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SHORT NOTE

Respiratory responses of arbuscular mycorrhizal roots to short-term alleviation of P deficiency

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Abstract Arbuscular mycorrhizal (AM) root respiration can impose a respiratory sink on host reserves under low P conditions, but it is not known how AM roots respond to short-term supply of sufficient P. Therefore, the effect of P stress alleviation on the respiration of AM roots was investigated in 5-week-old tomato plants. Plants were inoculated with *Glomus mosseae* in sand culture and grown hydroponically in a low P (2 μM) nutrient medium for 3 weeks. P stress was alleviated by the supply of 2 mM P for 72 h. With P stress alleviation, the improved root P status coincided with a decline in AM fungal activity and a reduction in root CO_2 and O_2 fluxes of the AM plants. During P stress alleviation, the AM roots had lower concentrations of organic acids, derived from root-zone CO_2 assimilation, in their root exudates. These results show that short-term alleviation of low P conditions in AM roots rapidly affects AM fungal symbiont activity, AM root respiration, and root-zone CO_2-derived organic acid metabolism.

Keywords Arbuscular mycorrhiza · P stress · Respiration · Organic acids

Introduction

During P limitation, plant roots employ several strategies to enhance P uptake, such as the arbuscular mycorrhizal (AM) symbiosis and the synthesis of root organic acids for exudation. Plants that form AM associations rely on the fungal symbionts to improve P uptake and plant growth in P poor soils (Bolan 1991; Graham 2000; Mortimer et al. 2005). The respiratory C cost to the host plant is a pivotal part of the AM symbiosis and although AM root respiration under low P conditions has been published (Pang and Paul 1980; Snellgrove et al. 1982; Harris et al. 1985; Peng et al. 1993; Jifon et al. 2002), several studies have also investigated the physiology of the AM symbiosis grown under conditions of long-term P sufficiency. Peng et al. (1993) found that P sufficient mycorrhizal *Citrus* plants invested 37% more C in root respiration than nonmycorrhizal *Citrus*, which led to a 10–20% decrease in specific C gain compared to nonmycorrhizal plants. Olsson et al. (2002) found reduced hyphal growth under P-sufficient conditions and proposed that it may be due to the host root reducing the C flow to the fungus. Jackson et al. (2002), in a study with *Lactuca sativa*, found a significant decrease in the frequency of arbuscule formation during P sufficiency. Although these studies elucidated some of the respiratory responses of AM roots grown at high P levels, very little is known about AM root respiration during short-term alleviation of low P supply.

In the absence of AM, the short-term resupply of P to P-starved cells results in the rapid recovery of adenylate and P_i levels (Dancer et al. 1990; Li and Ashihara 1990), or elevated levels of the glycolytic intermediates, which limit the tricarboxylic acid cycle (Weger 1993). Previous work on P stressed non-AM plants also found that high P nutrition results in a reduction of dark CO_2 incorporation (Theodorou and Plaxton 1996; Vance et al. 2003).

Although Pfeffer et al. (1999) found that AM fungi do not engage in dark CO_2 fixation in the symbiotic state, the host root component may still separately fix anaplerotic CO_2 and be influenced by short-term alleviation of low P supply. Consequently, root-zone CO_2 fixation may be

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influenced by the respiratory C metabolism of AM root systems during alleviation of P deficiency. The objective of this study was therefore to determine how AM root respiration at low P levels is affected by a short-term P supply. This was assessed by supplying P-deficient AM roots with sufficient P during 3 days and studying AM fungal activity and its consequent effect on the O_2 and CO_2 respiration as well as the dark CO_2 fixation of the AM root system.

Materials and methods

Mycorrhizal inoculation and plant sand culture

Tomato seeds (*Lycopersicon esculentum*, L. Mill F114) were germinated in 15-cm-diameter pots containing sterilized sand (grain size 2 mm) during May to June in an east-facing glasshouse in Stellenbosch, South Africa. The average maximum daily irradiance was 580 μmol m^{-2} s^{-1} and the average day/night temperatures and humidities were 21/14°C and 33/72%, respectively. Seeds were inoculated either with 5 g of a live culture (+AM treatment) of *Glomus mosseae* (Nicolson & Gerdemann) Gerd. & Trappe (Agricultural Genetics, UK) or an autoclaved (3 h at 110°C under steam pressure at 200 kPa) batch of *G. mosseae* inoculum (−AM treatment).

The inoculum, containing a mixture of spores and fragments of root in a clay support medium, was placed 1 cm below the seeds in each pot. The control seedlings were irrigated with a 0.5-g ml^{-1} of H_2O filtrate of the live inoculum to introduce mycorrhizal-associated microbes into the autoclaved inoculum. The filtrate was passed through a 30-μm (micron) mesh to exclude fungal material. Each pot received a total of 2 l of distilled H_2O per day during four 15-minute periods of drip irrigation. Upon germination, the seedlings were irrigated for the same periods per day with Long Ashton (Hewitt 1966) nutrient solution for 3 weeks. The nutrient solution was modified to contain 2 mM of NH_4^+, 2 μM of P, and 0.05 mM of 2-(*N*-morpholino)-ethane-sulfonate (MES) (pH 6).

Hydroponic plant culture

After 2 weeks in sand culture, the +AM and −AM seedlings were transferred to 22-l hydroponic tanks, containing the same low P Long Ashton nutrient solution (2 mM of NH_4^+, 2 μM of P, and 0.05 mM of MES, pH 6). The nutrient solutions were changed twice per week and the plants were cultured in the low P (2 μM of P and 0.05 mM of MES, pH 6) hydroponic medium for 3 weeks.

In the hydroponic culture, the hypocotyls of seedlings were wrapped with foam rubber at their bases and inserted through holes in the lids of the tanks. An airlift pump, consisting of an open-ended, 1-cm-diameter plastic tube, aerated at the bottom, and discharging at the surface of the solution, was inserted through each lid and gently bubbled air containing 5,000 ppm of CO_2. The CO_2 concentration in the air was measured with an ADC-225-MK3 (ADC, Hertz, UK) infrared gas analyzer calibrated to the required CO_2 concentration using a LiCor (LiCor, Model 6000-01, Lincoln, NE, USA) gas syringe containing a mixture of pure CO_2 and N_2 (Afrox, Republic of South Africa). The 5,000-ppm CO_2 was obtained by mixing industrial grade CO_2 (Afrox) with ambient air.

Short-term P resupply

After 3 weeks culture in the low P (2 μM of P and 0.05 mM of MES, pH 6) hydroponic medium, plants were separated for the various experiments. Before each experiment, the low P growing condition (2 μM of P and 0.05 mM of MES, pH 6) was alleviated by the supply of a sufficient P concentration at 2 mM P. This was done 72 h before each experiment by replacing the Long Ashton nutrient solution in the hydroponic tanks with a sufficient P nutrient solution (2 mM of NH_4^+, 2 μM of P, and 0.05 mM of MES, pH 6). After 72 h of sufficient P supply, the plants were separated for root respiration measurements, ^{14}C-labeling, mycorrhizal analysis, and vital staining.

Root respiration measurements

After 72 h of sufficient P supply in hydroponic culture, seedlings were transferred to 298-ml, temperature-controlled (20°C) chambers for the measurements of O_2 and CO_2 fluxes. The air temperature was maintained at 20°C and light was supplied at 350 μmol m^{-2} s^{-1}. The cuvettes contained three ports: one for the supply of air (containing either 5,000 or 0 ppm of CO_2), one for the addition of inhibitors in solution, and one for the sampling of gas. The cuvettes contained the same nutrient solution in which the plants were grown. The nutrient solutions were stirred in the cuvettes with magnetic stirrers at the base of each cuvette. The roots were aerated with air containing 5,000 ppm of CO_2 and delivered through precision needle valves at a rate of ca. 60 ml min^{-1}. The air with 5,000 ppm of CO_2 was supplied from a cylinder of compressed air mixed with industrial grade CO_2 (Afrox). CO_2 flux was measured with an ADC-225-MK3 (ADC) infrared gas analyzer and O_2 consumption with polarographic O_2 electrodes (YSI, Yellow Springs, OH, USA). Upon completion of the measurements, root and shoot components were harvested separately and oven-dried at 80°C for 48 h. The roots were weighed and respiration was expressed on a dry weight basis. The root respiratory gas exchange

quotient (Rq) was obtained by the ratio of CO_2 release rates/O_2 uptake rates.

^{14}C incorporation and fractionation

After the sufficient P supply period, seedlings were transferred to individual 300-ml temperature-regulated (20°C) cuvettes. These cuvettes contained the same nutrient solution as the hydroponic culture and were aerated with 5,000 ppm of CO_2. Light was supplied at 1,000 μmol m^{-2} s^{-1} PAR (photosynthetically active radiation) during the ^{14}C incorporation experiment. The 5,000-ppm CO_2 aeration was discontinued before the addition of 42 nmol of $NaHCO_3$ containing 0.093 MBq of $NaH^{14}CO_3$. The solution was aerated for 30 s every 15 min and the cuvettes were also swirled by hand every 5 min. After 1 h of ^{14}C exposure, the roots were rinsed twice in separate distilled water solutions, blotted dry, and separated into root and shoot components. These components were immediately weighed and quenched in liquid N before storage at −80°C. Plant components were homogenized with 80% (v/v) ethanol and separated into soluble and insoluble components. The soluble component was subsequently separated into water-soluble and chloroform-soluble components. The water-soluble component was further fractionated into amino acid, organic acid, and carbohydrate fractions, using ion exchange resins as described by Atkins and Canvin (1971).

For the determination of ^{14}C-labeled organic acids in the root exudation, a 20-ml sample of nutrient solution was evaporated under a continuous stream of ambient air for 24 h and resuspended in 1 ml of water. This solution was fractionated into organic acids using the ion exchange resins (Atkins and Canvin 1971).

Analysis of mycorrhizal colonization

Two sets of plants were harvested for mycorrhiza analysis before and after 72 h of sufficient P supply in hydroponic culture. One set of plants was used for determination of mycorrhizal colonization and the other set was used for vital staining of fungal structures in roots.

Roots were cut into 1-cm segments, rinsed, and cleared with 10% KOH for 5 min in an autoclave at 110°C under steam pressure of 200 kPa. The KOH was rinsed off and the segments acidified with 2 N of HCl for 10 min. Thereafter, the roots were stained with 0.05% (w/v) aniline blue for 10 min in an autoclave at 110°C under steam pressure of 200 kPa and then destained in lactic acid overnight. Frequency of arbuscules, vesicles, and intraradical hyphae in roots was determined according to Brundrett et al. (1994).

Vital staining was performed on fresh root fragments using succinate-NBT with counterstaining according to Smith and Gianinazzi-Pearson (1990).

The ratio of live/dead AM fungus was obtained from the percentage of live AM fungal structures/percentage dead AM fungal structures on a root length basis. The percentage of live AM fungus was calculated as: (all active structures/number of intersections counted)×100. The percentage of dead AM fungus was calculated as: (all nonactive structures/number of intersections counted)×100.

Statistical analysis

The percentage data were arcsine-transformed (Zar 1999). The effects of the factors and their interactions were tested with an analysis of variance (ANOVA) (SuperAnova, Abacus Concepts, USA); whereas the ANOVA revealed significant differences between treatments, the means were separated using a post hoc Student–Newman–Kuehls multiple range test ($P \leq 0.05$). Different letters indicate significant differences between treatments.

Results

Mycorrhizal colonization and P concentrations

The percentage colonization of +AM roots was 41%, while the −AM roots were not colonized (data not shown). The level of AM colonization did not change after 72 h of P stress alleviation, but there was a 75% decline in the ratio of live to dead AM (Fig. 1a). P stress alleviation caused significant increases in cellular P_i concentration for both +AM and −AM roots (Fig. 1b). At 72 h after P resupply, the P_i concentration in +AM roots increased to significantly higher levels than that of −AM roots at 72 h (Fig. 1b).

Root respiration

During low P stress (2 μM of P) the respiratory fluxes of O_2 (Fig. 1d) and CO_2 (Fig. 1c) were higher in +AM roots compared to −AM roots. After 72 h of P stress alleviation, +AM root O_2 flux declined to below that of −AM roots (Fig. 1d). The O_2 flux of −AM roots was 33% higher at 72 h after P stress alleviation than during P deficiency (Fig. 1d), while the +AM roots showed a 50% decline in O_2 consumption for the same period. Compared to the low P levels, the CO_2 release rates after 72 h of P stress alleviation had increased by 58% in −AM plants but decreased by 30% in +AM plants (Fig. 1c).

$^{14}CO_2$ incorporation

Under P deficient conditions, +AM roots had a higher Rq and correspondingly lower $^{14}CO_2$ incorporation than −AM roots (Fig. 2a). After 72 h of P stress alleviation the Rq of

+AM and −AM roots increased with a corresponding decline in root $^{14}CO_2$ incorporation (Fig. 2b). However, the +AM roots still maintained a higher Rq (Fig. 2a) and correspondingly lower $^{14}CO_2$ incorporation (Fig. 2b) than −AM roots during P stress alleviation.

In spite of the lower ^{14}C uptake by +AM roots, these roots had a greater percentage of ^{14}C incorporation into the organic fraction and a lower percentage of ^{14}C in the carbohydrate fraction than −AM roots during P stress (Fig. 2d). Under these P stress conditions, there was also a higher concentration of the ^{14}C-labeled organic acids in the exudation of +AM roots than −AM roots (Fig. 2c). After low P stress alleviation after 72 h, the +AM roots still had a higher percentage of ^{14}C incorporation into the organic fraction (Fig. 2d), but there was no difference in the ^{14}C-labeled organic acid concentration in the exudates of +AM roots and −AM roots (Fig. 2b). During the period of P stress alleviation, there was no difference in the percentage of ^{14}C in the carbohydrate fraction between +AM roots and −AM roots (Fig. 2d).

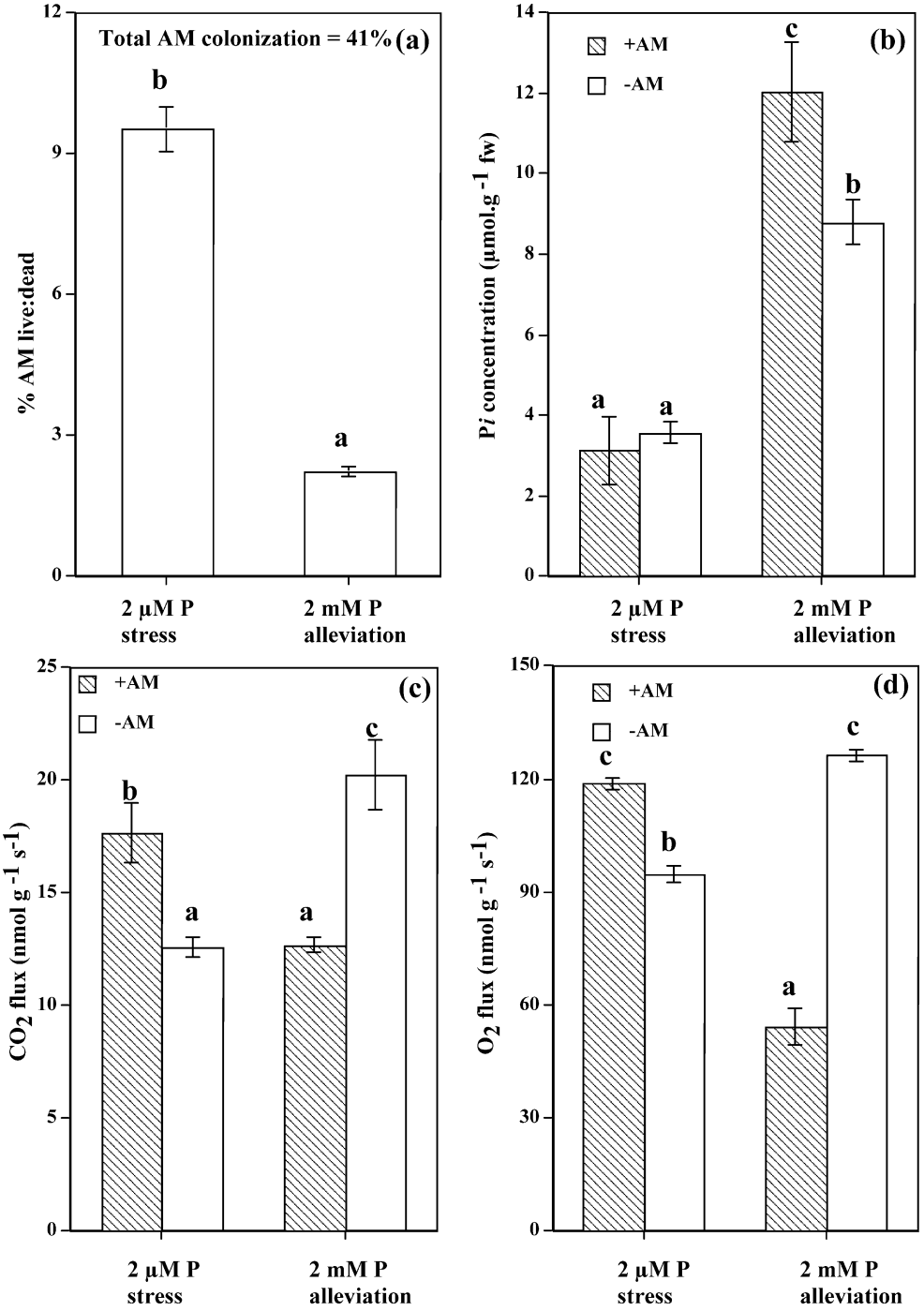

Fig. 1 Effects of 72 h of P stress alleviation (2 mM P) on the **a** ratio of live/dead AM structures, **b** cellular P_i concentrations, **c** root respiratory CO_2 flux, and **d** root respiratory O_2 flux of 5-week-old tomato plants under P deficiency (2 µM of P). Plants were either supplied with a live (+AM) or with an autoclaved AM inoculum (−AM) and grown hydroponically in a P-stressed nutrient solution (2 µM of P). P stress was alleviated by the resupply of 2 mM of P for 72 h. *Different letters* indicate significant differences between each treatment ($P \leq 0.05$). $n=5$

Discussion

A short period (72 h) of P stress alleviation influenced the fungal symbiont activity in +AM roots, which was consequently reflected in the root respiration and the organic acid exudation. In −AM tomato plants, the enhanced root P status could account for the increase in O_2 consumption and CO_2 release rates due to increased adenylate availability and P_i supply during alleviation of low P conditions, as reported for other plant species (Theodorou and Plaxton 1996). However, the decline in respiratory O_2 and CO_2 fluxes in +AM roots after 72 h of P supply was possibly due to the lower activity of the +AM symbiont caused by the high P supply. The greater root P_i concentration in +AM plants with high P supply can result from both AM fungus and host root uptake. This increased root P_i concentration after 72 h of P alleviation resulted in the reduction of AM fungal activity and AM root respiration, possibly due to the host reducing the C flow to the fungal symbiont. The reliance of the AM fungus on

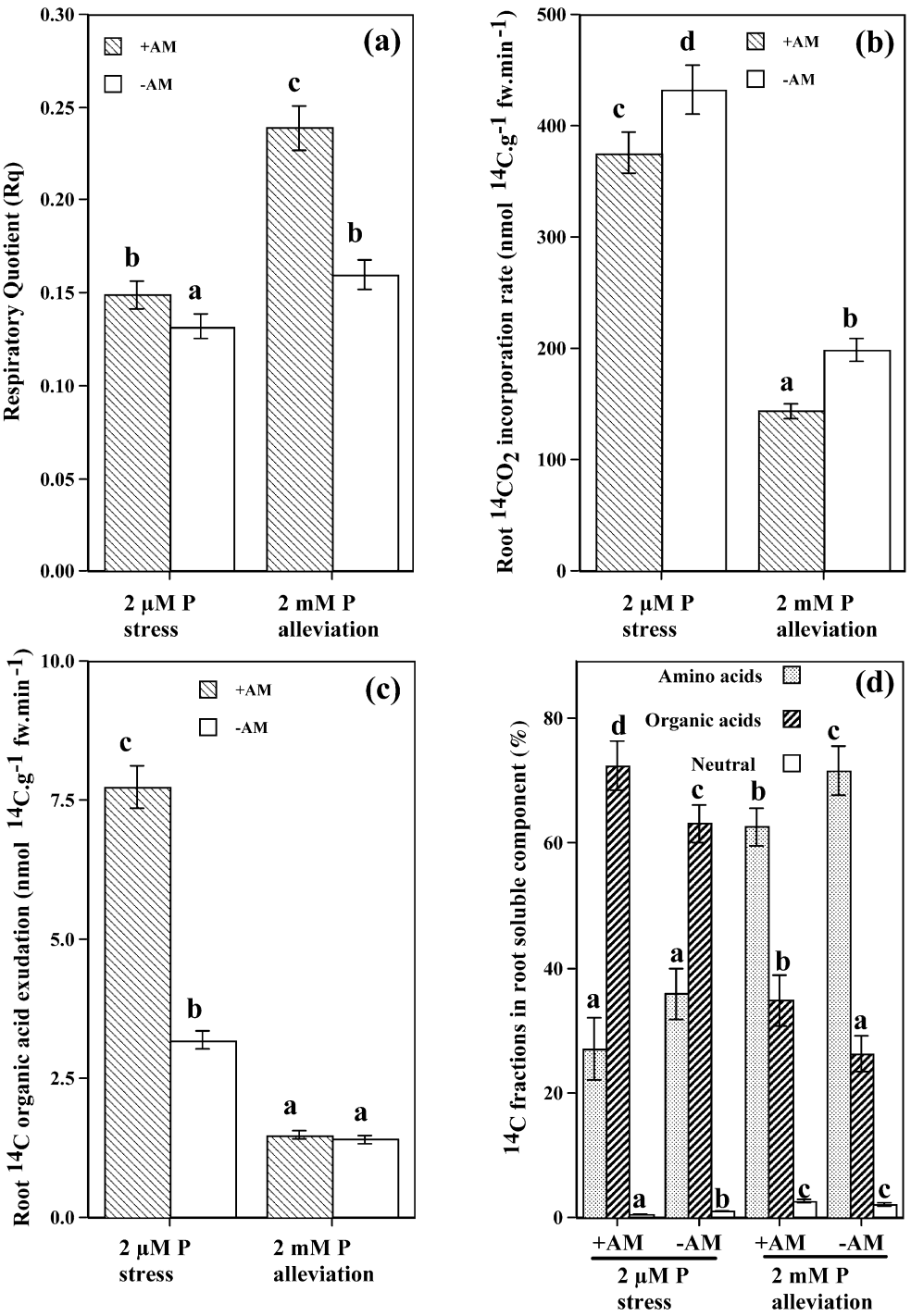

Fig. 2 Effects of P deficiency (2 µM P) on the **a** Rq, **b** the root $^{14}CO_2$ incorporation, **c** ^{14}C organic acid exudation, and **d** root $^{14}CO_2$ partitioning in the soluble fraction of 5-week-old hydroponically grown tomato plants. Plants were either supplied with a live (+AM) or with an autoclaved AM inoculum (−AM) and grown hydroponically in a P-stressed nutrient solution (2 µM P). P stress was alleviated by the resupply of 2 mM of P for 72 h. *Different letters* indicate significant differences between each treatment ($P \leq 0.05$). $n=5$

an optimal supply of host C may be largely related to the nutritional benefits, which the symbiont can provide to the host plant (Snellgrove et al. 1982). A high P supply would have reduced host dependency on the AM fungus for P uptake. A considerable proportion of mycorrhizal root respiration is related to P uptake and the maintenance of these uptake structures, which may impose a large C cost on the host roots (Snellgrove et al. 1982; Pang and Paul 1980; Jennings 1995). Recent estimates have placed this C drain to AM roots at 4–20% of host C budget (Douds et al. 2000; Graham 2000); a host reduction in C supply could therefore cause a decline in AM fungal activity. Baas et al. (1989) found that 13% of AM fungal C was used for nutrient uptake and 87% for fungal maintenance. Because AM fungi are obligate symbionts (Graham 2000), the host may be able to regulate the C supply to the fungus in function of the benefits that the fungal symbiont can supply. This suggestion of the host controlling C supply to the AM fungus concurs with findings that extraradical hyphal growth under P-sufficient conditions is reduced (Olsson et al. 2002) and that high P supply results in a significant decrease in arbuscule development (Jackson et al. 2002). Olsson et al. (2002) proposed that such effects may be due to the root reducing C flow to the fungus and concluded that the response of mycorrhiza to P sufficient conditions would depend on the P status of the root colonized.

The respiratory metabolism of C for organic acid production and exudation also showed signs of adapting to the prevailing P status of the +AM root system. A consequence of a lower Rq under P stress is that a larger proportion of dark CO_2 is incorporated from the root zone, normally into existing C skeletons, via phosphoenolpyruvate carboxylase for organic acid synthesis and P_i recycling (Theodorou and Plaxton 1996; Vance et al. 2003). With P alleviation the lower root-zone CO_2 incorporation in +AM roots compared to −AM roots, reflected in a higher Rq and lower ^{14}C-uptake, may be related to the fungal symbiont competing for organic C with the host root. The AM symbiont does not engage in dark CO_2 fixation in the symbiotic state (Pfeffer et al. 1999) so that the host component may have been limited in its CO_2 fixation by organic C skeletons flowing to the AM sink. In spite of the lower root-zone CO_2 assimilation in +AM roots, the higher percentage of ^{14}C-labeled organic acids in roots and ^{14}C-labeled organic acids in root exudates of mycorrhizal tomato plants growing under P stress suggest that AM roots partition the incorporated ^{14}C into fractions that would aid P uptake under P deficiency. However, after 72 h of P sufficiency AM roots started to change the allocation of incorporated ^{14}C so that there was no difference in levels of ^{14}C-labeled organic acids in root exudates of +AM and −AM roots.

In conclusion, the present data show that short-term alleviation of low P stress conditions can rapidly affect AM fungal symbiont activity and that this is reflected in the total respiration of the AM root system. The reduction in AM root activity was also evidenced by the decline in ^{14}C-labeled organic acids in root exudates.

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