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Direct effects of *Trichoderma harzianum* strain T-22 on micropropagated shoots of GiSeLa6® (*Prunus cerasus* × *Prunus canescens*) rootstock

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ABSTRACT

Trichoderma harzianum strain T-22 (T22) has the ability of enhancing root growth and plant development. The aim of this research is to explain the biochemical basis of the direct plant-growth-promoting activity of T22. Seven days after the transfer to root-inducing medium, *in vitro*-cultured shoots of GiSeLa6® (*Prunus cerasus* × *Prunus canescens*), an important *Prunus* rootstock for sweet and sour cherry varieties, were inoculated with T22. Indole-3-acetic acid (IAA), *trans*-zeatin riboside (*t*-ZR) and dihydrozeatin riboside (DHZR) were analyzed by a competitive enzyme-linked immunosorbent assay. Acidification of the medium by plant, T22, and plant + T22 were assessed by three pH indicators, whereas root morphological changes were observed by light and epifluorescence microscopic analysis. The results showed that after T22-inoculation, IAA in leaves and roots significantly increased by 148 and 122%, respectively, whereas DHZR decreased by 83%. Increases in *t*-ZR were found only in leaves (88%). The ratios auxin/cytokinins changed from 28.5 to 46.6 in leaves, and from 15.0 to 21.2 in roots of un-inoculated and T22-inoculated plants, respectively. Root activity determined a decline of medium acidity, and this effect was more marked in T22-inoculated plants (up to pH 4). Microscopic analysis revealed changes in root cell wall suberification in the exoderm and endoderm, with an increase in suberized cellular layers from 1 to 2–3, and an enhancement of cell wall epifluorescence. During the acclimatisation phase of nursery processes, all these T22-induced changes constitute an advantage, as inoculated plants could acclimatise better, so increasing plant survival in the absence of pesticides.

1. Introduction

Trichoderma harzianum has been successfully used for the biological control of many plant pathogens through chemiotropic mycoparasitic interactions with the target fungal organism (Thangavelu et al., 2004; Sahebani and Hadavi, 2008). Several studies demonstrated that *T. harzianum* produces and excretes mycolytic cell wall degrading enzymes (Carsolio et al., 1994; Haran et al., 1995; Cohen-Kupiec et al., 1999; Noronha and Ulhoa, 2000; Jensen and Shulz, 2004; Ferreira and Ferreira Filho, 2004; Belén Suárez et al., 2005; Yang et al., 2009). The genes encoding some of these enzymes are highly expressed during mycoparasitism (Carsolio et al., 1994; Haran et al., 1995; Cohen-Kupiec et al., 1999; Belén Suárez et al., 2005; Yang et al., 2009) and their transcription is induced by diffusible factors secreted by pathogenic fungi (Cortes et al., 1998). Therefore, *T. harzianum* is considered a source of genes for the control of plants diseases (Lorito et al., 1998).

On the other hand, it has been reported that *T. harzianum* has the ability to directly enhance root growth and plant development in the absence of pathogens (Harman, 2000), and it has been suggested that this could be due to the production of some unidentified growth-regulating factors by the fungus (Windham et al., 1986). All these findings indicate the versatility through which *T. harzianum* can directly manifest biological control activity. In spite of their theoretical and practical importance, the mechanisms responsible for the growth response due to the direct action of *T. harzianum* on agronomic plants have not been investigated extensively.

Our research group recently demonstrated that the application of *T. harzianum* strain T-22 (T22) during the rooting phase of two *Prunus* spp. rootstocks, under *in vitro* conditions with plants and T22 alone, resulted in greater shoot lengths, as well as increased numbers of leaves, roots, and stem diameters (Sofo et al., 2010). The same authors observed that the growth-promoting effects of T22 were particularly marked in the genotype GiSeLa6® (*Prunus cerasus* × *Prunus canescens*), one of the most important commercial rootstocks used for sweet and sour cherry varieties. Furthermore, *T. harzianum* strain T-22 is particularly important for agronomic

Abbreviations: DHZR, dihydrozeatin riboside; ELISA, enzyme-linked immunosorbent assay; IAA, indole-3-acetic acid; T22, *Trichoderma harzianum* strain T-22; *t*-ZR, *trans*-zeatin riboside.

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purposes, as it is able to colonise the roots of most plant species across a wide range of soil types (Harman et al., 2004).

It was confirmed that plant growth regulators, also called phytohormones, such as indole-3-acetic acid (IAA), abscisic acid (ABA), and several cytokinins (CKs) are reliable physiological markers of developmental stages and growth of *Prunus persica* L. (Moncaleán et al., 2001). In particular, it is well known that high values of the ratio IAA/CKs in plants promote root formation, whereas low contents induce shoot-bud formation. Another hypothesized positive direct effect of *T. harzianum* on plants is the solubilisation of some insoluble or sparingly soluble minerals by acidification of the medium, that could determine a better nutrient availability and uptake for the plants (Altomare et al., 1999; Kücük et al., 2008; Singh et al., 2010). Unfortunately, the acidification of the medium of a system containing both T22 and plants has never been so far demonstrated.

For all these reasons, the aim of this research is to explain the biochemical basis of the direct plant-growth-promoting activity of T22. On the basis of the results of previous experiments (Kleifeld and Chet, 1992; Yedidia et al., 2001; Singh et al., 2010), one of them carried out on GiSeLa6® plants (Sofo et al., 2010), we hypothesized that the higher shoot and root growth in the plants inoculated with T22, could be due to changes in phyto-hormonal balance and root morphology, and/or to the acidification of the medium due to redox fungal activity.

2. Materials and methods

2.1. Experimental design

A sample of 40-d-old *T. harzianum* strain T-22 (T22) was cultured in liquid potato dextrose broth (PDB; Oxoid Ltd., Cambridge, UK) for 20 d at 25 °C on a rotary shaker at 150 rpm. The culture was then filtered through two layers of Whatman No. 1 filter paper (Whatman Ltd., Maidstone, UK) to remove hyphal fragments, and then filtered through 0.20 μm Minisart SFCA sterile filters (Sartorius Stedim Biotech GmbG, Goettingen, Germany).

Genetically uniform shoots of GiSeLa6® produced by *in vitro* multiplication (mean heights of 2.0 cm) were cultured in sterile 400 mL transparent glass containers filled with 100 mL of agarized Murashige and Skoog (MS) medium without vitamins (Sigma–Aldrich, St. Louis, MO, USA) supplemented with 1.0 mg L^{-1} IBA (indole-3-butyric acid; Sigma–Aldrich). This root-inducing medium was called 'cherry medium'. During the rooting phase, micropropagated shoots were maintained under controlled conditions at a constant temperature of 25 °C with a 16 h photoperiod and a PAR of 350 μmol m^{-2} s^{-1}. The same conditions were used for micropropagated rooted shoots (successively called 'plants' throughout the text) during the following phases.

2.2. Auxin and cytokinins determination

Seven days after the shoot transfer to the 'cherry medium', plants in five containers were inoculated with T22, while the remaining five containers were not inoculated with T22, and were kept as controls. Before the fungal inoculation in the 'cherry medium', the liquid culture filtrate was suspended in 50 mL sterile water and shaken for 5 min. Fungal inoculation was carried out using a sterile syringe with 5 mL (approximately 50,000 conidiospores) of liquid culture filtrate of T22. Nine days after inoculation [this period was chosen on the basis of the significant effect of T22-inoculation observed by Sofo et al. (2010)] the levels of *trans*-zeatin riboside (*t*-ZR), dihydrozeatin riboside (DHZR), and indole-3-acetic acid (IAA) were determined on three groups of plants per container chosen at random ($n = 15$).

Both for leaves and root tissues, an aliquot of 250 mg was ground into powder with liquid nitrogen with a mortar and pestle and put in a tube. To each tube, 2.5 mL extraction solvent (2-propanol/H_2O/HCl 37%; 2:1:0.002, v/v/v) was added. The tubes were shaken at a speed of 100 rpm for 30 min at 4 °C. To each tube, 2.5 mL of dichloromethane was added and then the samples were shaken for 30 min at 4 °C and centrifuged at 13,000 × *g* for 5 min. After centrifugation, two phases were formed. An aliquot (1.0 mL) of the solvent from the lower phase was transferred using a Pasteur pipette into a screw-cap vial, and the solvent mixture was concentrated using an evaporator with nitrogen flow. Finally, the samples were re-dissolved in 0.1 mL methanol and stored at −20 °C.

The quantitative determinations of IAA, *t*-ZR and DHZR were carried out in both un-inoculated and inoculated plants by a competitive enzyme-linked immunosorbent assay (ELISA) using the following kits, respectively: Phytodetek® *t*-ZR Test Kit, Phytodetek® DHZR Test Kit, and Phytodetek® IAA Test Kit (Agdia Biofords, Evry, France). The means of the optical densities and binding percentage of duplicate standards or samples (100 μL) were calculated and plotted in a semi-logarithmical scale, in order to linearize the equation.

Samples of 'cherry medium' ($n = 15$), from un-inoculated and inoculated containers, were also analyzed for IAA, *t*-ZR and DHZR.

2.3. Medium pH changes

Solutions of three pH-indicators starting from their powder form were prepared: 10 mg L^{-1} bromothymol blue, 10 mg L^{-1} bromocresol green, and 10 mg L^{-1} methyl red (Sigma–Aldrich). Each indicator solution was filtered through 0.20 μm Minisart SFCA sterile filters and a 2 mL aliquot was inoculated to agarised MEA (Malt Extract Agar) medium implemented with streptomycin 0.03 mg mL^{-1} and prepared in 1/20 strength Hoagland solution supplemented with 1.0 mg L^{-1} IBA. This medium was called 'indicator medium'. On the basis of previous laboratory trials, it was observed that MEA can support T22 growth, avoiding its behavior as a saprophyte and promoting plant-T22 symbiosis, whereas the Hoagland solution and IBA are essential for plant growth and root development, respectively. The value of pH of the medium without T22 or plant was 7.2.

Both transparent test tubes and 400 mL transparent glass containers were filled with each of the three indicator media, and half of them (12 test tubes and four containers per indicator) were inoculated with T22, whereas the remainder were not inoculated. Before fungal inoculation of the indicator media, the liquid culture filtrate was suspended in 50 mL sterile water and shaken for 5 min. Fungal inoculation was carried out using a sterile syringe with 1 or 5 mL of liquid culture filtrate of T22 for each test tube or container, respectively. Twenty days after the transfer of micropropagated shoots to the cherry medium, plants were transferred from cherry medium to each of the three indicator media. The effect of T22-inoculation on pH changes in the media was followed for seven days after the transfer of plants in the indicator media ($n = 8$).

2.4. Microscopic analysis

Root samples, taken at random from T22-inoculated and un-inoculated plants grown in cherry medium (some of the plants used for hormone determination), were previous cleaned by heating in 10% (w/v) KOH for 45 min at 80 °C, then treated with 2.5% (v/v) HCl for 30 min, and stained with 0.05 (w/v) Trypan blue (Sigma–Aldrich). Root fragments were so mounted on slides and observed at different magnifications using a compound optical microscope (Eclipse 80i; Nikon, Tokyo, Japan) under transmitted light and then photographed (Digital Camera DS-Fi1 with NIS-Elements Imaging Software, Nikon).

Table 1
Levels of indole-3-acetic acid (IAA), *trans*-zeatin riboside (*t*-ZR), dihydrozeatin riboside (DHZR) and auxin/cytokinins ratio (IAA/CK) in GiSeLa6® plants (*Prunus cerasus* × *P. canescens*) inoculated with *Trichoderma harzianum* strain T-22 and in un-inoculated plants. Values (±standard deviation) are means of 15 replicates ($n = 15$). For each column, values followed by a different letter are significantly different at $P \leq 0.05$, according to Fisher's LSD test.

Sample	Organ	IAA (ng g^{-1} fresh weight)	*t*-ZR (ng g^{-1} fresh weight)	DHZR (ng g^{-1} fresh weight)	IAA/CK (ng g^{-1} fresh weight)
Plant	Leaves	85.9 ± 6.5 b	1.7 ± 0.0 c	1.3 ± 0.0 a	28.5 b
	Roots	67.9 ± 4.1 c	3.2 ± 0.2 a	1.3 ± 0.1 a	15.0 d
Plant + T22	Leaves	127.4 ± 12.6 a	1.7 ± 0.0 c	1.1 ± 0.0 b	46.6 a
	Roots	82.6 ± 8.8 b	2.8 ± 0.1 b	1.1 ± 0.0 b	21.2 c

Five fresh apical portions per treatment, at least, were cross-sectioned (≤1 mm), 10 and 20 μm thick, at approximately 6 mm from the tip, and observed by an optical microscope with a mercuric vapours lamp (HBO 50 Axiophot, ZEISS, Oberkochen, Germany) to check epifluorescence emissions.

2.5. Statistical analysis

Statistical analysis was performed by analysis of variance (ANOVA) with SAS software (SAS Institute, Cary, NC, USA). Significant differences were determined at $P \leq 0.05$, according to Fisher's LSD test.

3. Results and discussion

3.1. Hormone levels and ratios

Production of growth-promoting metabolites and/or induction of the production of these compounds in the plants have been proposed as direct mechanisms by which *T. harzianum* promotes plant growth (Windham et al., 1986; Kleifeld and Chet, 1992; Harman et al., 2004). It is well known that IAA is the most widely naturally occurring auxin in vascular plants, and it has great importance during lateral and adventitious roots initiation and emergence, and shoot development. In this work, the levels of IAA in both leaves and roots increased significantly (148 and 122% of un-inoculated plants, respectively) after the inoculation with T22 (Table 1). This could explain the higher root and shoot growth in T22-inoculated GiSeLa6® shoots observed by Sofo et al. (2010). In fact, the application of IAA to plant tissues elicits a multitude of effects including electrophysiologic and transcriptional responses, and changes in cell division, expansion and differentiation (Hedden and Thomas, 2006). The results also show that foliar IAA was higher than in roots (Table 1), as this phyto-hormone is mainly produced in leaves, accumulated at the base of the shoot, and then transported to the roots by energy-dependent transport (Srivastava, 2002). As IAA also regulates lateral foliar bud formation, it is likely related to the number of leaves, that Sofo et al. (2010) have demonstrated to be higher in T22-inoculated GiSeLa6® plants.

Trans-zeatin (*t*-ZR) and dihydrozeatin (DHZR), derived from the purine adenine, are two of the major naturally occurring and more active cytokinins in plants. They control cell division in plants, and exogenous cytokinin applications induce cell division in tissue cultures in the presence of auxins (Hedden and Thomas, 2006). Cytokinins are also involved in overcoming apical dominance by releasing inactive lateral buds, inhibiting xylem formation and root growth, promoting leaf expansion, delaying senescence, and promoting chloroplast development (Srivastava, 2002). The results show that the effects of T22 on *t*-ZR levels were different from those of IAA, as the fungus significantly decreased *t*-ZR levels in roots of inoculated plants (88% of un-inoculated), whereas *t*-ZR in leaves was not affected (Table 1). Furthermore, the levels of *t*-ZR were significantly higher in roots than in leaves (Table 1), probably because root tips are a major site of cytokinin production and the primary transport path to the site of action is in the transpiration stream (Kuroh et al., 2002). In our experiment, DHZR levels were not significantly different between leaves and roots (Table 1). Furthermore, T22-treated leaves and roots presented a significant decrease in DHZR (both 83% of un-inoculated) (Table 1). DHZR is a precursor of *t*-ZR in the biosynthetic pattern, used as 'zeatin storage' for plant cells (Hedden and Thomas, 2006). Thus, its decrease suggests that a part of DHZR could be transformed in *t*-ZR after T22-inoculation.

It is noteworthy that none of the three hormones was detected in cherry medium of both T22-inoculated and un-inoculated plants, suggesting that they have not been released by the fungus. Thus, T22 induced hormone synthesis *ex novo* in the plants likely by the secretion of elicitors diffused into the medium or directly transferred from the fungal hyphae to the root cells. Our results are in contrast with those of Contreras-Cornejo et al. (2009), who found that *Trichoderma virens* cultured with *Arabidopsis* is able to produce and release in the medium auxin-related compounds. Thus, it is probable that the up-regulation of key genes for auxin and cytokinins biosynthesis or the down-regulation of the genes involved in their catabolism is induced by T22 (Harman et al., 2004).

Both auxin and cytokinins are involved in shoot and root growth and morphology. In particular, root growth and rooting of cuttings is stimulated by auxins and inhibited by low concentrations of cytokinins and ethylene (Pallardy, 2004). For the study of hormonal regulation in plants, not only is the hormone level *per se* important but the balances of the various hormones (Srivastava, 2002). In this experiment, auxin/cytokinins ratios significantly increased in leaves (from 28.5 to 46.6) and roots (from 15.0 to 21.2) of the plants inoculated with T22 (Table 1). This hormonal ratio closely regulates lateral roots growth controlling the emergence of root primordia, so explaining the well developed root system of T22-inoculated plants (Fig. 1).

The results obtained also confirmed that the method used for hormone extraction was successful. In particular, the following two points are to be considered critical during the extraction procedures: (a) the maintenance of the temperature below 4 °C and (b) the evaporation of the solvent mixture (not to complete dryness) under nitrogen flow to avoid hormone oxidation. Furthermore, the competitive ELISA assay allowed a good quantitative profile of the three hormones examined both in leaves and roots (Table 1).

3.2. Medium acidification

The pH indicator bromothymol blue acts as a weak acid in solution. Thus, it can exist in a yellow protonated form or a blue deprotonated form, whereas it is blue/green in neutral solution. In our experiment, bromothymol blue (pH measuring range = 7.6–6.0) was the first indicator to change colour with increasing acidification of the medium due to T22 and root exudates (Fig. 2A). The results showed that colour change of the medium due to the synergistic action of T22 + plants was more intense than that due to T22 alone (Fig. 2A). We also used methyl red, a pH indicator showing a red colour for pH values under 4.4 and yellow for pH values over 6.2. In this case, the acidification effects of T22 alone and of T22 + plants were much more clearly evident (Fig. 2B). Finally, the experiment using bromocresol green, a dye of the

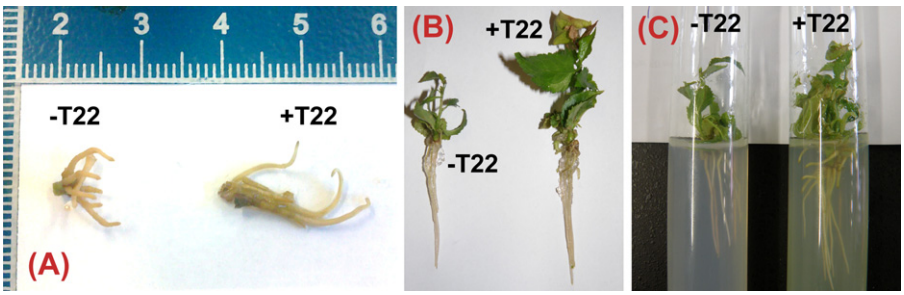

Fig. 1. (A) Morphological comparison of GiSeLa6® plants (*Prunus cerasus* × *P. canescens*) inoculated with *Trichoderma harzianum* strain T-22 (right) and un-inoculated plants (left). Inoculated roots appear more yellow-brownish in colour. Scale in centimeters. (B and C) Growth-promoting effects in T22-inoculated (right) and un-inoculated (left) GiSeLa6® plants.

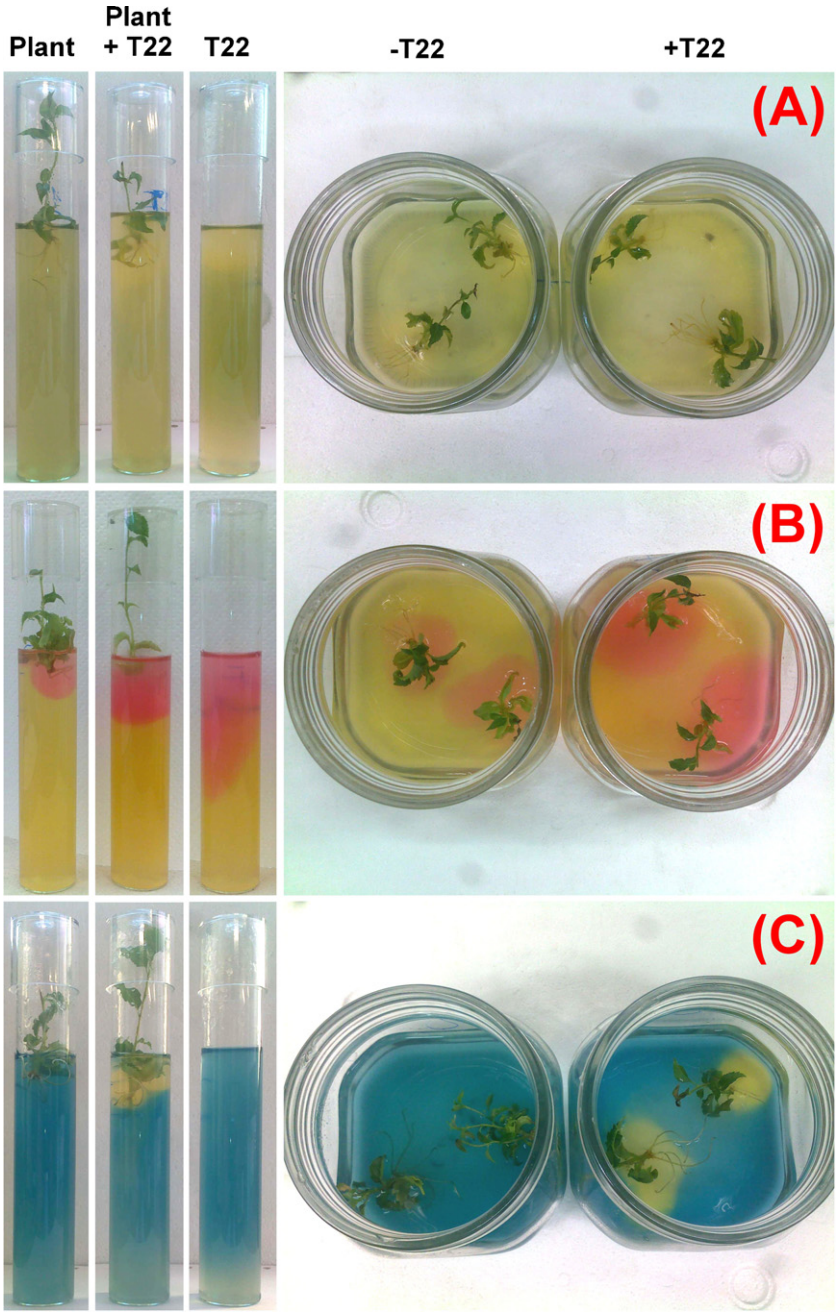

Fig. 2. Medium acidification by GiSeLa6® (*Prunus cerasus* × *P. canescens*) un-inoculated plants, GiSeLa6® plants inoculated with *Trichoderma harzianum* strain T-22, and T22 alone, as assessed by (A) bromothymol blue (pH measuring range = 7.6–6.0, with colour change from pale green to yellow), (B) methyl red (pH measuring range = 6.2–4.2, with colour change from yellow to red), and (C) bromocresol green (pH measuring range = 5.4–3.8, with colour change from blue to yellow).

Table 2
Values of pH of the indicator media in the three treatments: GiSeLa6® (*Prunus cerasus* × *P. canescens*) un-inoculated plants, GiSeLa6® plants inoculated with *Trichoderma harzianum* strain T-22, and T22 alone. Values (±standard deviation) are means of eight replicates ($n = 8$). For each column, values followed by a different letter are significantly different at $P \leq 0.05$, according to Fisher's LSD test.

Sample	pH		
	Bromothymol blue	Methyl red	Bromocresol green
Only plant	6.2 ± 0.1 a	6.3 ± 0.1 a	6.2 ± 0.1 a
Plant + T22	4.0 ± 0.4 c	4.2 ± 0.5 c	4.0 ± 0.1 c
Only T22	5.0 ± 0.1 b	5.0 ± 0.4 b	4.9 ± 0.1 b

triphenylmethane family, which is used as a pH indicator within the range 3.8 (yellow)–5.4 (blue) showed a significant acidification due to T22-inoculation of plants (Fig. 2C). In all the three cases, the acidified area remained around the root system (rhizosphere and surrounding areas) (Fig. 2).

Altomare et al. (1999) emphasised that the plant-growth-promoting capacity of *T. harzianum* was associated with *in vitro* solubilisation of certain insoluble minerals accomplished via production of chelating metabolites and fungus redox activity. In our experiment, we clearly demonstrated that a strong acidification in the medium inoculated with T22 occurred (Fig. 2). This acidification could determine the solubilisation of some salts and their higher availability for plants (Kücük et al., 2008; Singh et al., 2010). The experiment with methyl red revealed changes in pH values up to 4.4 both in the presence of T22 alone, the plant alone, and the plant + T22 (Fig. 2B). However, the use of bromocresol green (Fig. 2C) also indicated a higher acidification.

It is noteworthy that the values of pH of the indicator media showed a marked decrease also in the presence of T22 alone (Table 2), but it appears that the synergistic action by plant and T22 caused a greater acidification of the medium. This can be depend on the fact that T22 enhance the acidifying capacity of the root due to the proton extrusion of the root cells through the plasmalemma (Kücük et al., 2008). The pH decrease could allow the solubilisation of MnO_2, Fe_2O_3, metallic zinc, and calcium phosphate, and the reduction of Fe(III) and Cu(II), with evident benefits for plant nutrient uptake (Kücük et al., 2008; Singh et al., 2010).

The minimum pH values found by Altomare et al. (1999) in sucrose–yeast extract liquid cultures plus *T. harzianum* but without plants were approximately 5.0, so similar to our values (Table 2). In our experiment, we observed a further pH decrease of approximately 1 U of pH during T22-plants interaction (Table 2). The low pH values here observed could be probably due to the fact that the indicator media did not contain redox buffers and that the volume within the test tubes and containers was limited. We hypothesize that in natural soils, that usually have a pronounced buffering capacity, the acidification due to T22 could be less pronounced, as recently suggested by Singh et al. (2010).

3.3. Morphological analysis

Microscopical observations were carried out to compare root systems of T22-inoculated and un-inoculated plants. Plant overall morphology, and in particular root growth and distribution, of T22-inoculated and un-inoculated plants grown in cherry medium differed significantly (Fig. 1). Moreover, T22-colonised roots appeared yellow-brownish in colour (Fig. 1A).

Root tissues contain abundant alkaloids: berberine, chelerythrine, sanguinarine and chelidonine (along with other isoquinoline alkaloids), and some of them act as fluorochromes for suberin and lignin, providing numerous potential natural dye sources for fluorescence microscopic techniques (O'Brien and McCully, 1981; Brundrett et al., 1988). In our case, the observed cell

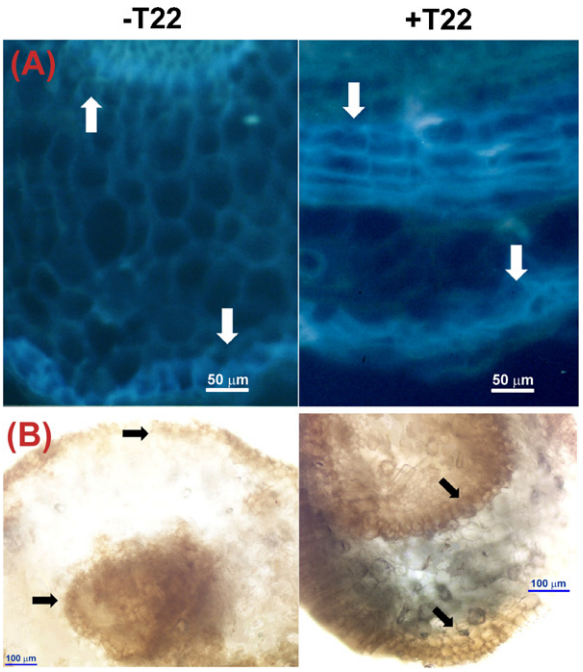

Fig. 3. (A) Root cross sections (diameter ≤1 mm; 6 mm from the root tip) of GiSeLa6® plants (*Prunus cerasus* × *P. canescens*) inoculated with *Trichoderma harzianum* strain T-22 (right) and un-inoculated plants (left) observed at 10× magnification with a mercuric lamp for epifluorescence detection. The arrows indicate the endodermic (inner) and exodermic (outer) layers. (B) Root cross sections (diameter ≤1 mm; 6 mm from the root tip) of GiSeLa6® plants inoculated with *Trichoderma harzianum* strain T-22 (right) and un-inoculated plants (left) under transmitted light at 10× magnification. The arrows indicate the endodermic (inner) and exodermic (outer) layers.

wall epifluorescence (Fig. 3A) indicated that T22 seems to induce the synthesis *ex novo*, of phenolic compounds in the plants, likely by the secretion of elicitors and the following induction of defense responses ('induced systemic resistance'; SAR), as suggested by Azcón-Aguilar and Barea (1996), and Mathivanan et al. (2008). Therefore, the biocontrol of fungal pathogens with *T. harzianum* could be also due to the induction of SAR in target plants, as indicated by De Meyer et al. (1998).

Among the compounds induced and involved in plant defence there are the enzymes of the phenylpropanoid pathway, involved in lignin biosynthesis (Gianinazzi-Pearson et al., 1994). The microscopic analysis of T22-inoculated plants highlighted a higher wall suberification and the increase in the number of suberized cellular layers from 1 to 2–3 both at the level of exoderm and endoderm (Fig. 3B). The increased lignification of root endodermal cells induced by mycorrhiza was already found by Dehne (1982) but it was never demonstrated for T22. We suggest that the accumulation of protective molecules, such as lignin and suberin, in plants inoculated with T22 could accelerate their hardening. The accumulation of structural substances may be of key importance in the resistance process, increasing the mechanical strength of the host cell walls (Spanu and Bonafante-Fasolo, 1988; Dalisay and Kuc, 1995).

4. Conclusions

Considering the positive effect on shoot and root growth in plants inoculated with T22 (Sofo et al., 2010), we hypothesise that changes in auxin/cytokinins ratios could be involved in this process. Several lines of evidence indicate that while auxins stimulate root formation, cytokinins inhibit it (Srivastava, 2002). In GiSeLa6® plants, the herein observed alterations in hormonal balance, with the consequent higher number of lateral roots and higher root

length (Sofo et al., 2010), could be an adaptative response induced by T22, that could benefit from a greater root surface area for colonisation, so reinforcing symbiotic behaviors with the plants. Furthermore, increased absorptive surface by branched roots may increase water and nutrient uptake capacity of plants.

The acidification of the medium could be one of the cause of the direct benefits of T22 on plants, as it this implies a reduction in the electrical potential within the cells that could favour the diffusion of cations from the medium ('soil' in the natural systems) to the root against the concentration gradient. Finally, infection of plants with T22 could make their roots more responsive to pathogen attacks, promoting a quicker and stronger reaction against the pathogens.

During the acclimatisation phase of nursery processes, all the observed biochemical and morphological changes induced by T22 constitute an advantage, as inoculated GiSeLa6® plants could acclimatise better to new and hostile environments, so increasing plant survival in the absence of pesticides.

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