The qid74 gene from *Trichoderma harzianum* has a role in root architecture and plant biofertilization

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The *Trichoderma harzianum* qid74 gene encodes a cysteine-rich cell wall protein that has an important role in adherence to hydrophobic surfaces and cellular protection; this gene was upregulated in *Trichoderma* high-density oligonucleotide (HDO) microarrays in interaction with tomato roots. Using a collection of qid74-overexpressing and disrupted mutants the role of this gene in cucumber and tomato root architecture was analysed in hydroponic and soil systems under greenhouse conditions. No significant differences were found in the pattern of root colonization and the length of primary roots of cucumber or tomato plants inoculated by *T. harzianum* CECT 2413 wild-type (wt) strain or any of the qid74 transformants. However, compared to the wt treatment, lateral roots were significantly longer in plants inoculated with the overexpressing transformants, and shorter in those treated with the disruptant strains. Microscopic observations revealed more and longer secondary root hairs in cucumber plants treated with the qid74-overexpressing mutants and fewer and shorter hairs in roots treated with qid74-disrupted transformants, compared to those observed in plants inoculated with the wt strain. qid74-induced modifications in root architecture increased the total absorptive surface, facilitating nutrient uptake and translocation of nutrients in the shoots, resulting in increased plant biomass through an efficient use of NPK and micronutrients.

INTRODUCTION

Some species of the genus *Trichoderma* are now considered as multifunctional endophytic plant symbionts due to their capacity to colonize intercellular root spaces and their multiple beneficial effects on plants (Harman *et al.*, 2004a; Shoresh *et al.*, 2010; Harman, 2011). Besides the ability of these strains to act as biocontrol agents they are able to penetrate and colonize plant roots (Yedidia *et al.*, 1999) and establish a molecular dialogue during the interaction with the plant (Lorito *et al.*, 2010). As a result of the symbiotic association, resistance to biotic, abiotic and physiological stresses as well as plant growth promotion can be induced (Mastouri *et al.*, 2010; Shoresh *et al.*, 2010). The positive effect observed is strongly dependent on the fungal and plant genotype (Harman *et al.*, 2004b; Tucci *et al.*, 2011) and the physiological status of the plant (Donoso *et al.*, 2008).

In order to better understand the complex interaction between *Trichoderma* spp. and plants, we developed a high-density oligonucleotide (HDO) microarray to examine the transcriptional response of *T. harzianum* CECT 2413 in contact with tomato roots (Samolski *et al.*, 2009). A number of fungal genes were identified to be overexpressed within the first hours of contact with the host. Among the significantly upregulated *Trichoderma* genes in the interaction with tomato plants in the absence of phytopathogenic fungi we identified the qid74 gene, encoding a cell wall protein with cysteine-rich repetitive regions with tri- (CXC) and penta-peptidic (CXXXC) motifs previously shown to be induced in *Trichoderma* by chitin (Rey *et al.*, 1998). This finding was of particular interest since qid74 had been previously related to mycoparasitic processes in this strain (Rosado *et al.*, 2007), but so far it had not been related to *Trichoderma*–plant interactions. Based on its induction under simulated mycoparasitism conditions (inclusion of chitin and mycelial cell wall of phytopathogens in the medium), the presence in its promoter sequence of a...
regulatory region characteristic of genes with a direct role on mycoparasitic interactions (MYC2 motif), and its important role in adherence to hydrophobic surfaces and cellular protection, it was hypothesized that the QID74 protein could have a specific role in adherence to prey cell surface and in protection against toxins and enzymes produced by the host during Trichoderma attack (Rosado et al., 2007).

Fungal–host recognition and adhesion during pathogenesis of other fungi, insects and plants, as well as in symbiotic associations, have been reported to be mediated by hydrophobins. These are small surface-active proteins, solely found in fungi, whose core structure consists of four $\beta$ strands, containing a signature of C-X$_n$-CC-X$_n$,C-X$_n$-CC-C$_n$,C-X$_n$-CC-C$_n$,C-X$_n$-CC-C$_n$, cross-linked by four disulfide bridges, creating in this way a structure enabling self-assembly at the interface between the hydrophilic cell wall and a hydrophobic environment (Whiteford & Spanu, 2002; Linder et al., 2005). Hydrophobins are very well represented in Trichoderma genomes (Kubicek et al., 2011). Hydrophobins and hydrophobin-like proteins (ceratoplatanins) have been reported to be involved in Trichoderma hyphal development and sporulation (Askolin et al., 2005; Muñoz et al., 1997), while others, as part of the plant interaction process, participate in root attachment and colonization (Viterbo & Chet, 2006) and systemic induction of disease resistance (Djonović et al., 2006; Seidl et al., 2006).

The deduced 704 aa sequence of QID74 (Rey et al., 1998) has 14 repetitions, of which eight are 59 aa in length and have the typical eight cysteine residues found in hydrophobins that could explain the role of this protein in cell wall protection and hydrophobic adherence. However, QID74 is a high-molecular-mass protein and its amino acid sequence repetitions do not fit into the typical consensus patterns between the first and eighth cysteine residues found in hydrophobins. The altered Trichoderma hydrophobicity previously related to this protein was therefore a consequence of QID74 modulation of other proteins rather than a hydrophobic nature per se.

Taking into account the involvement of qid74 in adherence to hydrophobic surfaces and cellular protection together with its overexpression in contact with tomato roots, a possible role of this gene in mediating Trichoderma–plant symbiosis at different stages, such as plant root attachment and colonization, could be envisaged. The aim of this work was to characterize the intimate relation of QID74 with plant roots at the rhizosphere competence level and evaluate whether its effect is reflected in a change of root architecture and/or plant growth. For this purpose T. harzianum CECT 2413 qid74-overexpressing and disrupted mutants were used to inoculate cucumber and tomato roots in axenic and in soil systems under greenhouse conditions.

**METHODS**

**Fungal strains and growth conditions.** Trichoderma harzianum wild-type strain CECT 2413 was obtained from the Spanish Type Culture Collection (Burjasot, Valencia, Spain). Mutants overexpressing the qid74 gene of this strain (T1 and T2) and their corresponding null mutants (A1 and A2) were obtained in previous work (Rosado et al., 2007). GFP-marked strains were obtained using the plasmid pZECA 1 carrying the gfp gene under the control of the constitutive promoter pki (pyruvate kinase) of T. reesei (Zeilingler et al., 1999), kindly provided by C. P. Kubicek (Vienna, Austria), and the pAN7-1 plasmid carrying the hph gene as selectable marker for hygromycin B resistance (Punt et al., 1987). The two plasmids were used at a 9:1 ratio respectively to co-transform qid74 mutants of T. harzianum CECT 2413 via a protoplast transformation method previously described (Cardoza et al., 2006). Strains that expressed gfp and showed normal growth were selected. The strains were maintained on potato dextrose agar medium (PDA, Sigma) at 28 °C in the dark until complete spor germination of the plate (~7 days). The spores were harvested and diluted in sterile distilled water. Fungal germlings were obtained in synthetic medium (SM) (Okon et al., 1973) according to Yedidia et al. (1999). Briefly, T. harzianum spores (1 × 10$^5$) were used to inoculate 100 ml SM. The cultures were shaken at 150 r.p.m. for 24 h at 30 °C to allow spore germination; germlings were separated from the growth medium by centrifugation and washed twice with sterile distilled water.

**Plant material and growth conditions.** Cucumber seeds (Cucumis sativus L. ‘Kfir’) from Gedera Seeds Co. were used for axenic hydroponic cultures. For greenhouse soil experiments cucumber (Cucumis sativus L. 'Marketmore 70') and tomato seeds (Solanum lycopersicum L. ‘Marmande Raf’), both from Semillas Batlle SA, were used. The same tomato variety was used for solid media assays. Seeds were surface sterilized in 70 % (v/v) ethanol followed by 2 % (v/v) NaOCl (2 min each step for cucumber or 10 min for tomato seeds), and finally thoroughly washed with sterile distilled water.

For hydroponic cultures, cucumber seedlings were grown in transparent polyethylene sterile containers (Phytatray II, Sigma) with 100 ml plant growth medium based on the growth system developed by Yedidia et al. (1999). Briefly, 20 surface-sterilized seeds per box were placed under aseptic conditions on a metallic screen covered by a gauze sheet, which held them 1.5 cm above the medium. Cultures were grown in a growth chamber with low agitation in a controlled environment: 25 °C, 75 % relative humidity and a photoperiod of 16 h of light.

For in vitro growth in solid media, surface-sterilized tomato seeds were germinated and grown on 14 cm side Petri dishes (six seeds per plate) containing 0.2 × Murashige & Skoog semi-solid medium (MS medium including vitamins and MES buffer; Duchefa Biochemie) with 0.8 % agar similarly as described by Contreras-Cornejo et al. (2009). Plates were sealed with tape (Micropore; 3M Healthcare), half-covered with aluminum foil to keep the roots in the dark, and incubated on their sides in a growth chamber under the same controlled conditions as mentioned above.

For greenhouse soil experiments, cucumber and tomato seeds (coated with a fungal spore suspension as further described below) were sown in sterilized soil mixed with vermiculite (3:1) in multi-cell growing trays (35 seeds per treatment) and kept for 4 weeks under controlled greenhouse conditions: 25 °C, 75 % relative humidity, and a photoperiod of 16 h of light. The plants were watered daily and supplemented with Hoagland nutrient solution (Hoagland & Arnon, 1950) to ensure nutrient availability. Experiments were arranged in a completely randomized design.

**Trichoderma–plant interactions.** The roots of seedlings grown in hydroponic cultures were inoculated with fungal germlings, obtained as described above, by adding, under aseptic conditions, 1 × 10$^5$ germinated spores ml$^{-1}$ to the plant growth medium of 7-day-old seedlings (Yedidia et al., 1999). The inoculated growth containers...
were maintained under the same conditions as described above and arranged in a completely randomized design.

For *Trichoderma*-plant interaction in solid media, 5-day-old tomato plants grown in Petri dishes were inoculated by placing 5 μl spore suspension containing 1 x 10⁶ spores 5 cm away from the root tips. Two plates containing six seedlings each were used per treatment. Plates were sealed and arranged in a completely randomized design in the growth chamber under the same conditions as used for growth.

Seeds used for soil experiments were surface sterilized as described above and incubated in a *T. harzianum* spore suspension (2 x 10⁶ spores ml⁻¹), then dried for 3 h in a fume hood to allow spore coating. Seeds were immediately used for sowing.

**Quantification of *Trichoderma* root colonization by real-time PCR.** A time-course experiment was performed with treated roots detached from each hydroponic growth container at 24, 48, 72, 96 and 120 h post-inoculation (p.i.) and extensively washed in water. After sterilization in 1 % (v/v) NaOCl for 1 min, the roots were washed with sterile distilled water, and total DNA extraction was performed according to Dellaporta et al. (1983). Two growth containers for each treatment were sampled and six roots were randomly chosen every time from each container and considered as a random sample for DNA extraction. In *plants* quantification was followed by amplification of a 72 bp fragment using *T. harzianum* CECT 2413 specific primers, Q2413f (5'-TGGCGTGAATTGAG-TTTGTG-3') and Q2413r (5'-CCCTCCGATGTTGGTTAAGGTTATG3'), designed from an 837 bp SCAR marker by Rubio et al. (2005). *C. sativus* actin gene primers, CSf (5'-GATGGTCTCTCTTCTTCTC-3') and CSr (5'-TCTGAGGCAACGCTCCTCCA-3'), were used as control reference for quantitative analysis. PCR was carried out in 96-well plates using 10 μl reaction containing 1x Kapa SYBR Fast qPCR kit Master Mix (2x) ABI Prism, 200 nM primers (for each forward and reverse) and 1 μl DNA extract. Non-template controls contained sterile water instead of DNA template. Amplifications were performed using the ABI Prism 7000 Sequence Detection System (PE Applied Biosystems) with standard PCR conditions of 95 °C for 15 s and 60 °C for 1 min for 40 cycles for absolute quantification. Melting curves were examined for primer-dimer formation by a dissociation step. Each sample was examined in triplicate using a relative quantification analysis by the standard curve technique as described by Suárez et al. (2005). Brieﬂy, standard curves for both *T. harzianum* and plant reference genes were generated using serial dilutions of the template by plotting the known DNA amounts (ng) against the Ct value exported from the Detection System for each plate. The Ct values for unknown samples were extrapolated from the standard curves and normalized amounts of *T. harzianum* obtained by dividing the amount of *T. harzianum* DNA (ng) by the plant DNA (ng) for each sample.

**In vivo microscopic analysis of cucumber root colonization.** Cucumber roots treated with *T. harzianum* GFP-marked strains were detached from the hydroponic cultures and evaluated for fungal–root interaction by confocal laser scanning microscopy (CLSM). The roots were mounted in 10 μM propidium iodide solution (Sigma) to label the plant cell walls. Samples were viewed using a TCS SP2 microscope (Leica), applying an excitation wavelength of 488 nm (Ar laser) and collecting the emitted light at 500–550 nm for GFP and at 600–700 nm for propidium iodide fluorescence. Digital images were acquired by using Leica confocal software LCS (v. 2.61.1537).

**Nutrient quantification in aerial parts of the plants.** Cotyledons from cucumber seedlings grown in the hydroponic system in contact with *Trichoderma* germings were detached at 7 days p.i.; seedlings from four axenic boxes were pooled, oven-dried at 65 °C for 2 h and pulverized using an electric blender. Shoots of cucumber and tomato plants grown under greenhouse conditions were collected at 28 days post-emergence of *Trichoderma*-coated seeds; three pools of 10 shoots per treatment were chosen randomly and each pool was considered as a random sample. Pooled shoots were dried and pulverized as described above. Dried tissue was sent to the Ionomics Service of the CEHAS-CSIC (Murcia, Spain), where inductively coupled plasma-optical emission spectroscopy (ICP-OES) and elemental analysis were performed for mineral content determinations; each plant pool was measured twice.

**Statistical analysis of experimental data.** One-way ANOVA and overall significance post-hoc determinations by Duncan’s test (P=0.05) were performed using SPSS Statistics software v. 17.0.

## RESULTS

**Selection of *qid74* transformants**

We previously identified 47 distinct *T. harzianum* CECT 2413 genes whose expression was increased at least two-fold in contact with tomato roots (Samolski et al., 2009). The *qid74* gene, encoding a cysteine-rich cell wall protein, showed a 4.4-fold increase in its expression and was chosen for further characterization of its role in *Trichoderma*-plant interactions. *qid74*-overexpressing (T2) and disrupted (Δ2) mutants obtained by Rosado et al. (2007), along with CECT 2413 wild-type (wt), were transformed with the *gfp* gene, encoding the green fluorescent protein (GFP). Several GFP-marked strains were obtained, and wt'-GFP, T'-GFP, T"-GFP, Δ'-GFP and Δ"-GFP were selected for CLSM analysis of root colonization. The selected strains showed the same growth and sporulation rates as the wt (data not shown).

**qid74** mutants retain cucumber root colonization capability

Early macroscopic examination did not reveal any differences in behaviour between the wt and *qid74*-gfp mutant strains in contact with cucumber seedling roots in hydroponic medium and all of them were able to attach to the roots to the same extent, showing at 10 h p.i. a profuse attachment over the root surface. CLSM observations at 24 h p.i. showed the *Trichoderma* hyphae anchored on the root surface (Fig. 1a) covering it mainly at the root tip level (Fig. 1b), with no apparent internal root colonization. At 48 h p.i. the fungus started to penetrate the root and some hyphae were detected in the intercellular spaces; at 120 h p.i. the fungus was able to profusely grow between the epidermal root cells (Fig. 1c). No differences in the morphology of root-interacting hyphae were noticed; neither papilla-like structures nor yeast-like cell differentiation were detected during the association in any of the strains tested.

For an accurate analysis of colonization, a time-course experiment was performed with the aim of quantifying the *in planta* fungal DNA of surface-sterilized roots at 24, 48, 72, 96 and 120 h p.i. by means of real-time PCR. No significant differences were found in the pattern of colonization between strains. Fungal DNA from each strain was detected at very low levels during the first
96 h, and at 120 h p.i. a remarkable increase was measured (Fig. 2), confirming the CLSM observations.

**Effect of qid74 mutants on root architecture and nutritional status in axenic systems**

No modification of cucumber primary root length was observed in the presence of qid74 transformant or wt strains; however, qid74-overexpressing mutants T1 and T2 were able to modify root architecture. Secondary roots in contact with these strains in hydroponic medium underwent morphological changes, developing abundant root hairs (Fig. 3a). In contrast, contact with the wt led to moderate density of hairs (Fig. 3b), and in the case of the disrupted mutants Δ1 and Δ2 root hairs were scarcely found and shorter (Fig. 3c). We also detected the presence of hyphal coiling around the root hair (Fig. 3d) as previously reported by Yedidia *et al.* (2000) for *Trichoderma asperelloides* interacting with cucumber roots.

As mentioned above, cucumber roots grown for 7 days in hydroponic medium in contact with *T. harzianum* wt, T1, T2, Δ1 or Δ2 strains did not show differences in the length of the main root, with the exception of those colonized by the disruptant Δ2, which showed a 24 % reduction in main root length relative to wt-colonized roots. An additional *in vitro* assay with tomato seedlings was carried out on solid medium (Contreras-Cornejo *et al.*, 2009) in order to analyse the primary and secondary root development. At 7 days p.i. no statistically significant differences among the five *T. harzianum* strains were found in the calculated measures of main root length (Fig. 4a). However, compared to the wt treatment the differences in lateral root length were statistically significant in plants inoculated with the qid74-overexpressing transformants: lateral root lengths increased by 42 and 30 % with mutants T1 and T2, respectively, and decreased by 35 and 27 % with the disruptant Δ1 and Δ2 treatments, respectively (Figs 4b and 5). The length increases observed with the T1 and T2 mutants were accompanied by a 34 % reduction in the mean number of lateral roots relative to that obtained with the wt strain (Fig. 4c).

Since QID74 was related to the elongation of lateral roots and the formation of root hairs, which have a role in increasing the surface available for nutrient uptake, a higher leaf NPK content could be expected. Cucumber plants inoculated with qid74-overexpressing T1 and T2 mutants indeed showed increased values of around 8 % and 11 % for N, 11 % and 22 % for P, and 23 % and 40 % for K, respectively, relative to the wt (data not shown). NPK concentrations of plants inoculated with disrupted mutants Δ1 and Δ2 were similar to those obtained for wt-inoculated plants.

**Growth promotion and nutritional status of plants in greenhouse soil experiments**

Since hydroponically grown cucumber seedlings were able to concentrate more NPK in the leaves when they were
root-inoculated with the *qid74*-overexpressing transfor-
mants, greenhouse assays were performed in which tomato
and cucumber seeds, coated with wt, T1 or T2 strains, were
sown in autoclaved soil supplemented with Hoagland
nutrient solution to avoid the presence of other micro-
organisms and guarantee enough mineral availability,
respectively. At 28 days p.i. with T1 or T2, cucumber
shoots were 14 and 9 % longer than those corresponding
to wt treatment, respectively. A shoot biomass increase of
22 % and 18 % relative to the wt strain was also detected in
plants inoculated with T1 and T2 mutants, respectively
(Fig. 6a). In tomato plants no significant differences were
observed in the shoot lengths but an increase of 27 % and a
statistically significant increase of 39 % were detected for
shoot biomass for the T1 and T2 mutants, respectively,
relative to the wt treatment (Fig. 6b).

Table 1 shows the mineral accumulation in cucumber and
tomato shoots per plant at 28 days p.i. with wt, T1 or T2
strains. Cucumber plants displayed higher accumulation
values for K, Li, Mn and C (total) when treated with the
mutants than with the wt. In tomato plants, both T1 and
T2 treatments gave higher accumulation of Ca, K, Mg, N,
P, B, Cu, Fe, Li, Mn, Na, Zn and C (total) relative to the wt
treatment.

**DISCUSSION**

The *T. harzianum* *qid74* gene encodes a cysteine-rich cell
wall protein with an unexpected homology to a salivary
protein of the dipteran insect *Chironomus* (Rey *et al.*, 1998). This gene is induced in cultures simulating myco-
parasitism conditions by inclusion of 2 % chitin or 0.1 %
fungal cell walls (Rosado *et al.*, 2007). However, *Tricho-
derma* microarray data showed that *qid74* displayed higher
expression levels in *T. harzianum*-tomato plant interaction
(4.4-fold) than in 1 % chitin cultures (1.5-fold) (Samolski
*et al.*, 2009). Furthermore the *qid74* homologous gene
FG03969 of *Fusarium graminearum* was also upregulated in

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**Fig. 3.** Microscopic analyses of cucumber seedling roots inoculated by *Trichoderma*. (a) Secondary root exhibiting abundant hairs in contact with *qid74*-overexpressing mutant T1. (b) Moderated density of root hairs in contact with the wt strain. (c) Shorter root hairs exhibited in contact with the *qid74*-disruptant mutant Δ1. (d) T1 hyphae growing around a root hair.

**Fig. 4.** Effect of *Trichoderma* inoculation on the root architecture of tomato seedlings. (a) Primary root length. (b) Lateral root length. (c) Lateral root number per plant. wt, wild-type; T1 and T2, *qid74* overexpressors; Δ1 and Δ2, *qid74* disruptants. Significantly different means are represented by different letters (*P*=0.05).
minimal medium containing plant cell walls (Carapito et al., 2008).

Besides its induction under simulated mycoparasitism conditions, QID74 protects fungal cells against lytic enzymes and enhances adherence to hydrophobic surfaces. Hence the suggestion of a specific role in adherence to pathogen cell surfaces and in protection of the invading fungus against defence compounds produced by the host during mycoparasitism (Rosado et al., 2007). In a similar way, considering its higher induction in contact with plant roots, QID74 might be mediating the attachment and protection of the growing hyphae from locally synthesized plant defence compounds during the first stages of plant colonization. The same role was proposed for the hydrophobin TasHyd1 of T. asperelloides (Viterbo & Chet, 2006). Nevertheless, despite its hydrophobin-like functions and the presence of eight-cysteine repetitive regions in its sequence, QID74 cannot be considered a hydrophobin due to its high molecular mass, different cysteine pattern and the predominance of polar amino acids in the protein. It has been previously shown that the homologous qid74 gene of T. virens (Tv-qid74) is positively regulated by the MAP kinase TVK1 (Mendoza-Mendoza et al., 2007) in a similar way to the homologous hydrophobin TasHyd1 gene of T. asperelloides (Viterbo & Chet, 2006) in T. virens (Tv-hfb3). Therefore, the altered hydrophobicity pattern previously observed in qid74 mutants (Rosado et al., 2007) seems to be a repercussion of qid74 regulation of hydrophobins or hydrophobin-like proteins. Considering that disrupted qid74 mutants produced only 75% of the extracellular proteins secreted by the wild-type, it is possible that qid74 expression leads to a higher secretion of molecules able to modify the hydrophobicity pattern and/or proteins, such as inhibitors, able to counteract the action of lytic enzymes, thus providing cellular adhesion and protection abilities.

In view of a possible plant-colonization role for QID74, a set of T. harzianum mutants, qid74-overexpressing or null, transformed or not with the gfp gene, was used in this study in order to explore the impact of QID74 on the plant-Trichoderma interaction. All the mutants were able to attach to cucumber roots, and similar behaviour was detected in a real-time PCR time-course analysis comparing T. harzianum wt and its corresponding gfp transformant, as a demonstration of the absence of negative effects due to gfp gene insertion. The increases in fungal DNA detected in the roots at 120 h p.i. confirm the capacity of all mutants to grow inside the root system. Since there was no significant difference between the mutants in root colonization ability we conclude that QID74 is not involved in the hyphal adherence to the roots nor in protection against the defence compounds produced by the plant. These findings suggest that qid74 expression has a different effect.

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**Fig. 5.** Lateral root length distribution of Trichoderma-inoculated tomato seedlings. wt, wild-type; T1 and T2, qid74 overexpressors; Δ1 and Δ2, qid74 disruptants.
from *TasHyd1* on *Trichoderma* spp. behaviour (Viterbo & Chet, 2006) and may be involved at other stages of interaction with the plant. Morphological modifications in the filamentous structure of *Trichoderma* such as the papilla-like or yeast-like cells described by Chaco´n *et al.* (2007) were not observed, at least in the first 120 h p.i. No significant differences were found in the length of primary roots of cucumber plants inoculated by wt or *qid74* transformants. However, microscopic observations revealed longer secondary roots with more and longer root hairs in cucumber plants treated with the *qid74*-overexpressing mutants and shorter secondary roots with fewer and shorter hairs in roots treated with disrupted transformants, compared to those detected in plants inoculated with wt strain. The modification in root architecture did not lead to an increased root colonization rate by *T. harzianum* (Fig. 1), demonstrating that root hairs are not essential for *T. harzianum* root infection as reported for rhizobacteria (Gage, 2004; Prieto *et al.*, 2011).

The regulation of root architecture and hair development in response to nutrient availability can be altered by plant growth regulators, such as auxins (IAA), cytokinins (CKs) and ethylene, suggesting that the nutritional control of root development may be mediated by changes in hormone synthesis, transport or sensitivity (López-Bucio *et al.*, 2003). Since nutrients were available for the plants, the QID74-related root hair modifications observed could be explained by alterations in hormonal balance. Conceivably, *qid74* expression could lead to a higher secretion of growth-promoting compounds that stimulate root hair proliferation. Although the production of CKs and their implication in *Trichoderma*–root interaction needs to be studied in

![Fig. 6. Effect of *qid74*-overexpressing mutants on shoot length and dry weight of (a) cucumber and (b) tomato plants grown in greenhouse soil experiments. Mean values relative to the wt strain are plotted. T1 and T2, *qid74* overexpressors; *, significantly different from the wt (P=0.05).](http://mic.sgmjournals.org)

Table 1. Mineral content per shoot of *Trichoderma*-inoculated cucumber and tomato plants grown in soil supplemented with nutrient solution under greenhouse conditions at 28 days post-emergence

<table>
<thead>
<tr>
<th>Plant and inoculant</th>
<th>C (mg)</th>
<th>Ca (mg)</th>
<th>K (mg)</th>
<th>Mg (mg)</th>
<th>N (mg)</th>
<th>P (mg)</th>
<th>B (µg)</th>
<th>Cu (µg)</th>
<th>Fe (µg)</th>
<th>Li (µg)</th>
<th>Mn (µg)</th>
<th>Na (µg)</th>
<th>Zn (µg)</th>
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<td><strong>Cucumber</strong></td>
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<tr>
<td>wt</td>
<td>312</td>
<td>11</td>
<td>44.5</td>
<td>4.8</td>
<td>38.2</td>
<td>8.1</td>
<td>23.7</td>
<td>5.7</td>
<td>70.3</td>
<td>7</td>
<td>77.8</td>
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<tr>
<td>T1</td>
<td>382.8</td>
<td>11.8</td>
<td>50.7</td>
<td>5.3</td>
<td>38.9</td>
<td>8.5</td>
<td>26.4</td>
<td>6.8</td>
<td>77.8</td>
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<td>93.3</td>
<td>773.5</td>
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<td>(% rel)</td>
<td>22.7*</td>
<td>7.6</td>
<td>14*</td>
<td>9.7</td>
<td>1.8</td>
<td>4.7</td>
<td>11.7</td>
<td>19.1</td>
<td>10.7</td>
<td>71.4*</td>
<td>19.9*</td>
<td>20.3</td>
<td>1.2</td>
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<td>T2</td>
<td>375.7</td>
<td>11.6</td>
<td>51.7</td>
<td>5.1</td>
<td>38.2</td>
<td>8.7</td>
<td>30.4</td>
<td>9.9</td>
<td>82.4</td>
<td>12.1</td>
<td>92.1</td>
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<td>(% rel)</td>
<td>20.4*</td>
<td>5.8</td>
<td>16.2*</td>
<td>5.9</td>
<td>0.1</td>
<td>7.9</td>
<td>28.3*</td>
<td>74.1*</td>
<td>17.2*</td>
<td>72*</td>
<td>18.3*</td>
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<tr>
<td>wt</td>
<td>103.8</td>
<td>4.3</td>
<td>21.8</td>
<td>1.3</td>
<td>19.6</td>
<td>2.8</td>
<td>8.2</td>
<td>2.9</td>
<td>31.9</td>
<td>1.8</td>
<td>33.5</td>
<td>282.2</td>
<td>18.7</td>
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<td>135.2</td>
<td>5.4</td>
<td>25.5</td>
<td>1.7</td>
<td>23.7</td>
<td>3.6</td>
<td>10.7</td>
<td>3.6</td>
<td>42.7</td>
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<td>364.6</td>
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<td>(% rel)</td>
<td>30.2*</td>
<td>24.9*</td>
<td>17*</td>
<td>35.9*</td>
<td>20.9*</td>
<td>29.2*</td>
<td>30.5*</td>
<td>21.4*</td>
<td>34.1*</td>
<td>29.3*</td>
<td>21.7*</td>
<td>29.2*</td>
<td>20.6*</td>
</tr>
<tr>
<td>T2</td>
<td>162.8</td>
<td>6.3</td>
<td>28.1</td>
<td>2</td>
<td>29.1</td>
<td>4</td>
<td>11</td>
<td>4.2</td>
<td>46.6</td>
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<td>45.9</td>
<td>421.2</td>
<td>28.1</td>
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<tr>
<td>(% rel)</td>
<td>56.8*</td>
<td>44.3*</td>
<td>28.8*</td>
<td>52.4*</td>
<td>48.6*</td>
<td>42*</td>
<td>34.6*</td>
<td>42.4*</td>
<td>46.2*</td>
<td>39.2*</td>
<td>37.3*</td>
<td>49.2*</td>
<td>50*</td>
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</table>

*Values significantly higher than the wt (Duncan’s test; P=0.05).
†C refers to total carbon content.
depth, it is known that CKs inhibit primary root elongation and lateral root formation but can promote root hair development (Richardson et al., 2009). In this case, no changes in primary root length were observed in the presence of qid74 transformants or wt strains, so QID74-related secondary root hair proliferation could perhaps be explained by alterations in the ethylene/IAA balance as was hypothesized for Azospirillum–tomato interaction (Ribau et al., 2006). Oscillations in extracellular pH and reactive oxygen species (ROS) modulate tip growth of Arabidopsis root hairs (Monshausen et al., 2007). It has been recently described that T. harzianum produces ROS through an NADPH oxidase activity (Montero-Barrientos et al., 2011) but also reduces damage resulting from accumulation of ROS in stressed plants (Mastouri et al., 2010). Further studies at proteomic and metabolomic level are needed to confirm these hypotheses. Root hair growth is not a constant process, instead it occurs as discrete episodes (Knight, 2007), and researchers in this area will have to integrate all these mechanisms of interaction to produce a reliable combined model.

Root hair cells are the major point of contact between the plant and the rhizosphere, constituting up to 70% of the total surface area (Richardson et al., 2009). QID74 influences lateral root elongation and hair formation and elongation, increasing the total surface for nutrient uptake and in consequence the capacity of plants to grow in soils in which nutrient resources are limiting. As mentioned above, the availability of nutrients and their external and internal concentrations regulate root architecture and hair development (López-Bucio et al., 2003). In this study we minimized the influence of nutrient availability by the use of Hoagland nutrient solution, which supplied sufficient macro- and micronutrients into the system. Since a preliminary test on hydroponic cucumber showed that roots treated with the qid74-overexpressing transformants concentrated more NPK in the leaves than the wild-type and disruptant strains, a greenhouse sterile soil experiment was performed with tomato and cucumber to compare plant growth and nutrient content in aerial parts of 28-day-old plants after seed coating inoculation with the qid74-overexpressing transformants and the wt strain. As shown in Fig. 4, the two qid74-overexpressing transformants had a similar effect: measured as shoot length and dry weight, compared to those inoculated with the wt, tomato and cucumber plants increased their size after inoculation with T1 or T2 strains. The differences found in length and weight increases between treatments could be due to the intrinsic variability of plants and Trichoderma strains (Tucci et al., 2011). Accumulation of nutrients in the aerial parts of tomato and cucumber (Table 1) was always higher in the cucumber plants and, as could be expected, both tomato and cucumber plants treated with the two qid74-overexpressing transformants absorbed more nutrients. Since these results were obtained with sufficient levels of minerals in the soil, the fertilization effect observed in tomato and cucumber inoculated with T. harzianum seems to be the result of improved nutrient uptake due to the increase in absorptive area observed in the plants treated with qid74-overexpressing mutants, rather than nutrient solubilization. T. harzianum is also able to solubilize phosphate and micronutrients (Altomare et al., 1999), but considering the design of our experiments, with supply of Hoagland nutrient solution, this fertilization mechanism was not tested. Yedidia et al. (2001) reported the effect of T. asperelloides on concentration of minerals and increased plant growth via higher nutrient uptake, but the mechanism was not established. As far as we know, this is the first report that explains the increased nutrient uptake shown in Trichoderma-treated plants by means of root hair proliferation. The higher nutrient levels acquired by root hairs must be translocated from roots to shoots, which was reflected in plant growth promotion. T. harzianum increases the efficiency of nitrogen use in maize and other crops, giving improved yields (Harman, 2000). Nitrogen content increases in Trichoderma-treated plants (Shoresh et al., 2010), although this was more evident in strains overexpressing qid74. Nitrogen concentrations in tomato and cucumber shoots were indicative of the efficient use of nutrients since the lower concentration values (expressed as mg nitrogen per g shoot) detected in plants inoculated with qid74-overexpressing mutants (data not shown) were accompanied by an increase of shoot biomass.

In summary, QID74 is a large cysteine-rich cell wall protein related to fungal adherence and cell protection that has an important role in lateral root growth and hair formation and elongation. Modification of root architecture increases the total absorptive surface, facilitating nutrient uptake and translocation of nutrients in the shoots, and resulting in increased plant biomass through an efficient use of mineral elements.

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