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SHORT NOTE

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Root respiratory quotient and nitrate uptake in hydroponically grown non-mycorrhizal and mycorrhizal wheat

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Abstract Oxygen and CO_2 fluxes were measured in hydroponically grown mycorrhizal and non-mycorrhizal *Triticum aestivum* L. cv. Hano roots. The NO_3^- uptake of the plants was used to estimate the amount of root respiration attributable to ion uptake. Plants were grown at 4 mM N and 10 μM P, where a total and viable mycorrhizal root colonisation of 48% and 18%, respectively, by *Glomus mosseae* (Nicol. and Gerd.) Gerd. and Trappe (BEG 107) was observed. The O_2 consumption and NO_3^- uptake rates were similar and the CO_2 release was higher in mycorrhizal than in non-mycorrhizal wheat. This resulted in a significantly higher respiratory quotient (RQ, mol CO_2 mol^{-1} O_2) in mycorrhizal (1.27 ± 0.13) than in non-mycorrhizal (0.79 ± 0.05) wheat. As the biomass and N and P concentrations in mycorrhizal and non-mycorrhizal wheat were the same, the higher RQ resulted from the mycorrhizal colonisation and not differences in nutrition *per se*.

Key words *Glomus mosseae* · Hydroponics · Nitrate uptake · Root respiration · *Triticum aestivum*

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Introduction

Root respiration can represent 30–50% of below-ground carbon allocation in plants (Burton et al. 1997). Since the roots of most plant species are often colonised with endomycorrhizal fungi and hyphae may represent an important source of C (as CO_2 loss) for microorganisms (Jakobsen and Rosendahl 1990), it is important to compare the extent of carbon allocation to root respiration in mycorrhizal and non-mycorrhizal plants. Various reports have shown that root and/or soil respiration of mycorrhizal plants is higher than that of non-mycorrhizal plants (Snellgrove et al. 1982; Jakobsen and Rosendahl 1990; Peng et al. 1993).

Root respiration of mycorrhizal plants has been estimated in various ways, i.e. using uprooted plants transferred to hydroponics (e.g., Baas et al. 1989) or soil-cultured plants where root respiration was estimated from bulk measurements of soil and root respiration (e.g., Peng et al. 1993). Uprooting plants may result in underestimation of fungal respiration due to the removal or damage of extraradical hyphae in the soil. Comparison of mycorrhizal and non-mycorrhizal plants using combined soil and root respiration as an estimation of root respiration does not take into account the effects of mycorrhizal fungi on the microbial population, which may be responsible for up to half of the CO_2 released from the rhizosphere (Barber and Martin 1976).

The aims of this present investigation were (1) to quantify in root O_2 consumption and CO_2 release of hydroponically grown non-mycorrhizal and mycorrhizal wheat of equal size and nutritional status under non-limiting light conditions, together with NO_3^- uptake, since a large portion of root respiratory energy is required for anion uptake (Clarkson 1985) and most of this is NO_3^- (Baas et al. 1989), and (2) to compare these results with those previously reported for soil culture or up-rooted plants placed in hydroponics.

Materials and methods

Plant and fungus culture

Plants (*Triticum aestivum* L. cv. Hano) and fungus [*Glomus mosseae* (Nicol. and Gerd.) Gerd. and Trappe (BEG 107)] were pre-cultured and subsequently grown in hydroponic culture (Hawkins and George 1997) using vermiculite for the pre-culture instead of perlite. The mycorrhiza inoculum comprised loess soil containing *G. mosseae* spores and maize root pieces colonised to about 50% by *G. mosseae*. During the pre-culture, plants in pots were irrigated daily with 40 ml modified Long Ashton nutrient solution (LANS, Hawkins and George 1997). Four-week-old plants were then transferred into hydroponics using black 20-l hydroponic containers (5 plants per container) for a further 3 weeks. Plants were cultured in a Conviron growth chamber (Controlled Environments Ltd, Winnipeg) with a PAR of 300–400 μmol m^{-2} s^{-1} (14-h day length), a day/night temperature of 25 °C/18 °C and a relative humidity of 60%.

Nutrient medium

Modified LANS was used for hydroponic culture as described by Hawkins and George (1997). The N and P in the nutrient solution was supplied as: 4 mM KNO_3, 9.4 μM $NaH_2PO_4.2H_2O$ and 0.6 μM $Na_2HPO_4.12H_2O$. A pH of 6.0 was maintained by 0.15 mM MES-KOH adjusted with 2 *N* HCl every third day as necessary. The nutrient solutions were changed when the N concentration dropped to one-third of the original concentration. The nutrient solutions were aerated via aquarium stones producing a fine stream of bubbles for 2-h periods, 4 times a day.

Mycorrhizal root colonisation

Mycorrhizal root colonisation was determined using the gridline-intersect method (Giovannetti and Mosse 1980) at a magnification of ×160 with roots stained with trypan blue (Koske and Gemma 1989) or for succinate dehydrogenase activity (Schaffer and Peterson 1993) to confirm the viability of the mycorrhizal structures. Colonisation was determined from the combined presence of arbuscules, vesicles or hyphae within the roots.

Nitrogen and phosphorus analysis

Nitrogen concentration was determined by dry oxidation of dried, milled shoot and root material (Macro N, Heraeus Holding Ltd., Hanau, Germany). Phosphorus concentration was determined using the molybdenum blue assay (Murphy and Riley 1962) using dried, milled shoot and root material after dry ashing and dissolving in 1:30 (v/v) HCl.

Oxygen consumption, CO_2 release and NO_3^- uptake

For measurement of NO_3^- uptake, O_2 consumption and CO_2 release by plant roots, single plants were transferred into five temperature-controlled (20 °C) cuvettes. Plant hypocotyls were inserted into screw-on lids of 288-ml opaque, closed cuvettes. The roots were hermetically sealed into the cuvettes using a closed-cell neoprene foam rubber and a layer of 1% Gelrite (Merck). The cuvettes were completely filled with nutrient solution and equipped with a magnetic stirrer, an inlet for aeration with ambient air and an outlet for sampling the gas stream. The plants were left to equilibrate in the light for 12 h in nutrient solution with an N concentration of 1 mM to avoid diurnal effects on NO_3^- uptake (Gojon et al. 1986). Before measuring, the nutrient solution (1 mM N) was renewed. Transpiration was compensated for during the measurements by adding recorded volumes of water. Measurements were performed at 20 °C at a light intensity of 380 μmol m^{-2} s^{-1}.

Root respiratory CO_2 release was measured by monitoring the CO_2 concentration of gas from the outlets of the cuvettes using an ADC MK3 (Analytical Development Corporation, Hoddeson, UK) infra red gas analyser (IRGA). The gas flow rate was set to 64.4 ml min^{-1} and the IRGA was calibrated with known CO_2 concentrations prepared by mixing pure CO_2 with N_2 in a gas mixing syringe (Li–Cor Inc. Model 6000-01, Lincoln, Neb., USA). Measurement of O_2 depletion began when the air supply was stopped. Any remaining air volume was replaced with nutrient solution. Oxygen consumption was monitored polarographically using Clark-type electrodes (Yellow-Springs Instrument Co. Inc., Yellow-Springs, Ohio, USA). An additional cuvette containing only nutrient solution was included as a reference. Oxygen consumption was calculated from the slope of O_2 depletion over time (about 40 min) until the concentration reached 80% of the starting concentration.

Nitrate uptake was measured separately by following NO_3^- depletion of the aerated nutrient solution over 360 min in the cuvettes. Samples (500 μl) were withdrawn from an opening on top of the cuvette and the NO_3^- concentration determined according to the method of Cataldo et al. (1975).

Statistics

Plant culture containers were placed randomly in the growth chamber and their positions changed regularly. Eight plants per treatment were used for determining biomass accumulation and colonisation rate of the roots and five of these eight plants were used for measuring root respiration and NO_3^- uptake. Student's *t*-tests were applied to determine differences between non-mycorrhizal and mycorrhizal plants. Results are presented as means ± standard error.

Results

Plant dry mass and mycorrhizal colonisation

There were no significant differences between the shoot biomass (3.34 ± 0.13 versus 2.83 ± 0.25 g) or root biomass (1.34 ± 0.08 versus 1.50 ± 0.11 g) of non-mycorrhizal and mycorrhizal plants grown in hydroponics.

No mycorrhizal root colonisation was observed in plants not inoculated with mycorrhizal fungi. In inoculated plants, the initial total root colonisation before transfer into hydroponics was 56 ± 5%, of which 20 ± 4% was viable mycorrhizal colonisation. Total root colonisation after 4 weeks in hydroponics was 48 ± 4%, of which 18 ± 4% was viable colonisation.

Nitrogen and P concentrations

The N and P concentrations were not significantly different ($P < 0.05$) between non-mycorrhizal (N: 17.85 ± 0.41 and P: 0.44 ± 0.07 mg g^{-1} dry mass) and mycorrhizal (N: 18.63 ± 0.41 P: 0.40 ± 0.04 mg g^{-1} dry mass) plants.

Oxygen consumption, CO_2 release and NO_3^- uptake

Root respiration in terms of O_2 consumption was not significantly different between non-mycorrhizal and mycorrhizal roots (Fig. 1). However, mycorrhizal roots had a significantly higher CO_2 release and, consequently, a significantly higher RQ than non-mycorrhizal roots (Fig. 1). The difference in the NO_3^- uptake of non-mycorrhizal (11.21 ± 0.72 μmol NO_3^- g^{-1} root dry mass) and mycorrhizal (11.46 ± 1.46 μmol NO_3^- g^{-1} root dry mass) roots after 360 min was not significant at $P < 0.05$.

Discussion

The total and viable root colonisation levels of wheat by *G. mosseae* were similar to those reported for plants grown on a solid substrate (e.g. Hawkins and George 1997).

Differences in plant P status result in P-mediated effects on root respiration (Theodoru and Plaxton 1993). In the present study, the non-mycorrhizal and mycorrhizal wheat plants did not differ in P or N concentrations or in plant biomass. This may have been due to unlimited diffusion of nutrients in hydroponics and, therefore, the elimination of one of the benefits of root-distant hyphae, namely nutrient scavenging. Thus, these plants were suitable for comparing root respiration of non-mycorrhizal and mycorrhizal roots with little interference of P/N or growth-mediated effects on root respiration.

Mycorrhizal colonisation in these plants resulted in a 32% and 61% increase in CO_2 release and RQ, respectively, while O_2 consumption was not significantly affected (Fig. 1). In this system, it can be assumed that the differences in root respiration between non-mycorrhizal and mycorrhizal plants were due to the presence of mycorrhiza and not other soil microorganisms or to N/P mediated effects. This increase in root respiration is in agreement with previous reports based on CO_2 release (Snellgrove et al. 1982; Baas et al. 1989; Jakobsen and Rosendahl 1990; Peng et al. 1993). The increase measured was slightly less than that found by Baas et al. (1989) with uprooted *Plantago major* L. plants placed in hydroponics (37%) and that found by Peng et al. (1993) with *Citrus volkameriana* Tan & Pasq. trees in soil (37%). Thus, these results are surprisingly similar considering the different experimental conditions and plant/fungal species used in the experiments with soil-grown plants reported previously.

Baas et al. (1989) attributed approximately 13% of the increased root respiration to increased ion (NO_3^-) uptake. In the present investigation with wheat, there was no significant difference between NO_3^- uptake of non-mycorrhizal and mycorrhizal roots. The rate of ATP consumption in non-mycorrhizal roots depends on the three major energy-requiring processes of root growth, maintenance of root biomass and ion uptake (Lambers et al. 1996). In mycorrhizal roots, the ion uptake, growth and maintenance of the fungus contribute to the root respiration. Since root growth and ion (NO_3^-) uptake were similar in non-mycorrhizal and mycorrhizal roots, fungal respiration should account for the difference in root respiration. It is possible that most of the 32% increase in CO_2 release from mycorrhizal roots was due to fungal respiration itself, in particular the relatively high decarboxylation processes needed, for example, for the synthesis of energetically expensive lipids (Cox et al. 1975; Cooper and Lösel 1978) as suggested by Peng et al. (1993) for *C. volkameriana*.

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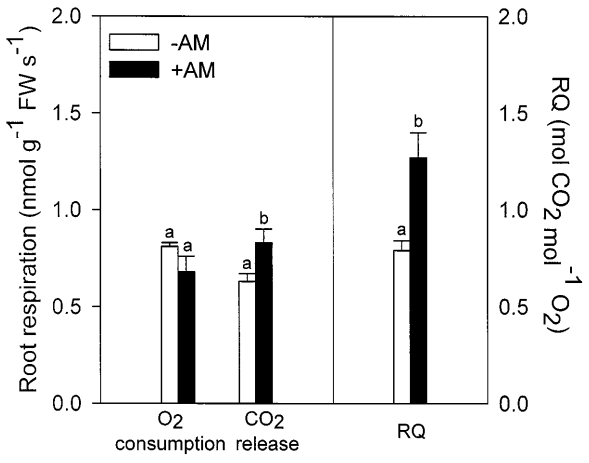

Fig. 1 Root respiration of non-mycorrhizal (*–AM*) and mycorrhizal (*+AM*) 7-week-old *Triticum aestivum* roots in terms of O_2 consumption, CO_2 release and respiratory quotient (*RQ*). Letters indicate statistical difference between –AM and + AM treatments ($P < 0.05$, Student's *t*-test)

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