The impact of mycorrhizal symbiosis on tomato fruit quality

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Abstract

The project investigates the potential impact of mycorrhizal fungi, which have been acknowledged as a new class of bio-fertilizers, on the quality of vegetables. To verify such a hypothesis, we selected tomato (Solanum lycopersicum) as a model plant to examine whether the beneficial effects of mycorrhizal fungi on plant development may be extended to some qualitative fruit features. As a second step, five genes related to carotenoid biosynthesis and volatile compounds were selected. Their expression was investigated through a real-time RT-PCR comparison of mycorrhized and non-mycorrhized plants.

Introduction

Arbuscular mycorrhizal fungi (AMF) represent a key-component of the rhizosphere, since they form a mutualistic association with the roots of 90% of land plants. They are known to carry out many ecosystem functions such as improvement of plant establishment and growth, enhancement of nutrient uptake and plant protection against biotic and abiotic stresses (Smith & Read, 1997). For these reasons, they are considered to play a fundamental role in natural as well as agricultural ecosystems together with other soil microorganisms, opening new employment perspectives in the frame of a low-input agriculture.

In order to check the hypothesis that in addition to an improved mineral nutrition AM fungi also benefit their host plants by influencing fruit traits, we selected tomato (*Solanum lycopersicum*) as a model. We first evaluated plant growth parameters and mineral content in order to verify whether tomato responds to AM fungi by stimulating its vegetative growth, phosphate and nitrogen accumulation and fruit productivity. Then the potential impact of AM fungi on fruit quality was assessed by considering carotenoid biosynthesis, a process which is strictly regulated during fruit development and ripening. As a second qualitative trait, the complex mixture of volatile and non volatile compounds that contribute to the overall aroma and taste of the fruit was considered. Real-time RT-PCR analysis was used to compare the expression of five genes related to carotenoids biosynthesis and volatile compounds in tomato fruit investigating mycorrhized versus non-mycorrhized plants.

Materials and methods

Germinating seedlings of *S. lycopersicum* cv. Pearson were inoculated with the AM fungus *Glomus mosseae* BEG12 purchased from Biorize (Dijon, France). The growth experiment consisted of 14 pots filled with sterilized quartz sand mixed with the *G.*

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mosseae inoculum (30% w/w) for the myc condition plants (seven pots). The plants were placed in a growth chamber and fertilized once a week with a modified Long-Ashton nutrient solution. After 90 days the roots were sampled and the growth parameters were evaluated as well as the P and N content. The shoots were frozen and ground to a fine powder which was sent to Floramo (Rocca de' Baldi CN) for the chemical analysis. The N content was expressed in concentration %P/P while the P content was expressed as mg of P for each Kg of biomass. In a second experiment, 20 *S. lycopersicum* cv Micro-Tom plants were brought to fruit production.

For the expression analysis, samples of fruit pericarps from myc and non myc tomato plants were ground to a fine powder in liquid N. Total RNA extraction was performed with a modified C-tab method.

For conventional and real-time RT-PCR, the selected genes were targeted with primers newly designed, except for primers LeF1/LeR2 (Botella-Pavia *et al*., 2004). The following genes were selected for expression analysis: deoxyxylulose 5-phosphate synthase gene (DXS), hydroxymethylbutenyl diphosphate reductase gene (HDR), phytoene synthase gene (PSY1), the carotenoid cleavage dioxygenase gene LeCDD1B and the lypoxygenase gene loxC.

First single-strand cDNA was obtained with the SuperscriptII reverse transcriptase kit (Invitrogen). Real-time RT-PCR reactions were performed with specific oligonucleotide primers (0.3 µM each), according to the conditions described by Siciliano *et al.* (2007).

Results

Impact of AM fungi on plant development

To verify the impact of the AM fungus on growth performance, mycorrhized and control plants were harvested and the fresh weight of shoot and root was evaluated. Mycorrhized plants displayed higher shoots and a wider root apparatus (Fig 1).

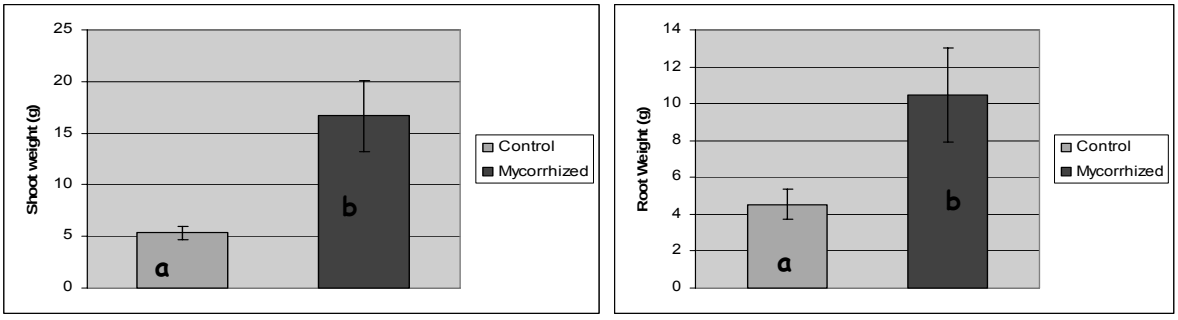

Fig. 1 Shoot and root weight evaluation. Different letters indicate significantly different values according to the Krukall–Wallis test of variance (*P* < 0.05).

To evaluate whether the increase in biomass was also coupled to a higher accumulation of mineral elements, the P and N content was evaluated. These elements are crucial for AM symbiosis, which is characterized by a nutrient exchange between the symbiotic partners.

No significant differences were found in N content, while a significant variation was detected when P was evaluated: the mycorrhized plants had the highest amount of P in their tissues.

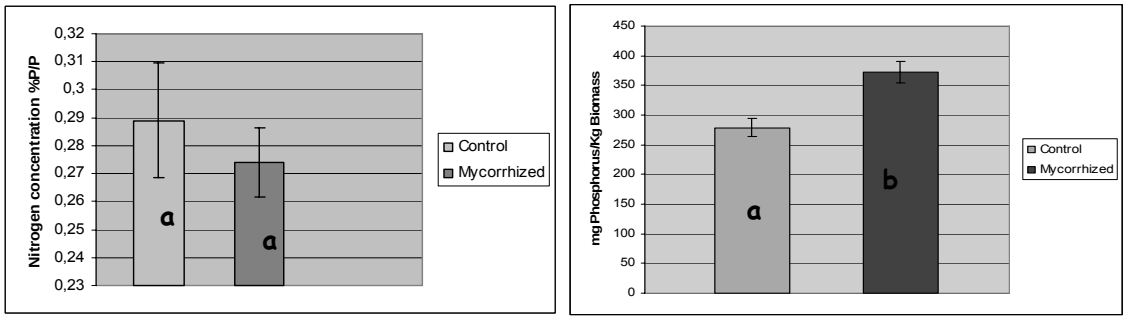

Fig. 2 Nitrogen and Phosphorus content. Different letters indicate significantly different values according to the Krukall–Wallis test of variance ($P < 0.05$).

Productivity of tomato plants

The first fruit was obtained for the myc condition 76 days after the sowing, while in the control condition, the first fruit was obtained 10 days later. The fruit was harvested from all the 10 inoculated plants, with an average productivity of 5.5 pieces of fruit per plant, while 9 out of 10 non mycorrhizal plants were productive, with an average of 2.2 fruit per plant. The inoculated plants produced fruit for a longer period (80 days of productivity, compared to 35 days for the control plants). Six months after seeding, 10 myc plants and 2 control plants were still viable.

Real-time RT-PCR assays

Real-time RT-PCR experiments were performed using cDNA from three independent biological replicates for each condition. The five genes were targeted in individual real-time assays. In all the cases a good amplification signal was detected, with threshold cycles (Cts) ranging from 16 to 21. The PCR efficiencies were comparable, and ranged from 98.1% to 105.6%. The Cts values obtained for the investigated genes were normalized by comparing them with the Cts obtained for the calibration genes (actin1 and 18S rDNA), according to the 'comparative threshold cycle' method. In all cases no significant differential expression was detected between the inoculated and control condition, with a maximal standard deviation of 0,3 on the mean values.

Discussion and perspectives

Here, we have shown that *G. mosseae* positively affects the growth development of tomato plants, the P content and fruit production. Inoculated tomato plants produced more fruit and their productive period was remarkably longer. This new finding can be explained thanks to the improved mineral nutrition, which is shown by the higher P content. However, a direct effect of mycorrhizal status on fruit development cannot be ruled out.

A number of plant carotenoids and derived compounds have an important nutritional value according to their activity as pro-vitamin A and their ability to act as antioxidants that help to prevent some types of human cancer and degenerative diseases (Fraser and Bramley, 2004). We monitored the expression in tomato fruit of two key genes of the MEP pathway, DXS and HDR, whose activity has been shown to be limiting for carotenoid biosynthesis during tomato fruit ripening (Lois *et al*., 2000; Botella-Pavia *et al*., 2004). At the same time, we analyzed the expression of the phytoene synthase gene (PSY1), which represents the first committed step of carotenoid biosynthesis in the strict sense (Fraser and Bramley, 2004). A significant accumulation of the PSY1

transcript was observed in ripening fruit, reaching the highest level at the orange stage (Lois *et al*., 2000).

Given that fruit aroma and taste is considered an important feature for good tomato quality (Baldwin *et al*., 2000), we targeted two genes involved in tomato aroma composition: the first one (loxC) is involved in the generation of fatty acid-derived C6 compounds (Chen *et al.*, 2004), while the second is involved in the formation of volatile terpenoids from carotenoids (Simkin *et al*., 2004).

We harvested the tomato fruit as the colour turned from yellow to orange, according to Gillaspy and colleagues (1993), who reported that carotenoid accumulation is concomitant with the fist visible colour change in fruit. Under our experimental conditions, all the target genes were well expressed in the considered developmental stage, confirming published data. However, the five genes considered did not reveal a differential expression between the mycorrhized and control conditions. Two reasons can be given to explain the result: the selected genes do not represent a target of the mycorrhizal impact, or such an effect is not evident at the analyzed developmental stage. Alternatively, mycorrhizal symbiosis does not influence the metabolic pathways here considered.

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