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Effect of *Trichoderma asperellum* applications and mineral fertilization on growth promotion and the content of phenolic compounds and flavonoids in onions

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ABSTRACT

The objective of this study was to evaluate the growth of onion bulbs and the content of flavonoids and phenolic compounds, when the onion plants had been inoculated with *Trichoderma asperellum* and grown with different doses of mineral fertilizer. Onion seeds of varieties Red Satan and Crystal White were inoculated with one of three *T. asperellum* isolates from *Allium cepa* L. (onion), *Solanum lycopersicum* L. (tomato) and *Mangifera indica* L. (mango) respectively, and the commercial product, PHC T-22®, based on *Trichoderma harzianum*, for comparison. Plants were grown in a greenhouse and 100% fertilizer doses applied; after 150 days, bulb mass and the content of phenolic compounds and flavonoids were measured. For all isolates, bulb mass and the content of phenolic compounds and flavonoids increased compared with uninoculated controls but the level of increase was dependent on onion variety and *Trichoderma* isolate. The *T. asperellum* isolate from onion, (To), had the greatest capacity for growth promotion, production of indole acetic acid (IAA) and induction of phenolic compounds and flavonoids. Onion plants of the two varieties were then inoculated with the To isolate and grown with 0, 25, 50, 75 and 100% fertilizer doses. The fertilizer requirements to obtain the maximum bulb mass was reduced by up to 50% when plants were inoculated with the To isolate compared to uninoculated plants. At all fertilizer doses, the content of phenolic compounds and flavonoids was higher in the bulbs of plants that had been inoculated with the To isolate, compared with uninoculated control plants. Fertilizer requirements to achieve the highest quantities of both compounds were also reduced by between 50 and 100% in inoculated plants. These data suggest that nutrient availability in onion plants was increased by inoculation with *T. asperellum* isolate To which modulated the synthesis of phenolic compounds and flavonoids without detrimental effects on the mass of onion bulbs.

1. Introduction

Trichoderma is a genus of fungus found associated with many root ecosystems. It is widely used as an antagonist of plant pathogens (Verma et al., 2007) and to induce plant resistance to plant pathogens (Harman et al., 2004) and abiotic stresses (Mastouri et al., 2010). *Trichoderma* species also have diverse beneficial effects on plant growth and development, they increase the proliferation of secondary roots, leaf area, shoot length, dry weight and crop yield (Mukherjee et al., 2013; Hermosa et al., 2013). The mechanisms involved in promotion of plant growth by *Trichoderma* species include production of metabolites with activity similar to the auxin, indole acetic acid (IAA). These IAA-like compounds stimulate plant growth and root development (Contreras-Cornejo et al.,

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2009). Another mechanism implicated in plant growth promotion by *Trichoderma* species relates to increases in nutrient availability. *Trichoderma harzianum* enhances the availability of P and Fe in the cucumber plant, leading to significant increases in dry weight, shoot length and leaf area (Yedidia et al., 2001). *T. harzianum* also produces soluble forms of phosphates, zinc and manganese, and metabolites called siderophores that are capable of reducing Fe (III) and Cu (II) so that they can be assimilated by the plant (Altomare et al., 1999). Likewise, *Trichoderma* species can influence the uptake and efficient use of nutrients, which could lead to a significant reduction in mineral fertilizers application. In maize, the application of *T. harzianum* improved grain and silage production, and nitrogen use efficiency under reduced rates of nitrogen fertilizer (Harman, 2000). The inoculation of *T. harzianum* and arbuscular mycorrizal fungi under conditions of reduced mineral fertilization also enhanced shoot weight and the nutritional status of melon crops (Martínez-Medina et al., 2011).

As a result of root colonization by *Trichoderma* species, there are changes to plant metabolism. Proteins involved in primary metabolism such as photosynthesis, photorespiration and carbohydrate metabolism, are differentially regulated by *Trichoderma asperellum* (Segarra et al., 2007). Synthesis of plant secondary metabolites, such as antimicrobial phenolic compounds and the expression of phenylalanine ammonia lyase (PAL) and hydroxyperoxide lyase genes, is also increased by colonization of the fungus (Yedidia et al., 2003). Phenolic compounds accumulated in *Trichoderma*-pathogen-co-treated plants may serve as electron and hydrogen donors thereby protecting root tissues against oxidative stress during pathogen attack (Singh et al., 2011).

Onions are the third most important vegetable produced in the world (FAO, 2013). From a viewpoint of human nutrition and health, onions are one of the richest sources of flavonoids and phenolic compounds with antioxidant activity (Prakash et al., 2007). It is known that the content of these chemicals is affected by variations in the rhizosphere, and nutrient availability (Charron et al., 2001). For these reasons, there is interest in increasing productivity and to understand the changes in the content of flavonoids and phenolic compounds in onions that occur through the application of *Trichoderma* species. However, in relation to onion productivity, results from the application of commercial products based on *T. harzianum* have been inconsistent. The commercial product KUEN 1585 did enhance bulb diameter but applications of the commercial product TrichoFlow WP TM, had no effect on onion yield or bulb characteristics (Coskuntuna and Özer, 2008). It has been suggested that the ability of *T. harzianum* to colonize the root system depends on the plant species (Altintas and Bal, 2008). Therefore, it is important to evaluate the growth promotion benefits of *Trichoderma* isolates that have been obtained from the target crop, onion, and also to consider potential effects of onion variety.

In contrast, Perner et al. (2008) reported that onion root colonization by a commercial product containing arbuscular mycorrhiza in conjunction with provision of a sufficient nitrate source, increased the flavonol glycoside content—but the total content of phenolic compounds did not change. The authors suggest that this effect is as a consequence of plant defense mechanisms and the increased uptake of nitrate (Perner et al., 2008). Other studies show that dual inoculation with arbuscular mycorrhizal fungi and saprotrophic fungi improves yield and antioxidant activity in onion bulbs (Albrechtova et al., 2012). Nevertheless, studies on the effect of treatments with *Trichoderma* species on the content of phenolic compounds and flavonoids in onion plants growing under reduced fertilizer rates are scarce and are the subject of this study. Also we determined whether the growth promotion and the synthesis induction of both compounds were dependent on the isolate of *Trichoderma* and the onion variety.

2. Material and methods

2.1. *Isolates of* Trichoderma asperellum *used in experiments*

Three isolates of *T. asperellum* (To, Tt and Tm) were selected for their known antagonistic activity against two pathogens of onion, *Sclerotium rolfsii* and *Alternaria porri* (Ortega-García et al., 2013). These isolates of *T. asperellum* were identified by species-specific characteristics of the ITS region of the nuclear rDNA and the *tef*1 gene. The NCBI GenBank accession numbers for the isolates are KP059112 (To), KP059113 (Tt) and KP059111 (Tm) for tef1; and KP059115 (To), KP059116 (Tt) and K059114 (Tm) for ITS. .

Of these three isolates, To and Tt were obtained from the culture collection of the Phytopathology Laboratory of the Centro de Desarrollo de Productos Bióticos, Yautepec, Morelos state, Mexico and isolate Tm was provided by Dr. Alejandro Casimiro Michel Aceves from the Colegio Superior Agropecuario del Estado de Guerrero, Iguala, Guerrero state, Mexico. Isolates To, Tt and Tm were isolated from *Allium cepa* L. (onion), *Solanum lycopersicum* L. (tomato) and *Mangifera indica* L. (mango) crops, respectively.

To produce spore suspensions for experiments each isolate was grown on potato dextrose agar (PDA, Bioxon) for 8 days, at $25 \pm 2\,^{\circ}C$, under ambient daylight conditions. Spore suspensions were prepared in sterile distilled water and adjusted to a concentration of 1×10^7 spores mL^{-1} based on counts in a Neubauer haemocytometer.

2.2. *Biochemical characterization of* Trichoderma asperellum *isolates (To, Tt and Tm)*

2.2.1. *Phosphate solubilization*

The phosphate solubilization capacity of *T. asperellum* isolates was evaluated using the method of Mehta and Nautiyal (2001). The assay is based on decreases in the pH and absorbance of blue bromophenol dye added to the culture medium. At neutral pH, the dye absorbs red light, transmits blue light and the culture medium appears blue. At low pH, the dye absorbs ultraviolet and blue light and the culture medium appears yellow. The absorbance changes are measured at 600 nm. Decreases in pH and absorbance at 600 nm are indicative of the capacity of phosphate solubilization by *T. asperellum*. The National Botanical Research Institute's phosphate growth medium (NBRIP) was used to cultivate each *T. asperellum* isolate. This medium is composed of sucrose (10 g), $Ca_3(PO_4)_2$ (5 g), $MgCl_2 6H_2O$ (5 g), $MgSO_4 7H_2O$ (0.25 g), KCl (0.2 g), $(NH_4)_2SO_4$ (0.1 g) and bromophenol blue (0.025 g) in distilled water (1 L). Erlenmeyer flasks (150 mL) with 50 mL of media (adjusted to pH 7) were inoculated with 100 μL aliquots of spore suspensions (1×10^7 spores mL^{-1}) of each *T. asperellum* isolate. The liquid cultures were incubated at 25 °C on a rotary shaker (150 rev min^{-1}) for 96 h under ambient daylight conditions; after this time, the pH of cultures was measured. The cultures were centrifuged at 4000 × g for 10 min and the supernatant recovered. The absorbance at 600 nm of supernatants was measured. Controls consisted of the uninoculated culture medium. There were five replicate flasks for each treatment.

2.2.2. *Siderophore production*

The siderophore production capacity of *T. asperellum* isolates was determined using the colourimetric reaction method of Alexander and Zuberer (1991). In this assay, formation of a yellow halo surrounding the *Trichoderma* spp. mycelium is indicative of siderophore production. Controls consisted of uninoculated plates of culture medium. There were five replicate dishes for each treatment.