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BOOK OF ABSTRACTS

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USE OF TRICHODERMA HARZIANUM STRAIN T-22 AS A BIOCONTROL AGENT IN PRUNUS SPP. NURSERY PROCESSES

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SUMMARY

Trichoderma harzianum strain T-22 (T22) is one of the most effective strain and is able to colonize roots of the most of plant species across a wide range of soil types. This fungus is utilized as a biocontrol agent for crop production purposes, and for the improvement of plant nursery processes. Inoculation of T22 were applied on *in vitro*-cultured shoots of GiSeLa6® (*Prunus cerasus* x *Prunus canescens)* and GF677 (*Prunus amygdalus* x *Prunus persica*), two important varieties utilized as commercial rootstocks. The results showed that fungus inoculation (7 days after shoot transfer in the root-inducing medium), both GiSeLa6® and GF677 plants survived and showed significant increases in shoot growth and root development, particularly in root length (180 and 136% of non-inoculated controls, respectively). The microscopical analysis allowed to observe hyphae spreading on root cortex surface of GiSeLa6® (fungus colonization frequency of 20%) but not on GF677 roots. Our results demonstrated that the application of T22 during the rooting phase permits to have higher shoot length and leaves, number of roots and stem diameter and could be considered a natural product-based biopesticide. These morphological characteristics could increase nursery plant material and give notable advantages during the following acclimation phase.

INTRODUCTION

Trichoderma spp. are among the most abundant culturable fungi in many soils, and they are able to colonize plant roots and debris. The fungi of this genus are genetically quite diverse, with a number of different capabilities between different strains [1]. The biocontrol mechanisms exercised by *Trichoderma* could be attributed to competition for nutrients, release of extracellular hydrolytic enzymes, and secondary metabolites toxic for plant pathogens at very low concentrations [2]. In particular, *T. harzianum* produces a variety of antibiotic antifungal peptides called 'peptaibols', that interact with cell

membranes of plant fungal pathogens, so inhibiting their growth [1]. Furthermore, *T. harzianum* has been shown to inhibit wood rots and other fungal plant pathogens by up to 60% through production of volatile antibiotics [3]. *T. harzianum* is utilized as inoculants for crop production purposes, and for the improvement of plant nursery processes [2]. It is necessary to increase the quality of nursery plant material, in terms of higher and faster root and shoot development. For this purpose, the growth substrate (e.g., peat) is usually inoculated with *Trichoderma* few days (4 to 1) before rootstocks transplanting at the concentration of about 1 kg m^{-3} of substrate [1]. The inoculation of *T. harzianum* during the rooting phase, when the plants are cultured *in vitro* under sterile conditions, could be another way to minimize plant losses. This method could avoid the competition of *T. harzianum* with the other microorganisms usually found in soil, so allowing a better interaction with plant roots, and a better induction of the plant growth. The aim of this work was to conduct trials on the presence and development of the *T. harzianum*, and verify its effects on the growth of GiSeLa6® (*Prunus cerasus* x *Prunus canescens*) and GF677 (*Prunus amygdalus* x *Prunus persica*), two of the most important commercial rootstocks utilized for stone fruits.

MATERIALS AND METHODS

A sample of 40-day-old *Trichoderma harzianum* strain T-22 (T22) was cultured in potato dextrose broth (PDB) for 20 days at 25°C, on a rotary shaker at 140-150 rpm. The liquid culture was firstly filtered through two layers of Whatman No. 1 filter paper to remove hyphal fragments, and successively filtered through a 0.22 µm membrane filter. The 40-day period of fungus growth was chosen after a microscopical analyses, that assessed the abundance of conidiospores, according to Klein and Eveleigh [4]. Genetically uniform shoots of GiSeLa6® and GF677, deriving from *in vitro* multiplication, with a mean total length (shoot + root) of 3-4 cm, were cultured in sterile 400 mL transparent glass containers filled with 100 mL of Cherry medium and GF medium, respectively, and maintained in controlled conditions. After 7 days from the inoculation, for each treatment, a part of the plants (10 containers) was inoculated with T22 (R7+T22), while the other part (10 containers) was not inoculated with the fungus, and kept as a control (R7). The effects of fungal inoculum on the growth characteristics (shoot length, mean root length, number of roots, number of leaves, and stem basal diameter) were evaluated 6 and 9 days after fungal inoculation in GiSeLa6®, and after 19 and 23 days in GF677, respectively. Measurement were carried out on each of 10 randomly chosen cultured plants per container. Roots of GiSeLa6® and GF677 were microscopically analyzed in order to estimate

the progressive colonization of T22. Root samples, taken randomly from *in vitro* plants, were previous cleared by heating in KOH 10% for 45 minutes at 80°C, treated with 2.5% HCl for 30 min, and successively stained with Trypan blue. The presence of fungal hyphae and the frequency of system colonization were estimated on 50 fragments (length= 1 cm) per treatment. Root fragments, mounted on slides, were observed using a compound optical microscope under transmitted light at different magnifications and photographed.

RESULTS AND DISCUSSION

The results show that both GiSeLa6® and GF677 shoots survived to fungal inoculation. In addition, T22 caused significant increases in shoot growth and root development, and particularly in root length. In GiSeLa6®, these effects were visible starting from 6 days after inoculation, and were more marked after 9 days (increases in inoculated plants of 122, 180, 134, 136 and 161% for shoot height, root length, number of lateral roots, stem basal diameter and number of leaves, respectively). In GF677, these effects were slower and less marked, starting from 19 days after inoculation and more evident after 23 days (increases in inoculated plants of 118, 136, 105, 110, 126% for shoot height, root length, number of lateral roots, stem basal diameter and number of leaves, respectively). Therefore, a co-metabolic system with mutual benefits for plant and fungus has likely been established. Specific *Trichoderma* spp. strains were capable of plant growth enhancement, and of the bio-control of a range of wood-rot fungi when grown on a low-nutrient medium. In our case, we used a simple MS medium, that is quite representative of fresh softwood, with a C/N ratio of 410:1, and an amino-acid and glucose levels analogous to that found in the sap of growing peach and cherry trees [1]. In this investigation, the colonization frequency (number of colonized root fragments/total root fragments*100) of the *in vitro* system GiSeLa6®-T22 was 20%, when the fungus was applied 7 days after shoot transfer in the root-inducing medium. Although the measured fungal colonization frequency was not high, the morphological effects of fungus colonization on GiSeLa6® plants were evident, and inoculation mass and hyphae of T22 along roots of GiSeLa6® rootstock were clearly observed. Hyphae colonization was not microscopically detected in the system GF677-T22, and the effects of T22 on GF677 shoots and roots were slower and less evident. The different behavior of GiSeLa6® and GF677 could be due to the different gene expression patterns during the interaction of T22 with the two varieties, or to the particular signal transduction pathways that the symbiosis induces.

CONCLUSIONS

Our results demonstrated that the application of *T. harzianum* strain T22, 7 days after the beginning of the rooting pre-acclimation phase, permits to have a better shoot and root development. In particular, the fungus strongly enhances root length and stem basal diameter, and these could give notable advantages during the following nursery processes. A better quality of nursery plant material could promote a faster *in vitro* growth of micropropagated shoots and an increase of plant survival during the acclimation phase.

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