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AM symbiosis alters phenolic acid content in tomato roots

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Arbuscular mycorrhizal (AM) fungi colonize the roots of most plants to establish a mutualistic symbiosis leading to important benefits for plant health. We have recently shown that AM symbiosis alters both transcriptional and hormonal profiles in tomato roots, many of these changes related to plant defence. Here, we analytically demonstrate that the levels of other important defence-related compounds as phenolic acids are also altered in the symbiosis. Both caffeic and chlorogenic acid levels significantly decreased in tomato roots upon mycorrhization, while ferulic acid increased. Moreover, in the case of caffeic acid a differential reduction was observed depending on the colonizing AM fungus. The results confirm that AM associations imply the regulation of plant defence responses, and that the host changes may vary depending on the AM fungus involved. The potential implications of altered phenolic acid levels on plant control over mycorrhizal colonization and in the plant resistance to pathogens is discussed.

Plants have to cope with numerous environmental factors, including abiotic and biotic stresses, to ensure survival, growth and development. Regarding biotic stress, plants have evolved complex and integrated systems to defend themselves against a variety of deleterious organisms. These mechanisms include preformed physical and chemical barriers, but also inducible defences triggered upon non-self recognition, such as strengthening of the cell wall and activation of several biochemical pathways for the synthesis of defence-related enzymes and diverse secondary metabolites.[1-3] Upon recognition of the invader, the plant alters the levels of some phytohormones—mainly salicylic acid, ethylene and jasmonates—which in turn activate the appropriate defence mechanisms.[4-6] The responses triggered usually include the biosynthesis of compounds with biocidal activity such as alkaloids, terpenoids, flavonoids and phenolic acids.[2,7] Phenolic acids (PAs) are metabolites with a diverse structure characterized by hydroxylated aromatic rings. Despite some constitutive expression, their relative location and concentration may vary in response to cell invasion by microorganisms. PAs can fulfil different functions, as constituents of plant cell walls regulating cell wall plasticity as antimicrobial compounds, and they may act as signaling molecules modulating plant-microbe interactions.[3,7,8] In tomato, the PAs vanillic, caffeic, chlorogenic and ferulic acids have been shown to display biocidal activity and/or associated with resistance to pathogens.[7] Interestingly, ferulic acid has been also related with the induction of plant resistance by biocontrol agents.[9]

Arbuscular mycorrhizal (AM) symbioses are widespread mutualistic associations established between certain soil fungi and most terrestrial plants.[10,11] In the symbiosis, the AM fungi (AMF) obtain carbohydrates from their host plants and help them to acquire water and mineral nutrients. In addition, they may also increase plant resistance to biotic and abiotic stresses.[11] To establish the symbiosis, AMF extensively colonize the root cortex inter and intracellularly, process that requires the modulation of plant defence responses. Accordingly, evidences of both induction and repression of different defence-related responses have been reported.[11-13] Surprisingly, despite the importance of the phenolic compounds in plant defence, very little information is available regarding these metabolites in mycorrhizal systems. We have recently shown that AM symbiosis alters both hormonal and transcriptonal profiles in tomato roots, including changes associated to defence responses, and a regulatory role of jasmonates and other oxylipins in the interaction has been pointed out.[13] Here, we complement those results with the analysis and quantification in the same mycorrhizal systems of the phenolics caffeic, chlorogenic and ferulic acids by ultra pressure liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) in order to get new insights on the involvement of such compounds in the AM symbiosis.

LC-MS/MS Analysis and Quantification of Caffeic and Chlorogenic Acids in Mycorrhizal Roots

To assess whether AM symbiosis alters the levels of phenolic compounds in host roots, non mycorrhizal tomato plants were compared with plants colonized by two different AMF.[13] Nine weeks after inoculation with the AMF, 41% of the root system was colonized by *Glomus intraradices*, whereas *Glomus mosseae* colonized tomato roots to a lesser extent (*c.* 23%). For the analysis of PAs in mycorrhizal plants, root extracts from non-mycorrhizal control or AMF-colonized plants were obtained and analyzed by UPLC-MS/MS as described before.[14] Interestingly, the levels of chlorogenic acid (caffeoyl quinic acid) (**Fig. 1A**) were significantly ($p < 0.01$) lower in roots colonized by both AMF compared to non-mycorrhizal roots. The reduction, about 40%, was similar in both mycorrhizal associations (**Fig. 1A**). Reduced levels of chlorogenic acid were previously observed in roots of tobacco plants colonized by *G. intraradices*.[15] However, the authors suggested that this decrease in the steady-state of defence-related compounds in the roots was not a direct consequence of mycorrhization.

As for chlorogenic acid, a clear decrease in caffeic acid (**Fig. 1B**) was detected upon mycorrhization by both AMF. However, in this case the reduction on the levels of caffeic acid was different in both interactions (**Fig. 1B**). The reduction in *G. mosseae*-colonized roots was about 40% compared with non-mycorrhizal plants, whereas in *G. intraradices*-colonized roots the reduction was significantly ($p < 0.01$) higher (*c.* 75%). Interestingly, *G. intraradices* showed higher root colonization than *G. mosseae,* suggesting therefore an inverse correlation between the steady-state levels of caffeic acid and the level of colonization. As far as we know, there is no previous data on the endogenous levels of this compound in mycorrhizal roots. However, Douds and co-workers[16] reported that caffeic acid showed antifungal activity against two different AMF. In addition, treatments of *Digitaria sanguinalis* plants with caffeic acid decreased mycorrhizal colonization.[17] Thus, an inverse correlation between the levels of some PAs (particularly caffeic acid) and the levels of mycorrhization is likely. In this scenario, we speculate that the host plant use defence mechanisms to control AM fungal colonization and that individual AMF display differential capacity to overcome or regulate plant defences in order to colonize the host roots. Supporting this hypothesis, we have recently shown a higher induction of defence-related compounds (SA, JA-Ile and 9-LOX oxylipins) in roots colonized by *G. mosseae*, with a lower colonization capacity, compared to *G. intraradices*,[13] suggesting a more exhaustive control of *G. mosseae* by the plant.

In contrast to the reduction of caffeic and chlorogenic acids, the levels of ferulic acid (**Fig. 1C**) in mycorrhizal roots colonized by any of the AMF were significantly ($p < 0.01$) higher than in the non-mycorrhizal control plants (**Fig. 1C**). Induction of ferulic acid in mycorrhizal roots has been previously reported.[18] Remarkably, ferulic acid may be induced by fungal elicitors and jasmonates.[19,20] Our previous analysis pointed to jasmonates and related compounds as key regulators on the changes associated to the symbiosis in tomato,[13] and this may be the case also with ferulic acid levels. Increased ferulic acid has been associated to plant resistance to pathogens in resistant cultivars and upon treatment with some biocontrol agents.[9] Therefore, it is tempting to speculate that elevated ferulic acid levels may contribute to the enhanced resistance of mycorrhizal roots to soil-borne pathogens.

In summary, we show here that in addition to the altered hormonal and transcriptional profiles reported previously,[13] the levels of the major phenolic acids are also altered in tomato roots upon mycorrhization, confirming the regulation of plant defence mechanisms during the symbiosis. The differences observed in the host response to particular AMFs may account for the different mycorrhization levels achieved in each interaction, and their differential impact on plant resistance to root pathogens.

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References

1. Bruce TJ, Pickett JA. Plant defence signalling induced by biotic attacks. Curr Opin Plant Biol 2007; 10:387-92.
2. Thatcher LF, Anderson JP, Singh KB. Plant defence responses: what have we learnt from Arabidopsis? Funct Plant Biol 2005; 32:1-19.
3. Mandal SM, Chakraborty D, Dey S. Phenolic acids act as signaling molecules in plant-microbe symbioses. Plant Signal Behav 2010; 5:359-68.
4. Pozo MJ, Van Loon LC, Pieterse CMJ. Jasmonates—Signals in plant-microbe interactions. J Plant Growth Regul 2004; 23:211-22.
5. Pieterse CMJ, Leon-Reyes A, Van der Ent S, Van Wees SCM. Networking by small-molecule hormones in plant immunity. Nat Chem Biol 2009; 5:308-16.
6. Wasternack C. Jasmonates: An update on biosynthesis, signal transduction and action in plant stress response, growth and development. Ann Bot 2007; 100:681-97.
7. Ruelas C, Tiznado-Hernandez ME, Sanchez-Estrada A, Robles-Burgueno MR, Troncoso-Rojas R. Changes in phenolic acid content during *Alternaria alternata* infection in tomato fruit. J Phytopathol 2006; 154:236-44.
8. Nicholson RL, Hammerschmidt R. Phenolic compounds and their role in disease resistance. Annu Rev Phytopathol 1992; 30:369-89.
9. Panina Y, Fravel DR, Baker CJ, Shcherbakova LA. Biocontrol and plant pathogenic *Fusarium oxysporum*-induced changes in phenolic compounds in tomato leaves and roots. J Phytopathol 2007; 155:475-81.
10. Parniske M. Arbuscular mycorrhiza: the mother of plant root endosymbioses. Nat Rev Microbiol 2008; 6:763-75.

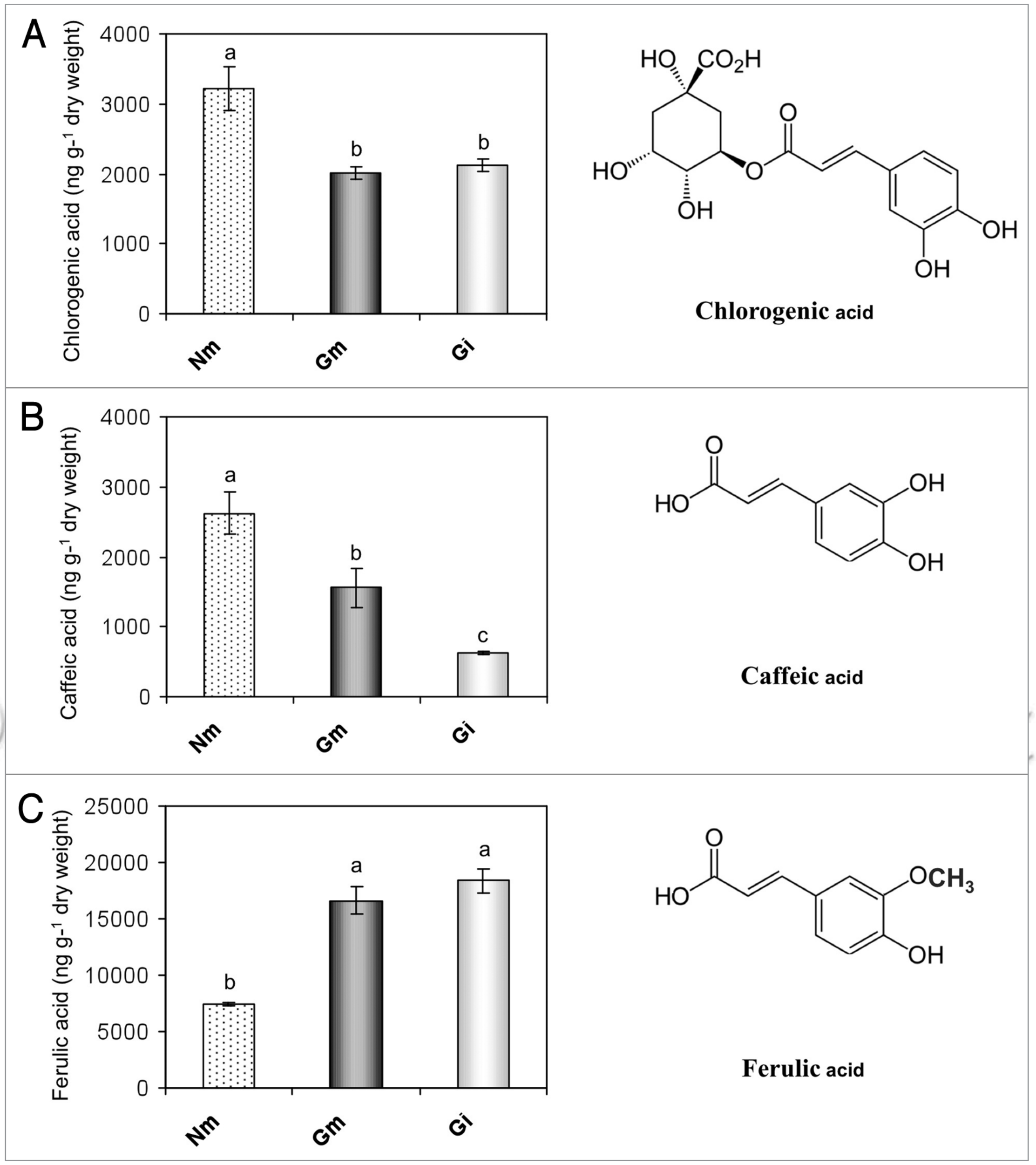

Figure 1. Phenolic content in tomato roots colonized by arbuscular mycorrhizal fungi and chemical structure for different phenolic compounds. Levels of chlorogenic acid (A), caffeic acid (B) and ferulic acid (C) were determined by UPLC-MS/MS in the roots of non-mycorrhizal plants (Nm, dashed bars) and mycorrhizal plants colonized by *G. mosseae* (Gm, closed bars) or *G. intraradices* (Gi, open bars). Data points represent the means of five independent replicates ± SE. Data not sharing a letter in common differ significantly ($p < 0.01$) according to the Fisher's LSD test.

11. Pozo MJ, Azcón-Aguilar C. Unravelling mycorrhiza-induced resistance. Curr Opin Plant Biol 2007; 10:393-8.
12. Garcia-Garrido JM, Ocampo JA. Regulation of the plant defence response in arbuscular mycorrhizal symbiosis. J Exp Bot 2002; 53:1377-86.
13. López-Ráez JA, Verhage A, Fernández I, García JM, Azcón-Aguilar C, Flors V, et al. Hormonal and transcriptional profiles highlight common and differential host responses to arbuscular mycorrhizal fungi and the regulation of the oxylipin pathway. J Exp Bot 2010; 61:2589-2601. DOI: 10.1093/jxb/erq089.
14. Erb M, Flors V, Karlen D, de Lange E, Planchamp C, D'Alessandro M, et al. Signal signature of aboveground-induced resistance upon belowground herbivory in maize. Plant J 2009; 59:292-302.
15. Schaarschmidt S, Kopka J, Ludwig-Muller J, Hause B. Regulation of arbuscular mycorrhization by apoplastic invertases: enhanced invertase activity in the leaf apoplast affects the symbiotic interaction. Plant J 2007; 51:390-405.
16. Douds DD, Nagahashi G, Abney GD. The differential effects of cell wall-associated phenolics, cell walls and cytosolic phenolics of host and non-host roots on the growth of two species of AM fungi. New Phytol 1996; 133:289-94.
17. Yun KW, Choi SK. Mycorrhizal colonization and plant growth affected by aqueous extract of *Artemisia princeps* var. orientalis and two phenolic compounds. J Chem Ecol 2002; 28:353-62.
18. Grandmaison J, Olah GM, Vancalsteren MR, Furlan V. Characterization and localization of plant phenolics likely involved in the pathogen resistance expressed by endomycorrhizal roots. Mycorrhiza 1993; 3:155-64.
19. Kim HJ, Fonseca JM, Choi JH, Kubota C. Effect of methyl jasmonate on phenolic compounds and carotenoids of romaine lettuce (*Lactuca sativa* L.). J Agric Food Chem 2007; 55:10366-72.
20. Mandal S, Mitra A. Reinforcement of cell wall in roots of *Lycopersicon esculentum* through induction of phenolic compounds and lignin by elicitors. Physiol Mol Plant Pathol 2007; 71:201-9.