Article

Induction of the defense response of onion plants in interaction with *Trichoderma asperellum* and *Alternaria porri*

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Abstract

Alternaria porri causes purple blotch disease in onion and beneficial fungi of the genus *Trichoderma* can be used for its biocontrol, but knowledge of the mechanisms of the defense response in the interaction of onion plants with *Trichoderma* and *A. porri* is limited. In this study, the activity of defense enzymes: glucanases, chitinases, catalases and peroxidases in onion plants in the interaction with the isolate To of *Trichoderma asperellum* and *A. porri* was evaluated. The isolate To of *T. asperellum* was selected because it stood out for its antagonistic activity against *A. porri* compared to that of another isolate of *T. asperellum* (TC1 and TC2) and of *T. harzianum* and *T. atroviridae*. With the method of dual culture and cellophane paper, the isolate To inhibited the mycelial growth of *A. porri* in 56 and 53%, respectively and showed mycoparasitic activity. The activity of enzymes depended on the interaction of onion plants with the isolate To of *T. asperellum* and *A. porri.* The activity of glucanases and chitinases increased with the isolate of *T. asperellum* and was repressed with *A. porri.* The activity of catalases was induced with *A. porri* and the activity of peroxidases increased with both microorganisms. In conclusion, the defense mechanisms of onion plants are regulated by the interaction with *T. asperellum* and *A. porri* and both microorganisms; aspect to be consider in the biocontrol of onion pathogens with the isolate To of *T. asperellum.*

Keywords: antioxidant enzymes, chitinases, glucanases.

Reception date: February 2021
Acceptance date: April 2021

Introduction

Plants during their life cycle constantly interact with beneficial microorganisms and pathogens. According to their lifestyles, plant pathogens are classified into biotrophs and necrotrophs. Biotrophs obtain the nutrients necessary for their development from living host plant cells; examples of this type of pathogens are the fungi *Phytophthora parasitica* and *Erysiphe* spp. (Glazebrook, 2005).

While necrotrophic pathogens damage plant tissue to use it as a source of nutrients causing tissue necrosis, some examples are the fungi *Alternaria brassicicola*, *Botrytis cinerea*, *Fusarium oxysporum* and *Sclerotinia sclerotiorum* (Glazebrook, 2005; Birkenbihl and Somssich, 2011). Plant necrosis is caused by an increase in reactive oxygen species (ROS) produced by the plant in response to infection by a necrotrophic pathogen. In plant tissue, the antioxidant capacity increases to tolerate infection. On the contrary, in interactions with biotrophs, with mutualistic and endophytic microorganisms, ROS are signal molecules to induce a systemic response in plants.

In this way, pathogenic and beneficial microorganisms modify the network of signals of the defense of plants through changes in the levels of phytohormones: salicylic acid, jasmonic acid and ethylene (Glazebrook, 2005; Barna *et al*., 2012; Vos *et al*., 2015).

The defense mechanisms that are induced in plants involve the strengthening of the cell wall, where the enzymes peroxidases and polyphenol oxidases participate; the degradation of the cell wall of the pathogen by glucanase and chitinase enzymes; and of antioxidant enzymes to counteract the levels of ROS, such as catalases, superoxide dismutases and peroxidases (Sepúlveda-Jiménez *et al*., 2005; Kumar *et al*., 2018; Jain and Khurana, 2018).

Among the beneficial microorganisms, fungi of the genus *Trichoderma* establish a symbiosis relationship with the roots of plants, which leads to promote their growth and development and induce a defense response in plants against the attack of pathogens and even pest insects (Mendoza-Mendoza *et al*., 2018; Guzmán-Guzmán *et al*., 2019). *Trichoderma* presents direct antagonistic activity against pathogens through mechanisms such as competition for space and nutrients, mycoparasitism and antibiosis (Harman 2006; Guzmán-Guzmán *et al*., 2019).

For these beneficial effects, *Trichoderma* is a biological control agent of phytopathogens, and the products based on this fungus are used to improve crop productivity, nutritional quality and resistance of plants to biotic and abiotic stress and its use can be a viable and sustainable strategy to reduce the use of fertilizers in horticultural crops (Ortega-García *et al*., 2015; Fiorentino *et al*., 2018; Sesan *et al*., 2020).

Among vegetables, the onion stands out for its largest market volume worldwide (FAOSTAT, 2020) and *Alternaria porri* is one foliar pathogenic fungus that causes the disease ‘purple blotch of onion’, which affects leaves, stems and bulbs. Symptoms begin in the leaves with lesions of an elliptical shape and color between yellow and brown, but as the fungus develops, the lesions turn to a reddish-purple color.

When the infection is strong, the plant loses the foliage and, in the bulb, a semi-aqueous rot is caused, causing losses in the crop. For the control of *A. porri*, non-systemic fungicides such as mancozeb and propineb, and systemic fungicides such as propiconazole and hexaconazole are used (Priya *et al*., 2015), but the pathogen generates resistance (Chethana *et al*., 2012; Rodríguez, 2014). An alternative for biological control of *A. porri* is the use of *Trichoderma*, and it is known that *T. harzianum* shows antagonistic capacity against *A. porri* in *in vitro* assays (Imtiaj and Lee, 2008), and that foliar application of *T. harzianum* before and after inoculation with *A. porri* reduces the incidence and severity of purple blotch in onion plants grown in the field (Prakasam and Sharma, 2012) and in greenhouse (Abo-Elyousr *et al*., 2014).

However, studies showing the activity of enzymes involved in the defense of onion plants in their interaction with *A. porri* and *T. asperellum* are scarce. Bayoumi *et al*. (2019) report that the treatment of onion plants with a combination of *T. viride* and sulfur reduces the incidence of purple blotch disease; and is associated with an increase in the activity of catalases and peroxidases.

Previously, in our working group, it was reported that the isolate To of *T. asperellum* obtained from an onion crop promotes the growth of the bulbs of two varieties of onion, with the advantage of reducing the use of fertilizers by up to 50% (Ortega-García *et al*., 2015). The isolate To of *T. asperellum* is also a potential agent for the biological control of *Stemphylium vesicarium* that causes leaf blight in onion crops of the state of Morelos (Zapata-Sarmiento *et al*., 2019).

Based on these results, in this research, the activity of glucanase, chitinase, catalase and peroxidase enzymes in the interaction of onion plants with the isolate To of *T. asperellum* and *A. porri* was evaluated. The antagonistic activity of the isolate To of *T. asperellum* against *A. porri* was compared with two other isolates of *T. asperellum* obtained from onion crops and *T. harzianum* and *T. atroviridae* from macadamia trees.

Materials and methods

Origin of fungal isolates

The isolate To of *Trichoderma asperellum* from onion roots was obtained by Ortega-García *et al*. (2015). While, in this work, the isolates TC1 and TC2 of *T. asperellum* from the roots of onion plants grown in the municipality of Ayala, state of Morelos, Mexico was obtained and identified. In the same place the isolate *Alternaria porri* from infected leaves of onion plants was obtained and identified. Likewise, isolates of *T. harzianum* and *T. atroviridae* were obtained and identified from the roots of *Macadamia* sp. trees cultivated in the municipality of Tlalnepantla, state of Morelos, Mexico.

Antagonistic activity of *Trichoderma* spp. isolates against *Alternaria porri*

Dual culture method: in Petri dishes with culture medium of potato, dextrose and agar (PDA, Bioxon), a disc (5 mm in diameter) of culture medium with mycelium of each isolate of *Trichoderma* was placed at one end, and at the opposite end of the Petri dish, another disc of the

same size, but of the pathogen *A. porri*, was placed. The control was Petri dishes inoculated with only the disc of culture medium with mycelium of the pathogen that was placed in the same position as in the dual culture treatment with *Trichoderma*. Petri dishes were incubated at 28 ±2 °C with a photoperiod of 12 h light/12 h dark. For each isolate of *Trichoderma* in dual culture with *A. porri* and the control, 6 repetitions were performed.

Method with cellophane paper: in Petri dishes with Czapek Dox culture medium added with Marmite, a cellophane paper disc was placed on top of the culture medium and then, in the center of the dish, a disc (0.5 mm in diameter) with culture medium and mycelium of each *Trichoderma* isolate was placed. The dishes were incubated at 28 ±2 °C with a photoperiod of 12 h light/12 h dark for 3 days. The cellophane paper was removed and a disc (0.5 mm in diameter) with culture medium and mycelium of *A. porri* was placed in the center of the dish.

The control was Petri dishes with culture medium inoculated only with the disc (0.5 mm in diameter) with culture medium and mycelium of *A. porri*. The cultures were incubated at 28 ±2 °C with a photoperiod of 12 h light/12 h dark until in the control, it was observed that the mycelium of *A. porri* completely covered the culture medium of the Petri dish. For each isolate of *Trichoderma* in confrontation with *A. porri* and the control, 5 repetitions were performed.

The data on the mycelial growth of the pathogen that were obtained in dual culture and cellophane paper treatments were used to calculate the percentage of inhibition of mycelial growth according to El-Katatny *et al*. (2001). The percentage of inhibition of the mycelial growth of the pathogen is defined as the difference between the growth of the pathogen in the presence of *Trichoderma* and the growth of the pathogen without *Trichoderma* multiplied by 100. Data were analyzed using analysis of variance (Anova) and comparison of means was performed using Tukey's test and Rstudio program (version 1.2.1335) with the Agricolae package.

Mycoparasitism test: inside a Petri dish, a V-shaped glass rod was placed and on it a slide with two PDA discs, one of the PDA discs was inoculated with mycelium of *A. porri* and the other disc with mycelium of the isolate To of *T. asperellum* and both discs were covered with a sterile coverslip. The dishes were incubated at 28 ±2 °C with a photoperiod of 12 h light/12 h dark.

When contact between the mycelium of the two fungi was observed, the preparation of the smears was carried out (Quiroz-Sarmiento *et al*., 2008), they were observed with an optical microscope and photographed at 20X and 40X magnification.

Inoculation of onion plants with *Trichoderma asperellum*

Onion seeds of the Crystal White variety were purchased from the distributor 'Rancho los Molinos', located in Tepoztlán, state of Morelos, Mexico. As a substrate for the germination of the seeds, a mixture of Peet Most and Metromix (Professional Growing Mix, Sunshine, Proveedores Horticolas de Mexico) was used in a ratio 1:3 (p:p), sterilized in an autoclave at 15 lb cm^{-2} for 30 min and stored at 4 °C until use. The substrate was poured into germination trays of 96 cavities, a seed was placed in each cavity and the trays were incubated in a greenhouse at 29 ±3 °C, with irrigation every third day.

At 20 days, the seedlings were transplanted into 1 L plastic pots with the same substrate and kept under the same greenhouse conditions. A suspension of 1.7 x 10^7 spores ml^{-1} of the isolate To of *T. asperellum* was used as an inoculum and the plants were inoculated at three times: at the time of sowing, at the transplantation and at the third month after planting. For inoculation during sowing, 1 ml of the spore suspension was placed on the seeds and on the substrate around them; in the transplantation, the seedlings were placed inside the spore suspension for 15 min and 1 ml of the spore suspension was placed on the substrate that surrounded the roots; and on the third inoculation, 1 ml of the spore suspension was placed around the root. The control was uninoculated plants, but sterile distilled water was applied to the seeds and plants. The plants were kept until three months of age and were used for infection with *A. porri*.

Inoculation of onion plants with *Alternaria porri*

The inoculation was performed on the leaves, where three discs of culture medium with mycelium (5 mm in diameter) of *A. porri* were placed, then they were covered with surgical gauze moistened in sterile distilled water. The plants were kept for 72 h in a greenhouse with micro sprinkler irrigation at a relative humidity of 90%, at 29 ±4 °C with natural light.

Afterwards, the plants were kept with a relative humidity of 50% for 24 h. After 4 days of inoculation with *A. porri*, the onion plants were moved to the laboratory where the leaves were cut with scissors, weighed and stored at -4 °C until use for the determination of enzyme activity. The treatments were plant leaves: a) uninoculated (control); b) inoculated with *T. asperellum* at the time of sowing, at transplanting and at the third month after sowing; c) inoculated with *A. porri*; and d) inoculated with *T. asperellum* and with *A. porri*, six leaves were used for each treatment.

Evaluation of enzyme activity

Determination of the activity of glucanases and chitinases: the tissue of the leaves was macerated with liquid nitrogen until a fine powder was obtained and mixed with 1 ml of sodium acetate 50 mM at pH 5. The mixture was centrifuged at 13 000 rpm at 4 °C for 30 min., the supernatant was separated and used as the enzymatic extract. The protein content of the extract was evaluated by the Bradford (1976) method and the activity of glucanases and chitinases was determined by the method described by El-Katatny *et al*. (2001). For the assay of the glucanase activity, the test mixture contained 200 µl of enzymatic extract and 500 µl of 5% laminarin (Sigma-Aldrich) dissolved in sterile distilled water; the mixture was incubated at 37 °C for 30 min at 300 rpm. For chitinase activity, the test mixture (1 ml) was prepared with 500 µl of the enzyme extract and 500 µl of colloidal chitin (1%); the mixture was incubated at 37 °C for 7 h at 300 rpm.

The product of glucanase and chitinase activity are reducing sugars such as glucose and N-acetyl glucosamine respectively, which were determined by a colorimetric reaction with dinitrosalicylic acid (DNS) according to Adney and Baker (2008). Also, to calculate the activity of enzymes, a standard curve was constructed with glucose for glucanases and with N-acetyl glucosamine for chitinases. Glucanase activity was expressed in nmol glucose min^{-1} mg^{-1} protein and chitinase activity in nmol N-acetyl glucosamine min^{-1} mg^{-1} protein.

Determination of catalase activity: catalase activity was evaluated according to the methodology of Beers and Sizer (1952). The leaves were macerated with liquid nitrogen and homogenized with 2 ml of sodium phosphate buffer 100 mM at pH 7, added with 1 mM ethylenediaminetetraacetic acid, 1 mM phenylmethylsulfonyl fluoride and 1 mM dichlorodiphenyltrichloroethane. The extract was mixed with 5 mg of polyvinylpolypyrrolidone: activated carbon 1:1 (p:p), it was centrifuged at 13 000 rpm at 4 °C for 30 min and the supernatant was recovered and used as an enzymatic extract. Catalase activity was measured by the decrease in absorbance at 240 nm of H_2O_2 used as substrate.

The activity of catalases was calculated considering the extinction coefficient of H_2O_2 (0.04 mM cm^{-1}) and expressed in μmoles H_2O_2 min^{-1} mg^{-1} protein. Determination of peroxidase activity: the activity was evaluated using the methodology of Stasolla and Yeung (2007). The test mixture contained 100 μl of the protein extract and 800 μl of sodium acetate buffer 50 mM (pH 5.2), H_2O_2 (0.3%) and guaiacol 1 M.

The oxidation of guaiacol in the presence of H_2O_2 was measured at an absorbance of 470 nm in a spectrophotometer. Enzyme activity was expressed as μmoles tetraguaiacol min^{-1} mg^{-1} protein. Tetraguaiacol μmoles were calculated considering the tetraguaiacol extinction coefficient (26.6 mM cm^{-1}).

Data on the activity of chitinases, glucanases and catalases were analyzed using analysis of variance (Anova) and comparison of means was performed using the Tukey test. Data on the activity of peroxidases were analyzed using the Kruskal Wallis test and the Dunn test ($p< 0.05$), because the data did not present a normal distribution or homogeneity of variances. Statistical analyses were performed with the Rstudio program (version 1.2.1335) with the Agricolae package.

Results and discussion

Antagonistic activity of *Trichoderma* spp. isolates against *Alternaria porri*

By the dual culture method, a significant difference was found in the inhibition of the growth of *A. porri* presented by the three isolates of *T. asperellum* (TC1, TC2 and To) and the isolate of *T. harzianum* compared to *T. atroviridae.* The three isolates of *T. asperellum* and *T. harzuianum* inhibited more than 40% the mycelial growth of *A. porri*. Whereas the isolate of *T. atroviridae* inhibited in 20% the mycelial growth of the pathogen. The To isolate of *T. asperellum* stood out for its greater ability to inhibit the growth of the pathogen (56%) (Figure 1).

Similarly, several studies show that *Trichoderma* has antagonistic activity against *A. porri* and that there is a difference between species and even between the same species of *Trichoderma.* Prakasam and Sharma (2012) show that isolates of *T. viride* and *T. harzianum* obtained from different plant cultures inhibit the radial growth of *A. porri* from 18.9 to 55.7% and from 19.7 to 61.5%, respectively.

The antagonistic activity of *Trichoderma* is due to various mechanisms, such as its high capacity for competition for nutrients compared to that presented by other fungi, to the production of compounds called siderophores that catch iron and stop the growth of other fungi and by its ability to generate ATP for its growth from different carbon sources (Gajera *et al*., 2013; Contreras-Cornejo *et al*., 2016).

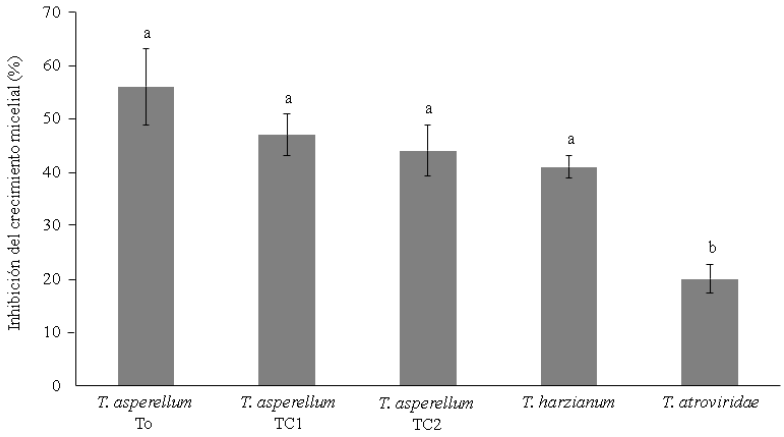

Figure 1. Inhibition of the mycelial growth of *Alternaria porri* with different isolates of *Trichoderma* by the dual culture method. The values correspond to the mean and standard deviation (n= 6). Different letters indicate significant differences between treatments according to Tukey's HSD test ($p< 0.05$).

By the cellophane paper method, it was found that there are significant differences in the antagonistic activity of the isolates, but the isolate To of *T. asperellum* stood out again because it inhibited the mycelial growth of *A. porri* in 53%, while the other *Trichoderma* isolates inhibited less than 26% the growth of the phytopathogenic fungus (Figure 2). The variability of the antagonistic capacity of *Trichoderma* isolates observed in this study may be due to the fact that they produce different antimicrobial compounds that inhibit the mycelial growth of *A. porri.*

Compounds with antibiotic activity can be alkyl pyrones, isonitriles, polyketides, peptaibols, diketopiperazines, sesquiterpenes and steroids (Harman, 2006; Gajera *et al*., 2013; Contreras-Cornejo *et al*., 2016). In addition to the species, the production of these compounds depends on factors such as temperature, pH and the substrate in which the fungus is found (Benítez *et al*., 2004).

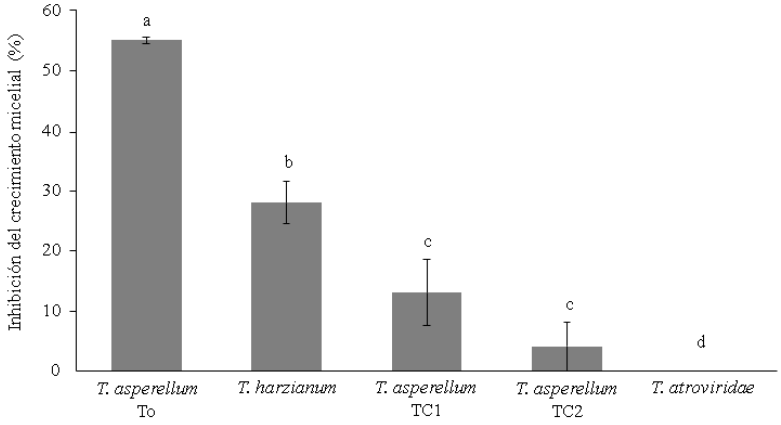

Figure 2. Inhibition of the mycelial growth of *Alternaria porri* with different isolates of *Trichoderma* by the cellophane paper method. The values correspond to the mean and standard deviation (n= 5). Different letters indicate significant differences between treatments according to Tukey's HSD test ($p< 0.05$).

In the process of mycoparasitism of *T. asperellum*, four events were observed: contact, invasion, penetration and destruction of the mycelium of *A. porri* (Figure 3). *Trichoderma* has the potential to attack and destroy by mycoparasitism pathogenic fungi of agricultural importance such as *Alternaria alternata*, *Botrytis cinerea*, *Rhizoctonia solani*, *Sclerotinia sclerotium*, *Phytium* and *Fusarium* spp. (Harman *et al*., 2004; Druzhinina *et al*., 2011).

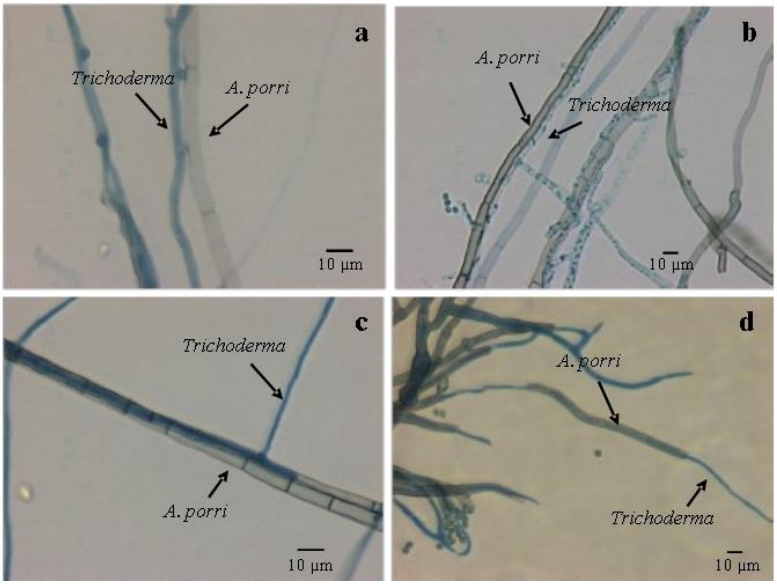

Figure 3. Mycoparasitism process of the isolate To of *Trichoderma asperellum* on *Alternaria porri*. a) Contact; b) invasion; c) penetration; and d) destruction.

The events that were observed in the mycoparasitism of *T. asperellum* on *A. porri* have been described in other interactions of *Trichoderma* with pathogenic fungi and it is known that it is a complex process involving the synthesis and recognition of molecules, the formation of infective structures and the production of antibiotic enzymes and metabolites that result in the death of the pathogen (Contreras-Cornejo *et al*., 2016; Guzmán-Guzmán *et al*., 2019).

In the first moments of the interaction there is a chemotrophic growth of the hyphae of *Trichoderma* towards the pathogen, where signals such as oligochitins can participate (Zeilinger and Omann, 2007), when the hyphae of *Trichoderma* and the pathogen have contact, they form hook-like structures to bind to the pathogen; in the penetration and destruction of the mycelium of the pathogen, *Trichoderma* produces glucanases and chitinases to degrade the cell wall of the pathogen (Almeida *et al*., 2007). For its outstanding antagonistic activity, the isolate *T. asperellum* To was selected to study the interaction of *T. asperellum* and *A. porri* with onion plants.

Enzymatic activity in onion plants treated with *T. asperellum* and *A. porri*

In plants inoculated with *T. asperellum*, significant differences in the activity of chitinases and glucanases in relation to control plants were found. These plants had the highest activity of chitinases and glucanases followed by untreated plants (control). While in plants infected with *A. porri* and plants inoculated with *T. asperellum* and infected with *A. porri*, a significant decrease in the activity of chitinases and glucanases was found with respect to the control (Figure 4).

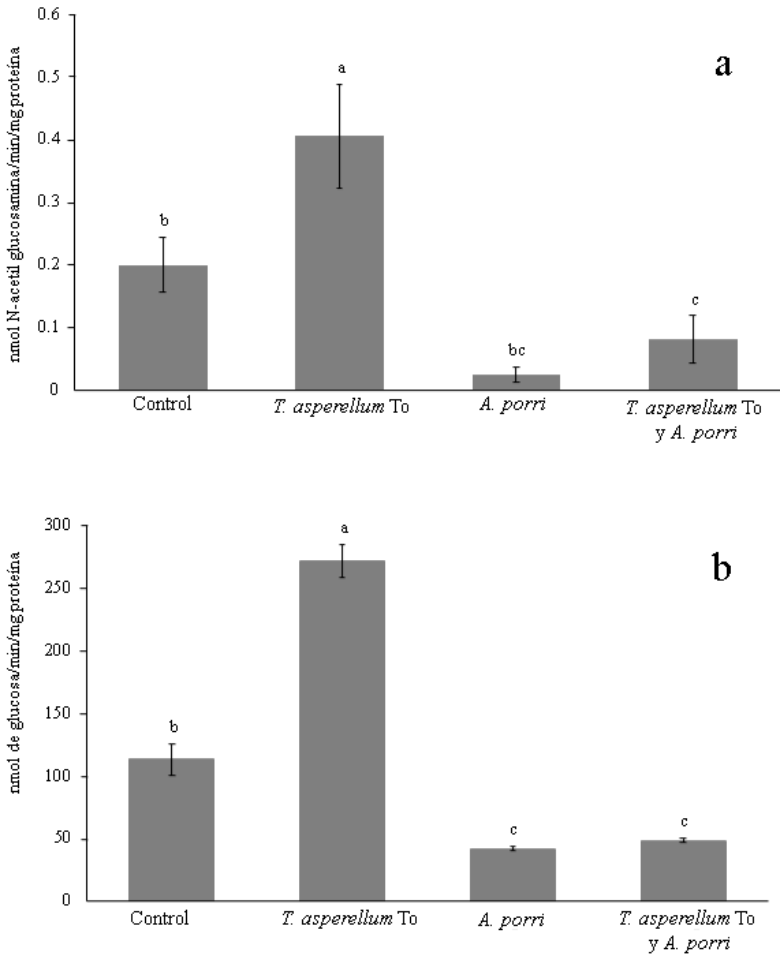

Figure 4. Activity of; a) chitinases; and b) glucanases in onion leaves inoculated with the isolate To of *Trichoderma asperellum*, *Alternaria porri* and with the two fungi. Controls are uninoculated plants. The values correspond to the mean and standard deviation (n= 4). Different letters indicate significant differences between treatments according to Tukey's HSD test ($p< 0.05$).

These results indicate that prior inoculation of *T. asperellum* in onion plants induces the activity of glucanase and chitinase defense enzymes for cell wall degradation of pathogenic fungi. Similarly, in other studies, it is reported that one of the defense responses in plants that is induced by the inoculation of *Trichoderma* is the activity of the enzymes glucanase and chitinases (Yedidia *et al*., 2000; Harman *et al*., 2004).

Likewise, it was shown that the isolate TC74 of *T. asperellum* obtained from cultures of *Capsicum annuum* (chili), when inoculated in plants of three varieties of onion, induces an increase in the basal activity of glucanases, chitinases and peroxidases in all organs, and this activity was related to a reduction in the severity of the disease caused by *Sclerotium rolfsii* (Guzmán-Valle *et al*., 2014). However, infection with *A. porri* counteracts the effect of *Trichoderma* in inducing the activity of glucanase and chitinase enzymes, in addition to causing a reduction in the basal activity of the two enzymes found in control plants, suggesting that *A. porri* has mechanisms to inhibit the basal defense response and that induced by *T. asperellum* in onion plants.

Necrotrophic fungi of the same genus as *A. brassicicola* and *A. alternata* produce specific toxins that cause host cell death and inhibit the defense response of plants (Tsuge *et al*., 2011; Cho, 2015; Pandey *et al*., 2016). *A. porri* also produces toxins and the chemical structure has been characterized, for instance, tentoxin, silvaticol and porritoxinol (Suemitsu *et al*., 1993; 1988), toxins inhibit the elongation of seedling roots and sulfonic acid porritoxin has a chemical structure that correlates with its phytotoxic activity (Horiuchi *et al*., 2003). Nevertheless, there are no studies of the involvement of toxins in the pathogenesis of *A. porri* or in the inhibition of the defense response of plants; so, it could be a topic for future studies.

Catalase activity showed significant differences in different treatments (Table 1). The plants inoculated with *A. porri* had the highest catalase activity, followed by the control plants, those inoculated with *T. asperellum* and those inoculated with *T. asperellum* and with *A. porri*.

Table 1. Activity of catalases and peroxidases in onion leaves inoculated with the isolate To of *Trichoderma asperellum*, *Alternaria porri* and with the two microorganisms.

Treatment	Catalase activity (μmole H_2O_2 min^{-1} mg^{-1} protein)	Peroxidase activity (nmol tetraguaiacol min^{-1} mg^{-1} protein)
T. asperellum	2.14 ±0.8 c	9.93 (7.5, 12.6) b
A. porri	6.05 ±0.5 a	17.76 (14.8, 20.9) ab
T. asperellum + *A. porri*	0.87 ±0.2 d	35.76 (34.1, 38.1) a
Control	4.94 ±0.2 b	7.12 (5.5, 8.6) b

These results indicate that infection with *A. porri* induces the activity of catalases in onion leaves; the increase in the activity of these enzymes could be an advantageous event for *A. porri*, as it could prevent the death of the pathogen itself. In this regard, it is reported that necrotrophic fungi of the same genus such as *A. alternata*, *A. brassicae* and *A. citri* produce specific toxins (Pandey *et al*., 2016) that induce lipid peroxidation, accumulation of reactive oxygen species and cell death in the plant.

However, the pathogen to avoid its own death by ROS coordinates the signals to detoxify the oxidizing environment that is generated between them, through the activation of antioxidant enzymes such as catalases and superoxide dismutases (Chung, 2012).

The activity of peroxidases showed significant differences in the different treatments. In plants inoculated with *T. asperellum* and with *A. porri*, the activity of this enzyme was found to be higher, followed by plants inoculated with *A. porri*, plants inoculated with *T. asperellum* and the control (Table 1). The results indicate that the presence of both microorganisms in onion plants induces the activity of peroxidases. These enzymes are involved in plant cell wall strengthening processes (Jain and Khurana, 2018).

Root colonization by *Trichoderma* induces changes in the proteome of plants and the expression of peroxidases is regulated as part of plant defense mechanisms (Shoresh and Harman, 2008). This increase in the activity of peroxidases by *Trichoderma* is related to an increase in plant resistance to phytopathogens (Patel and Saraf, 2017).

In onion plants, it was found that the greatest increase in the activity of peroxidases in bulbs and roots occurred when the plants were inoculated with the isolate of *T. asperellum* TC74 and the pathogen *Sclerotium rolfsii* and was related to a reduction in the severity of the disease. In the non-pigmented onion variety, there is a greater increase in the activity of peroxidases than that detected in pigmented onion varieties (Guzmán-Valle *et al*., 2014).

Conclusions

The isolate To of *T. asperellum* showed the greatest antagonistic activity against *A. porri* compared to other isolates of the same species and the same onion crop and the isolates of *T. harzianum* and *T. atroviridae*. In onion plants, different defense response mechanisms that depend on their interaction with the isolate To of *T. asperellum* and *A. porri* or both microorganisms are induced. Inoculation with the isolate To of *T. asperellum* increases the activity of glucanases and chitinases; but the inoculation with *A. porri* suppressed the activity of both enzymes. Inoculation with *A. porri* induces the activity of catalases, but inoculation with *T. asperellum* and A. *porri* increases the activity of peroxidases. Based on these results, some of the important aspects to consider in the future for the application of the isolate To of *T. asperellum* as a biological control agent of onion pathogens could be the variety of onion, nutrition and the type of pathogen (biotrophic or necrotrophic).

Acknowledgements

The research received financial support from the Research and Postgraduate Secretariat of the National Polytechnic Institute (SIP-IPN, SIP 20200701 project). MR-M and GS-J thank EDI-IPN and SIBE-IPN. VCL is a grantee of CONACYT (Mexico) to carry out doctoral studies and received the support of the Institutional Stimulus Grant for the Training of Researchers (BEIFI-IPN) program.

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