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Short communication

Arbuscular mycorrhizal symbiosis decreases strigolactone production in tomato

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ABSTRACT

Strigolactones are a new class of plant hormones emerging as important signals in the control of plant architecture. In addition, they are key elements in plant communication with several rhizosphere organisms. Strigolactones are exuded into the soil, where they act as host detection signals for arbuscular mycorrhizal (AM) fungi, but also as germination stimulants for root parasitic plant seeds. Under phosphate limiting conditions, plants up-regulate the secretion of strigolactones into the rhizosphere to promote the formation of AM symbiosis. Using tomato as a model plant, we have recently shown that AM symbiosis induces changes in transcriptional and hormonal profiles. Using the same model system, here we analytically demonstrate, using liquid chromatography–tandem mass spectrometry, that strigolactone production is also significantly reduced upon AM symbiosis. Considering the dual role of the strigolactones in the rhizosphere as signals for AM fungi and parasitic plants, we discuss the potential implications of these changes in the plant interaction with both organisms.

Introduction

Arbuscular mycorrhizal (AM) symbiosis is a mutualistic association established between certain soil fungi and most terrestrial plants, and it is considered a key step in the evolution of aquatic into terrestrial plants (Parniske, 2008). During the symbiosis, AM fungi obtain carbohydrates from their host plant and, in return, the plants obtain water and mineral nutrients (mainly phosphorous and nitrogen) from their fungal partners. In addition to improving the nutritional status, the symbiosis enables the plant to perform better under stressful conditions (Pozo and Azcón-Aguilar, 2007; Parniske, 2008). AM symbiosis establishment and functioning requires a high degree of coordination between the two partners based on a finely regulated molecular dialogue that orchestrates the complex symbiotic program (Paszkowski, 2006; Hause et al., 2007; Requena et al., 2007). The chemical dialogue starts in the rhizosphere, before there is any contact between the partners. Strigolactones are exuded by the plant roots into the soil and act as host detection signals for the AM fungi, stimulating their metabolism and hyphal branching (Akiyama et al., 2005; Parniske, 2008). In the rhizosphere, the strigolactones are also germination stimulants for root parasitic plant seeds of the family Orobancheaceae, such as *Striga*, *Orobanche* and *Phelipanche* spp. (Bouwmeester et al., 2007). In accordance with their AM fungi attracting role, under low nutrient conditions (mainly phosphorous and nitrogen), plants produce more strigolactones (Yoneyama et al., 2007; López-Ráez et al., 2008a), but this also increases the risk that the plant is abused by the parasitic weeds to establish a parasitic interaction (Bouwmeester et al., 2007). Recently, strigolactones have also been recognized as a novel class of plant hormones because, in addition to their role as signaling molecules in the rhizosphere, they also control plant architecture signaling inhibition of shoot branching (Gómez-Roldán et al., 2008; Umehara et al., 2008). Other phytohormones, such as ethylene, salicylic acid, abscisic acid and jasmonates have been proposed to be important regulators in the mutualistic interaction between plants and AM fungi (Herrera-Medina et al., 2003; Hause et al., 2007; Herrera-Medina et al., 2007; Riedel et al., 2008). We have recently shown that AM symbiosis alters both hormonal and transcriptional profiles in tomato roots, especially the oxylipin pathway (López-Ráez et al., 2010). With respect to strigolactones, there are indications that regulation of their levels occurs in the host plant once the symbiosis has been established. It was shown that AM fungal inoculation of maize and sorghum led to a reduction of *Striga hermonthica* infection (Lendzemo et al., 2005), and it was proposed that this reduced infection was caused, at least partially, by a reduction in the production of strigolactones in the mycorrhizal plants (Lendzemo et al., 2007; Sun et al., 2008), although no analytical evidence was provided. In the present work, a reduction in strigolactone production during AM symbiosis is also shown in tomato plants, indicating a conserved response in dicotyledonous and monocotyledonous plant species. Moreover, the reduction in strigolactones by AM symbiosis is, for the first time, analytically demonstrated by liquid chromatography–tandem mass spectrometry (LC–MS/MS). The

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potential use of AM fungi as a sustainable alternative in programs for parasitic weed control is discussed.

Materials and methods

Plant growth and AM fungal inoculation

Tomato seedlings (*Solanum lycopersicum* L. cv. BetterBoy) were grown in pots with a sterile sand:soil (4:1) mixture and inoculated or not with a 10% (v:v) soil-sand-based inoculum containing *Glomus mosseae* (BEG12) or *Glomus intraradices* (BEG 121) propagules. Five plants per treatment were used. Plants were randomly distributed and grown in a greenhouse and watered three times a week with Long Ashton nutrient solution (Hewitt, 1966) containing 25% of the standard phosphorous concentration (López-Ráez et al., 2010). Plants were harvested after eight weeks. For assessment of mycorrhizal colonization, roots were cleared and stained with trypan blue (Phillips and Hayman, 1970). The percentage of total root colonization was determined by the gridline intersection method (Giovannetti and Mosse, 1980).

Germination bioassay and strigolactone analysis using liquid chromatography–tandem mass spectrometry

For strigolactone analysis, root exudates were collected, purified and concentrated as described previously (López-Ráez et al., 2008b). Briefly, the substrate in the pots was rinsed to remove any accumulated strigolactones. After 5 h, 500 ml nutrient solution was applied to the pots, the root exudates were collected and the roots harvested. Then, the crude exudates were concentrated and purified by solid phase extraction on a C_{18} SEPAK cartridge. The exudate solution was loaded onto the pre-equilibrated column and the active fraction eluted with 60% acetone:water. Within each experiment, the exudates were diluted to the same ratio of root fresh weight per ml of root exudate before analysis. For strigolactone analysis in root extracts, 0.5 g of roots were ground in a mortar with liquid nitrogen and then extracted twice with 0.5 ml of acetone in a 2 ml tube. Tubes were vortexed for 2 min and centrifuged for 5 min at 8000 × *g* in a table top centrifuge. The organic phase was carefully transferred to 2 ml glass vials and stored at −20 °C until use.

Germination bioassays with *Phelipanche ramosa* seeds, as well as strigolactone analysis and quantification by LC–MS/MS, were performed as described in López-Ráez et al. (2008a,b), with the exception that LC–MS/MS analyses were performed using a Xevo tandem quadrupole (TQ) mass spectrometer (Waters) equipped with an ESI source.

Statistical analysis

Data for mycorrhization levels and strigolactone content were subjected to one-way analysis of variance (ANOVA) using SPSS Statistics v. 14.1 for Windows. To analyze the results of germination bioassays, ANOVA after arcsine[square root (*X*)] transformation was used.

Results and discussion

Germination stimulatory activity of root exudates from AM fungi-colonized tomato plants

The regulation of strigolactone production during AM symbiosis was assayed using tomato plants and two different AM fungi. In the case of *G. intraradices*, the percentage of colonized roots reached 75% after eight weeks of growth, whereas *G. mosseae* colonized only about 5% of the roots in this experiment. Strigolactone levels in root exudates from mycorrhizal and non-mycorrhizal plants were estimated using a germination bioassay for *P. ramosa* seeds (López-Ráez et al., 2008a). A clear reduction (of about 50%) in the germination stimulatory activity of the exudates from roots colonized by *G. intraradices*, but not *G. mosseae*, was observed (Fig. 1A), revealing an inverse correlation between mycorrhization level and stimulation of germination. A similar reduction in germination of *Striga hermonthica* seeds was observed with root exudates from mycorrhizal sorghum (Lendzemo et al., 2007) and maize plants (Sun et al., 2008). The results show that AM symbiosis can induce a reduction in the germination stimulatory activity of the exudates of tomato plants, as previously shown for monocotyledonous plants (Lendzemo et al., 2007; Sun et al., 2008), suggesting that

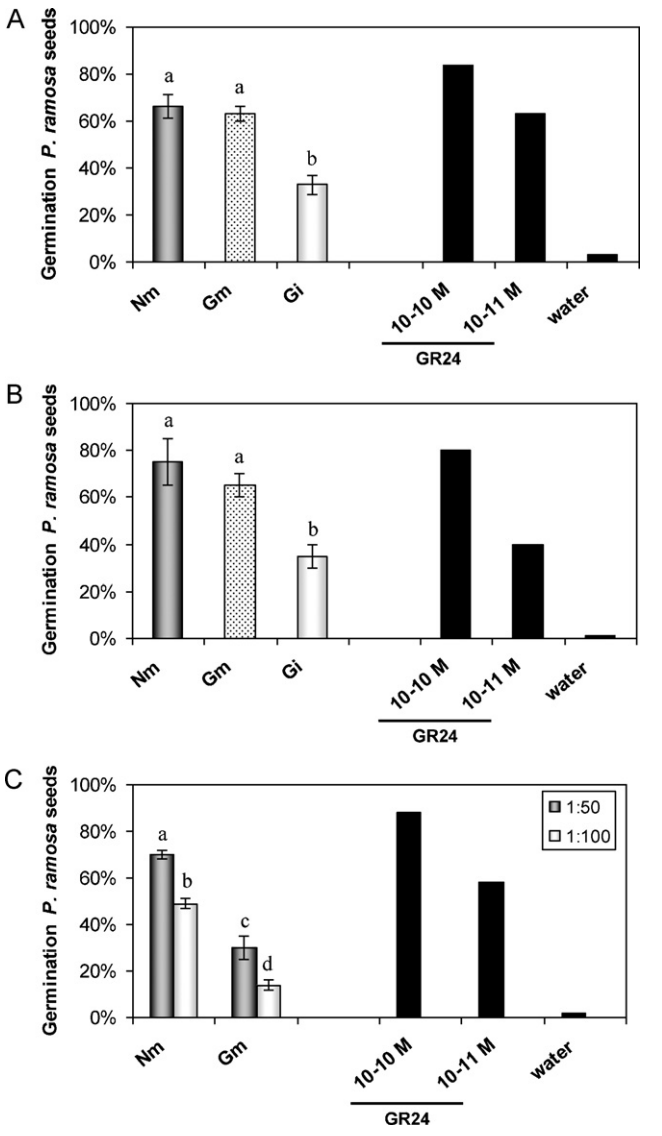

Fig. 1. Effect of mycorrhizal colonization of tomato roots (cv. BetterBoy) inoculated with *G. mosseae* or *G. intraradices* on germination stimulatory activity of the root exudates and root extracts. Germination of *P. ramosa* seeds induced by the root exudates (A) or root extracts (B) of tomato plants colonized by *G. mosseae* (Gm) or *G. intraradices* (Gi) (5% and 75% colonization, respectively) or non-colonized (Nm). (C) Germination of *P. ramosa* seeds induced by the root exudates of tomato plants colonized by *G. mosseae* (Gm) (55% colonization) or non-colonized (Nm). Two different dilutions (1:50 and 1:100) of the root exudates were used. GR24 (10^{-10} and 10^{-11} M) and demineralized water (closed bars) were used as positive and negative controls, respectively. Bars represent the mean of five independent replicates ± SE. Different letters indicate statistically ($P < 0.01$) significant differences according to Fisher's LSD test.