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The interaction with arbuscular mycorrhizal fungi or *Trichoderma harzianum* alters the shoot hormonal profile in melon plants

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ABSTRACT

Arbuscular mycorrhizal fungi (AMF) and *Trichoderma harzianum* are known to affect plant growth and disease resistance through interaction with phytohormone synthesis or transport in the plant. Cross-talk between these microorganisms and their host plants normally occurs in nature and may affect plant resistance. Simultaneous quantification in the shoots of melon plants revealed significant changes in the levels of several hormones in response to inoculation with *T. harzianum* and two different AMF (*Glomus intraradices* and *Glomus mosseae*). Analysis of zeatin (Ze), indole-3-acetic acid (IAA), 1-aminocyclopropane-1-carboxylic acid (ACC), salicylic acid (SA), jasmonic acid (JA) and abscisic acid (ABA) in the shoot showed common and divergent responses of melon plants to *G. intraradices* and *G. mosseae*. *T. harzianum* effected systemic increases in Ze, IAA, ACC, SA, JA and ABA. The interaction of *T. harzianum* and the AMF with the plant produced a characteristic hormonal profile, which differed from that produced by inoculation with each microorganism singly, suggesting an attenuation of the plant response, related to the hormones SA, JA and ethylene. These results are discussed in relation to their involvement in biomass allocation and basal resistance against Fusarium wilt.

1. Introduction

Beneficial rhizosphere microorganisms that improve plant nutrition and health include arbuscular mycorrhizal fungi (AMF) and *Trichoderma* spp. Alleviation of damage caused by soil-borne pathogens, such as *Phytophthora, Fusarium, Pythium, Rhizoctonia, Sclerotium* and *Verticillium* has been reported widely in mycorrhizal plants (Barea et al., 1997; Bi et al., 2007; Whipps, 2004). The AM establishment and functioning result from a complex molecular dialogue between the plant and the AM fungus (Harrison, 2005; Parniske, 2008; Paszkowski, 2006a; Requena et al., 2007). Some processes occurring in this dialogue are known to be mediated by phytohormones on the plant side (Hause et al., 2007). The establishment of the AM symbiosis has been reported to induce changes in the phytohormone balance in the roots of the host plants, with respect to cytokinins, gibberellins, ethylene, abscisic acid (ABA) and jasmonates (Allen et al., 1980, 1982; Drüge and Schönbeck, 1992; Hause et al., 2002, 2007; López-Ráez et al., 2010; Ludwig-Müller, 2000, Ludwig-Müller et al., 2002; Riedel et al., 2008), but there is only limited evidence about the systemic effects of this particular symbiosis in the shoots of AM plants (Pozo et al., 2009; Toussaint, 2007). In this regard, Taylor and Harrier (2003) observed that AM-tomato plants showed different gene expression patterns in leaf and root tissues, which could have been the result of an alteration of the hormonal balance in the host plants. There have been also several studies related to the induction of resistance by AMF, focusing especially on the activation of plant defence mechanisms in roots (Avis et al., 2008; Garcia-Garrido and Ocampo, 2002; Pozo and Azcón-Aguilar, 2007; Pozo et al., 2009). However, among the studies that have explored the role of phytohormones in AM–plant interactions, the results are inconsistent (Hause et al., 2007; Ludwig-Müller, 2000; Pozo and Azcón-Aguilar, 2007) and are focused on understanding the biology of the AM symbiosis, mainly at the root level. Therefore, there is still a lack of information on the physiological implications of the symbiosis in the shoot of the host plant (Toussaint, 2007). In this regard, accumulation of insect anti-feedant compounds (Gange, 2006) and transcriptional up-regulation of defence-related genes (Liu et al., 2007) have been described recently in the shoots of mycorrhizal plants.

Trichoderma (teleomorph *Hypocrea*) is a genus of asexual fungi found in the soils of all climatic zones. These fungi are opportunistic, avirulent plant symbionts and function as parasites and antagonists of many phytopathogenic fungi, thus protecting plants from diseases (Benítez et al., 2004; Harman et al., 2004; Howell, 2003; Vinale et al., 2008). Some strains can penetrate plant roots and colonise the epidermis and outer cortex, causing substantial changes in plant metabolism. It is well documented that some

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strains promote plant growth, increase nutrient availability, improve crop production and enhance resistance to pathogens, even in the shoot (Elad, 2000; Harman et al., 2004; Korolev et al., 2008; Shoresh et al., 2005; Vinale et al., 2008; Yedidia et al., 2003). It has been proposed that the phytohormones jasmonic acid (JA), ethylene and salicylic acid (SA) play a major role in the resistance induced by several *Trichoderma* isolates. *Trichoderma asperellum* isolate T203 has been shown to induce resistance in cucumber to *Pseudomonas syringae* pv. *Lachrymans*, through the pathway mediated by the signal phytohormones JA and ethylene in the plant (Shoresh et al., 2005). Further evidence for a role of JA and ethylene in *Trichoderma*-induced resistance was provided by Korolev et al. (2008), who demonstrated that mutants of Arabidopsis with a defect in ethylene or JA signalling were unable to elevate their systemic resistance levels to *Botrytis cinerea* after colonisation by *Trichoderma harzianum* isolate T39. Moreover, a number of mechanisms have been proposed to explain the growth enhancement by *Trichoderma* spp. (Benítez et al., 2004; Harman et al., 2004), among them, fungal interactions with phytohormonal signalling and induction of resistance against pathogens (Vassilev et al., 2006). A possible role of indoleacetic acid (IAA) in the growth stimulation of tomato plants produced by inoculation with *Trichoderma aureoviride* was proposed by Gravel et al. (2007).

We hypothesised that this wide range of plant responses induced by each of these beneficial microorganisms could be modified by their simultaneous co-inoculation, and that this could be reflected in the overall metabolism of melon plants. This study was conducted to investigate the alteration of the hormonal profile of melon shoots as a consequence of the plant response to two AMF (*Glomus intraradices* and *Glomus mosseae*), which showed different colonisation patterns and functionalities in previous studies, and to the beneficial fungus *T. harzianum*, following their individual and co-inoculation. Such studies are crucial, not only to the development of an integrated understanding of complex plant-beneficial microorganism relationships, but also for the development of rational strategies for improving pathogen resistance using beneficial microorganisms.

2. Results

2.1. Plant growth

Inoculation with the AMF alone did not change shoot fr. wt. compared with control plants, while inoculation with *T. harzianum* alone increased shoot fr. wt. by 20% ($P < 0.001$) (Table 1). The combined application of *T. harzianum* with the AMF resulted in an increased shoot fr. wt. relative to plants inoculated with the AMF alone. Although no changes in the shoot fr. wt. due to AMF colonisation were observed, an increased shoot/root ratio was observed in AMF-inoculated plants (23–25% higher) compared to control plants (Table 1). The same effect was observed after inoculation with *T. harzianum*. A significant interaction between the factors AMF and *T. harzianum* was observed regarding the shoot/root ratio. The shoot/root ratio of *G. intraradices*–*T. harzianum* co-inoculated plants was higher than that for plants inoculated with these microorganisms separately.

2.2. Arbuscular mycorrhizal root colonisation, T. harzianum proliferation and disease incidence

A significant interaction between the factors AMF and *T. harzianum* was observed regarding AM root colonisation. The presence of *T. harzianum* increased AM root colonisation relative to plants inoculated with the AMF alone, co-inoculation with *G. intraradices* and *T. harzianum* producing a higher percentage of colonisation than any other treatment (Table 2). No differences in the number of *T. harzianum* colony forming units (CFU) recovered from the substrate were observed for the treatments involving co-inoculation with either *G. intraradices* or *G. mosseae*, with respect to inoculation with *T. harzianum* alone (Table 2). *T. harzianum* decreased the disease incidence (by 50%, $P < 0.001$) with respect to control plants (Table 2). Inoculation with *G. intraradices* or *G. mosseae* alone also reduced the disease incidence (by 25% and 50%, respectively, $P < 0.001$). Although a significant interaction was found between the factors AMF and *T. harzianum* regarding disease incidence, the combination of *T. harzianum* with the AMF produced different patterns of disease incidence. Plants co-inoculated with *T. harzianum* and *G. intraradices* showed a lower disease incidence than plants inoculated with either microorganism singly, while no differences in disease incidence were found following the combined inoculation with *T. harzianum* and *G. mosseae*, compared with either microorganism inoculated singly (Table 2).

Table 1
The shoot and root fr. wt. (g) and the shoot/root ratio of melon plants inoculated with *Trichoderma harzianum* and/or *Glomus intraradices* or *Glomus mosseae*, 6 weeks after planting.

	Treatment	Shoot fr. wt. (g)	Root fr. wt. (g)	Shoot/root ratio
	Control	1.63 ± 0.08	0.66 ± 0.08	2.48 ± 0.10^{d}
	G. intraradices	1.45 ± 0.09	0.47 ± 0.10$^{¥}$	3.11 ± 0.07^{b}
	G. mosseae	1.63 ± 0.07	0.53 ± 0.06$^{¥}$	3.07 ± 0.09^{b}
	T. harzianum	1.96 ± 0.02$^{¥}$	0.67 ± 0.10	2.92 ± 0.13^{c}
	G intraradices + *T. harzianum*	1.77 ± 0.07	0.52 ± 0.06$^{¥}$	3.43 ± 0.12^{a}
	G. mosseae + *T. harzianum*	1.99 ± 0.10$^{¥}$	0.67 ± 0.12	2.98 ± 0.07^{b,c}
Two-way ANOVA				
	AMF inoculation	*	***	***
	T. harzianum inoculation	***	NS	**
	AMF × *T. harzianum*	NS	NS	*

The data are the means of five replicates (± SE).
¥ Means are significantly different from the control according to Dunnett's test ($P < 0.05$). Data not sharing a letter in common differ significantly ($P < 0.05$) according to Fisher's LSD test.
NS, not significant; * $P < 0.05$; ** $P < 0.01$. *** $P < 0.001$.

2.3. Hormonal profiling

Significant interactions between the factors AMF and *T. harzianum* were observed for all phytohormones except zeatin.

Table 2
The arbuscular mycorrhizal (AM) root colonisation (%) and the *Trichoderma harzianum* population (colony forming units × 10^6 g^{-1} of peat) 6 weeks after planting, and the Fusarium wilt incidence (% of infected plants) 3 weeks after pathogen inoculation in melon plants inoculated with *Glomus intraradices* or *Glomus mosseae*, alone or co-inoculated with *Trichoderma harzianum*.

	Treatment	AM root colonisation	*T. harzianum* colony forming units	Fusarium wilt incidence
	Control	<5	N.d.^{A}	80^{d}
	G. intraradices	67.33 ± 4.83^{b}	N.d.	60^{c}
	G. mosseae	56.03 ± 4.39^{c}	N.d.	41^{b}
	T. harzianum	<5	2.10 ± 0.97^{a}	40^{b}
	G intraradices + *T. harzianum*	84.72 ± 4.72^{a}	1.90 ± 0.56^{a}	13^{a}
	G. mosseae + *T. harzianum*	70.27 ± 3.42^{b}	1.02 ± 0.49^{a}	47^{b}
Two-way ANOVA				
	AMF inoculation	***	–	***
	T. harzianum inoculation	NS	***	***
	AMF × *T. harzianum*	*	–	**

The data are the means of five replicates (± SE).
A N.d., not detected. Data not sharing a letter in common differ significantly ($P < 0.05$) according to Fisher's LSD test.
NS, not significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.