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Effect of onion (*Allium cepa*) root exudates on the hyphal growth of *Gigaspora margarita*

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Abstract The effect of root exudates from onions differing in P status on spore germination and hyphal growth of arbuscular mycorrhizal fungi was investigated. Onion (*Allium cepa*) was grown in solution culture at different phosphorus concentrations (0, 0.1, 1.0, 8.0 and 24.0 mg P l^{-1}) and root exudates were collected. When spores of the arbuscular mycorrhizal fungus, *Gigaspora margarita* were incubated with these root exudates, spore germination was only slightly affected but hyphal growth was greatly affected, particularly with exudates from P-deficient plants. This suggests that the P nutrition of host plants influences the composition of root exudates and thereby the hyphal growth of arbuscular mycorrhizal fungi.

Key words Arbuscular mycorrhizae · *Gigaspora margarita* · *Allium cepa* · Phosphorus · Root exudate

Introduction

Infection of plants by arbuscular mycorrhizal (AM) fungi, which improves phosphorus (P) uptake and plant growth, is reduced by the addition of phosphate fertilizer to soil (Mosse 1973). However, the mechanisms bringing about such a decrease in infection are not well understood. The P concentration of host plants may affect the quantity or quality of root metabolites or root exudates and thus influence infection. The application of phosphate to citrus (Ratnayake et al. 1978), sudangrass (Graham et al. 1981) and white clover (Tawaraya et al. 1994) decreased root membrane permeability and with it the concentration of amino acids and reducing sugars in root exudates. It has also been demonstrated that root exudates from P-deficient Troyer citrange (Graham 1982) and white clover (Elias and Safir 1987) have a positive effect on the hyphal growth of *Glomus* spp. However, root exudates were collected from plants grown at only two P levels and the effects of root exudates from seedlings grown at a wide range of P concentration have not been reported.

In this present study, we tested the effects of root exudates from onion (*Allium cepa*) grown in culture solution at several P concentrations on the germination and hyphal growth of *Gigaspora margarita*.

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Materials and methods

Plant growth and collection of root exudates

Onion (*Allium cepa* L. cv. Sensyuchukoki) seeds were sown in a moist vermiculite spread. One week after sowing, culture solution was added to immerse the roots. The culture solution contained the following mineral nutrients (mg l^{-1}): 40 N (NH_4NO_3), 20 N ($NaNO_3$), 60 K (K_2SO_4), 80 Ca ($CaCl_2$), 40 Mg ($MgSO_4$), 2 Fe ($FeSO_4$), 1 Mn ($MnSO_4$), 0.01 Cu ($CuSO_4$), 0.005 Mo [$(NH_4)_6Mo_7O_{24}$], 0.4 B (H_3BO_3), 0.2 Zn ($ZnCl_2$) (Wagatsuma et al. 1988). The pH of the solution was adjusted daily to 5.0 ± 0.05 with dilute H_2SO_4 and NaOH. The P concentrations of the test solutions were adjusted to 0 (P0), 0.1 (P1), 1 (P2), 8 (P3), or 24 (P4) mg P l^{-1} with NaH_2PO_4. All solutions were aerated continuously and replaced weekly. Each treatment was replicated three times.

Onion seedlings were grown in the glasshouse for 28 days. The seedlings were then carefully lifted out of the vermiculite and washed with sterile water to remove all debris. Ten seedlings per treatment were fixed for 12 h in a conical flask containing 500 ml of aerated, sterile deionized water. The resulting solutions were used as root exudates and were stored at 4 °C until used.

Analysis of plant material

Shoot and root dry weights were determined after drying at 70 °C for 72 h. Dry material was ground and digested in a HNO_3–$HClO_4$–H_2SO_4 solution (Jones and Case 1990). The P content in the digest was determined colorimetrically using the vanadomolybdate-yellow assay.

Table 1 Effect of phosphate in culture solution on dry weight and P concentration of onion. The phosphate concentrations were 0 (P0), 0.1 (P1), 1 (P2), 8 (P3), and 24 (P4) mg P l^{-1} nutrient solution. Means within a column followed by the same letter are not significantly different ($P=0.05$) according to the Tukey test

P level	Dry weight (mg/plant)		P concentration (g kg^{-1})	
	Shoot	Root	Shoot	Root
P0	14.9 a	3.9 a	0.9 a	0.9 a
P1	19.0 a	4.1 a	0.9 a	0.9 a
P2	61.1 b	7.0 a	0.7 a	1.4 a
P3	112.8 c	6.9 a	7.2 b	10.9 b
P4	154.9 d	8.8 a	10.0 c	13.4 b

Fungal growth

Azygospores of *G. margarita* Becker & Hall were collected by wet sieving (Gerdemann and Nicolson 1963) from a commercial inoculum (Central Glass, Saitama, Japan). Spores were washed in distilled water with an ultrasonic cleaner (Branson 1200) for 5 min, surface sterilized with a solution containing 20 mg l^{-1} chloramine T, 200 mg l^{-1} streptomycin and 3 ml l^{-1} Tween 80 for 15 min (Mosse 1962) and then rinsed with sterile distilled water. Root exudates were passed through a membrane filter (0.2 μm) and 2-ml aliquots of the filtrates were added to pads on the bottom of 50-mm Petri dishes. Sterile distilled water was used as a control. A nitrocellulose membrane filter (A080M047A, Advantec) was placed on the pad and surface-sterilized spores were transferred onto the membrane filter, 5 spores per Petri dish. The spores were incubated in the dark at 27 °C. Germination was assessed by scoring the emergence of a germ tube at 7, 11, 13 and 16 days after incubation. Hyphal lengths were determined by the grid-line intersect method (Giovannetti and Mosse 1980).

Statistical analysis

Experiments were conducted with five replicate Petri dishes per treatment. The data were subjected to an analysis of variance. When a significant ($P<0.05$) treatment effect was found, the mean values were compared using the Tukey test ($P<0.05$).

Results and discussion

Table 1 shows onion shoot and root growth in the different culture solutions. Increasing the P concentration of the culture solution produced a significant increase in shoot dry weight. Root dry weight also increased with increasing P concentration, but the effect was lower than for shoot growth. Shoot and root P concentrations increased significantly with increasing culture solution P concentration. The shoot and root P concentrations at the P0 and P1 levels were lower than 1.0 g kg^{-1} and these seedlings were considered to be P deficient (Piggott 1986; Jones et al. 1991). On the other hand, the tissue P concentrations of plants grown at P4 were higher than 10.0 g kg^{-1} and these plants were considered to be P rich. Thus root exudates were collected root from plants of clearly different P status.

The germination of AM fungal spores was higher than 70% after 7 days of incubation, except in the controls, and remained constant from 11 to 16 days in all treatments (Fig. 1). The germination rate under all treatments was higher than that in the control, with PO showing the highest rate.

There were significant differences in hyphal length between treatments at 13 and 16 days, but no differences at 7 and 11 days (Fig. 2). The differences were greatest at 16 days in the following order: P0 ≈ P1 > P2 ≈ P3 > P4 ≈ control. Thus, root exudates from P0, P1, P2, and P3 appear to contain metabolites which accelerated hyphal growth, and these metabolites were affected by the P nutrition of the host plant. However, root exudates from P4 did not promote hyphal growth, and thus the quality of compounds contained in root exudates changed significantly between P3 and P4. Schwab et al. (1983) found that P had little influence on the spectrum of carboxylic acids and amino acids in root exudates. Some flavonoids have been shown to stimulate hyphal growth (Gianinazzi-Pearson et al. 1989; Tsai and Phillips 1991), and isoflavone formation in clover leaves increased under phosphate deficiency (Rossiter and Beck 1966). Furthermore, it is well accepted that the decrease in mycorrhizal infection in response to phosphate application arises from the resulting increase in tissue P concentration (Sanders 1975; Menge et al. 1978). Therefore, the P status of host plants may affect the metabolism of compounds that can accelerate fungal growth.

In conclusion, we collected root exudates from plants grown under five different nutrient regimes (P

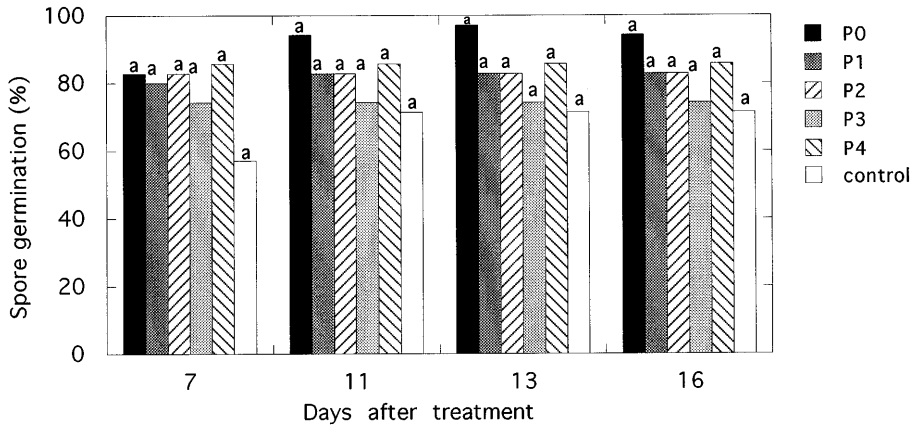

Fig. 1 Effect of root exudates on spore germination of *Gigaspora margarita*. For each harvest, the means followed by the same letter are not significantly different ($P=0.05$) according to the Tukey test. *P0–P4* refer to the phosphate concentrations described in Materials and methods

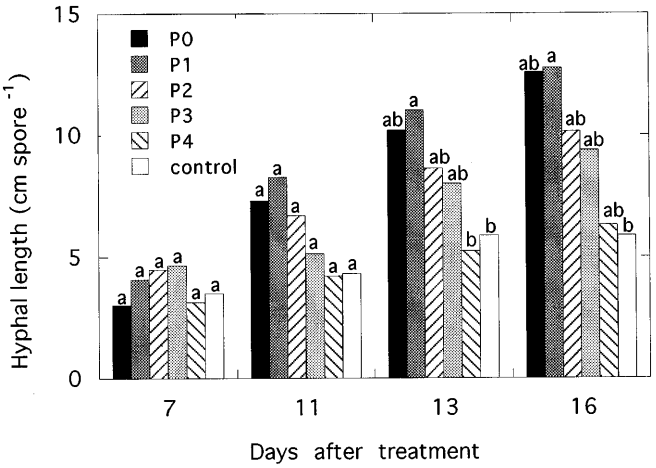

Fig. 2 Effect of root exudates on hyphalgrowth of *G. margarita*. For each harvest, the means followed by the same letter are not significantly different ($P=0.05$) according to the Tukey test. *P0–P4* refer to the phosphate concentrations described in Materials and methods

deficient to P rich). We found that root exudates from P-deficient or P-adequate onions stimulated hyphal growth whereas those from P-rich roots had no effect. The P concentration of host plants thus appears to influence the quantity and/or quality of root exudates, which can further affect the hyphal growth of AM fungi.

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