.3 t ha^{-1}) and grafted 'Dali' on CM-334 with *T. harzianum* and auxin applications at 1200 ppm every 20 days (46.9 t ha^{-1}) (Table 2). Meanwhile, the control treatments without auxin or *T. harzianum* applications (both grafted and non-grafted 'Dali') had lower yields of 41.6 and 41.5 t ha^{-1}, respectively. These results were similar to those observed in treatments with auxin applications every 45 days, suggesting that exogenous auxin applications to the roots at this frequency had no effect on fruit weight, whereas more frequent applications (every 15 and 30 days) did (Table 2).

The increase in fruit yield with auxin use may be attributed to their role as plant hormones that regulate various aspects of growth and development, including tissue and organ formation, by inducing cell division, growth, and differentiation (Jordán and Casaretto, 2006). In this regard, Salas *et al*. (2009) reported increased fruit yield in bell pepper when auxins were applied through irrigation systems. However, for the same crop, Pérez-Jiménez *et al.* (2015) found no significant differences between plants treated with indole-3-acetic acid (IAA) and the untreated control.

Regarding the use of *T. harzianum*, Kumari *et al.* (2019) reported a higher number of fruits per plant in bell pepper, and Khan *et al*. (2017) even observed up to a 300% increase. These positive effects of *Trichoderma* applications are associated with its production of phytohormones, which lower soil pH and enhance the availability of phosphates and other essential minerals for plant metabolism (Sharma and Gothalwal, 2017).

When comparing the effect of the CM-334 rootstock on manzano chili plants, 75% of the 12 Student's *t*-test comparisons (Table 2) showed only a 2% decrease in yield, with no incidence of *P. capsici* observed in any case. In contrast, 73% of the non-grafted plants died between 100 and 290 days after transplantation. This indicates that grafting onto CM-334 is a viable and sustainable alternative for reducing *P. capsici* incidence in manzano chili. None of the grafted plants exhibited wilting symptoms, and their yield was similar to that of non-grafted plants during the first production cycle. Additionally, grafted plants continued producing for multiple cycles (at least four years) due to their perennial growth habit (Pérez and Castro, 2008).

Partially similar results were reported by García-Rodríguez *et al*. (2010), who found that in ancho rebelde chili, plants grafted onto CM-334 did not develop *P. capsici*-related symptoms; however, plant vigor and yield were significantly lower. Leal-Fernández *et al.* (2013) also noted that CM-334 rootstock significantly reduced yield in the sweet pepper cultivar Triple Star, which could be influenced by factors such as nutrient absorption, root system efficiency, or increased endogenous hormone production. Zhao *et al.* (2011) attributed yield reductions in grafted plants to irrigation, fertilization, rootstock-scion combinations, and harvest timing. In the present study, grafted plants began producing marketable fruits three weeks later than non-grafted hybrids.

Pintado-López *et al*. (2017) also reported that the Camino Real cultivar from Harris Moran is highly susceptible to *P. capsici*, but when grafted onto CM-334, the plant can produce fruits without yield reduction. Similarly, Pérez-Grajales *et al*. (2021) concluded that there were no significant differences in fruit yield between non-grafted and CM-334-grafted manzano chili hybrids ('Maruca,' 'Jhos,' and 'Dali') grown under greenhouse-hydroponic conditions.

Additionally, it was found that applying *T. harzianum* every 20 days, combined with auxins at 1200 ppm, increased yield by 8% in grafted plants compared to non-grafted ones (Table 2). These results suggest that using grafted manzano chili plants on CM-334 is a viable alternative for commercial crop management, as it allows production without *P. capsici*-induced damage while maintaining high fruit yields, particularly when supplemented with *T. harzianum* and auxin applications.

Root biomass of grafted and non-grafted plants with auxin and *T. harzianum* applications. Due to the incidence and severity of *P. capsici* affecting both foliage and roots, not all treatments for non-grafted 'Dali' plants were evaluated. Only 6 out of 24 treatments were assessed, as symptoms of stem, collar, and root rot, as well as crown, leaf, and fruit blight caused by *P. capsici*, led to potential losses of up to 100% (Jiménez-Camargo *et al*., 2018).

Applications of auxins and *T. harzianum* in grafted and non-grafted 'Dali' manzano chili plants increased root length, volume, fresh weight, and dry weight (Table 3). The highest root biomass was observed in non-grafted plants treated with *T. harzianum* alone or in combination with IBA at 1200 ppm every 20 days, showing a root length of 47.4 cm, a volume of 1,367 mL, a fresh weight of 1,604 g, and a dry weight of 593.3 g (Table 3). This treatment also achieved the highest fruit yield, reaching 47.5 t ha^{-1} (Table 1).

Table 3. Root biomass of non-grafted and CM-334-grafted manzano chili plants, with and without the application of IBA and *T. harzianum*, in Chapingo, Mexico. 2020–2022 cycle.

Treatments	Length (cm)	Volume (mL)	Fresh weight (g)	Dry weight (g)
D control without Th and AIB	53.00 ab*	933.33 bc*	1104.0 bcdef*	550.00 ab*
D^i control without Th and AIB	37.33 abc	283.33 i	363.0 j	112.00 d
D Th+AIB 1200 ppm every 20 days	47.33 abc^{ns}	1366.67 a*	1604.0 a*	593.00 a*
D^i Th+AIB 1200 ppm every 20 days	36.00 abc	708.33 cdef	812.0 defgh	154.67 cd
D Th 1200 ppm every 20 days	54.67 a*	1266.67 a*	1420.3 ab*	587.33 a*
D^i Th 1200 ppm every 20 days	37.00 abc	666.67 ef	773.3 efghi	94.67 d
D^i AIB 1600 ppm every 15 days	34.00 bc	283.33 i	349.3 i	116.67 d
D^i AIB 1200 ppm every 15 days	37.00 abc	683.33 def	869.7 cdefg	180.33 cd
D^i AIB 800 ppm every 15 days	46.33 abc	625.00 fg	734.3 fghij	118.00 d
D^i AIB 1600 ppm every 30 days	31.667 c	383.33ghi	448.3 hij	105.33 d
D^i AIB 1200 ppm every 30 days	36.00 abc	750.00 bcdef	867.7 cdefg	164.33 cd
D AIB 800 ppm every 30 days	48.00 abc*	966.67 b*	1239.0 abc*	401.67 ab*
D^i AIB 800 ppm every 30 days	32.667 c	333.33 hi	411.0 ij	97.67 d
D AIB 1600 ppm every 45 days	36.33 abc^{ns}	875.00 bcde*	1174.7 bcd*	443.33 ab*
D^i AIB 1600 ppm every 45 days	36.67 abc	550.00 fgh	662.0 ghij	112.33 d
D AIB 1200 ppm every 45 days	44.00 abc^{ns}	925.00 bcd*	1130.0 bcde*	346.67 bc*
D^i AIB 1200 ppm every 45 days	39.00 abc	616.67 fg	587.0 ghij	103.00 d
D^i AIB 800 ppm every 45 days	35.00 bc	400.00 ghi	510.7 ghij	128.00 d
Coefficient of variation	15.52	11.42	15.33	28.35

D: non-grafted 'Dali' hybrid, Di: 'Dali' hybrid grafted onto the CM-334 rootstock, Th: *T. harzianum*, AIB: 4-indole-3-butyric acid, ^{z}Same letters indicate no statistically significant differences (Tukey, 0.05), Significant and ns: Not significant according to Student's *t*-test (P≤0.05).

In contrast, the lowest root biomass was found in grafted plants without auxin and *T. harzianum* applications, with values of 37 cm, 283 mL, 363 g, and 112 g for root length, volume, fresh weight, and dry weight, respectively, along with a fruit yield of 41.5 t ha^{-1}. These results demonstrate the effectiveness of auxin and *T. harzianum* applications in enhancing the root biomass of grafted manzano chili plants, specifically increasing the biomass of the CM-334 rootstock, which was reflected in higher fruit yield.

Grafted 'Dali' plants treated with IBA at 1200 ppm combined with *T. harzianum* every 20 days yielded 46.91 t ha^{-1}, representing an 11% increase compared to untreated grafted plants, which yielded 41.55 t ha^{-1}. Gamboa-Angulo *et al*. (2020) reported an increase in root volume in *Capsicum annuum* var. xcat'ik chili plants treated with *T. harzianum*, although they found no significant differences in fruit number and yield. Similarly, Larios *et al*. (2019) documented a 41.57% increase in root length and a 55% increase in root volume in habanero chili following *Trichoderma* spp. applications. These increases are attributed to *Trichoderma* spp.'s ability to produce auxins, which stimulate plant growth, particularly root system development (Contreras-Cornejo *et al*., 2009). According to Tucci *et al*. (2011), *Trichoderma* spp. can solubilize phosphates, micronutrients, and mineral cations essential for plant metabolism.

In the comparison between grafted and non-grafted plants (Table 3), it was found that in 50% of the six comparisons (treatments), non-grafted plants had 32% greater root length than those grafted onto the CM-334 rootstock. However, in 100% of the comparisons, non-grafted plants exhibited 50% greater root volume and fresh weight, as well as 76% more dry weight. These findings are consistent with the results obtained in the first experiment of this study for the three hybrids ('Maruca,' 'Jhos,' and 'Dali') grafted onto CM-334, as shown in Table 1.

This highlights the necessity of applying auxins and *T. harzianum* when grafting manzano chili onto the CM-334 rootstock. On one hand, these applications increase root biomass, which is associated with better water and nutrient uptake, ultimately leading to higher fruit yield (Table 1). On the other hand, the use of CM-334 as a rootstock ensures resistance to *P. capsici* (Figure 4). Therefore, using high-yield, high-quality manzano chili hybrids (Pérez and Castro, 2008) for commercial production is a viable and sustainable alternative when grafted onto CM-334. This approach guarantees *P. capsici* resistance while enhancing root biomass when combined with auxin and *T. harzianum* applications (Table 3).

CONCLUSIONS

The root biomass of the manzano chili hybrids 'Maruca,' 'Jhos,' and 'Dali' grafted onto the CM-334 rootstock showed 50%, 53%, and 75% lower root volume, fresh weight, and dry weight, respectively, compared to non-grafted hybrids.

Non-grafted plants exhibited a 70% incidence of *P. capsici* when grown in *P. capsici*-infested soil. In contrast, plants grafted onto CM-334 showed no incidence of the oomycete. This confirms that grafting manzano chili hybrids (*Capsicum pubescens*) onto CM-334 (*Capsicum annuum*) is a viable and sustainable alternative for growers, as most of them cultivate in naturally infested soils.

The fruit yield of the 'Dali' manzano chili hybrid decreased by an average of only 2% when grafted onto CM-334. However, an 8% increase in yield was observed when *T. harzianum* was applied alone or in combination with auxins at 1200 ppm every 20 days.

Grafted 'Dali' plants on CM-334 exhibited 32% shorter root length, 50% lower root volume and fresh weight, and 76% lower dry weight compared to non-grafted plants. Therefore, targeted applications of *T. harzianum* (1.25 kg ha^{-1}) combined with IBA (1200 ppm) every 20 days are recommended to enhance root biomass in the rootstock.

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