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Effect of root exudate fractions from P-deficient and P-sufficient onion plants on root colonisation by the arbuscular mycorrhizal fungus *Gigaspora margarita*

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Abstract The effect of root exudates from P-deficient onion on root colonisation by an arbuscular mycorrhizal fungus was examined. Onions (*Allium cepa* L.) were grown in solution culture at phosphorus concentrations of 0 (P0) and 2 (P2) mg P l^{-1}. Root exudates were collected and fractionated with Amberlite XAD-4 resin to give EtOH and water soluble fractions. Onions inoculated with the arbuscular mycorrhizal fungus *Gigaspora margarita* Becker & Hall were grown with or without (control) root exudates and exudate fractions in a growth chamber. After 24 days, arbuscular mycorrhiza levels and appressoria formation had increased in plants treated with P0-root exudate or the P0-EtOH fraction when compared to corresponding P2 treatments or control plants. P0 and P2 water-soluble fractions did not significantly affect either aspect of fungal development. These results suggest that hydrophobic compounds found in root exudates from P-deficient onion increase appressorium formation and, therefore, enhance mycorrhiza development.

Key words *Allium cepa* · Appressorium · Arbuscular mycorrhiza · Root colonisation · Phosphorus

Introduction

Phosphorus in host plant tissues (Sanders 1975; Menge et al. 1978; Tawaraya et al. 1994) and in the soil solution influences root colonisation by arbuscular mycorrhizal fungi. Phosphorus nutrition of host plants affects both the quantity and quality of root exudates. P-deficiency in plants increases root exudation of amino acids, sugars and organic acids (Ratnayake et al. 1978; Graham et al. 1981; Schwab et al. 1983), and root exudates of P-deficient *Citrus* (Graham 1982), clover (Elias and Safir 1987), onion (Tawaraya et al. 1996a) and carrot (Nagahashi et al. 1996) have been shown to increase hyphal growth of arbuscular mycorrhizal fungi. In addition, formation of secondary appressoria by *Gigaspora margarita* is increased on roots of P-deficient onion (Tawaraya et al. 1994). However, the root exudate components affecting colonisation are not well known.

In this present experiment, we studied the effect of root exudates from P-deficient and P-sufficient onion and of fractions separated by resin on appressorium formation and root colonisation by *G. margarita*.

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Materials and methods

Solution culture and collection of root exudates

Onion (*Allium cepa* L. cv. Sensyuchukoki) seeds were sown in a moist vermiculite spread. One week after sowing, 80–120 seedlings were transferred to 8-l plastic containers with a culture solution added to root immersion. The culture solution contained mineral nutrients (mg l^{-1}) according to Wagatsuma et al. (1988) : 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 ($ZnCl_2$). P concentrations of the solution were adjusted to 0 (P0) or 2 (P2) mg P l^{-1} with NaH_2PO_4. The pH of the solutions was adjusted daily to 5.0 ± 0.05 with dilute H_2SO_4 and NaOH. Solutions were aerated continuously with vinyl chloride tubes connected to an air pump and were replaced weekly. Each P treatment was replicated three times.

Onion seedlings were grown in the glasshouse of Yamagata University (38°44′N, 139°50′E) from 20 August to 13 September 1994. The mean ambient temperature during this period was 21.9 °C with a mean minimum of 15.4 °C and mean maximum of 28.2 °C. Ten seedlings per container were carefully lifted out of the vermiculite and washed to remove all debris with sterile water. They were then fixed with sponge in an Erlenmeyer flask containing 500 ml of sterile deionized water for 12 h. Solutions were aerated with vinyl chloride tubes connected to an air pump. The solutions collected (root exudates) were stored at –15 °C until use.

Fractionation of root exudates

Aliquots (100 ml) of root exudates were passed through Amberlite XAD-4 resin (6 g) for fractionation (Tang and Young 1982). Compounds which did not adsorb to the resin were collected and referred to as the water-soluble fraction. Adsorbed compounds were extracted with 100 ml of 99.9% ethyl alcohol, concentrated below 40 °C in a rotary evaporator, redissolved in 100 ml distilled water and referred to as the EtOH-soluble fraction. The two fractions, along with the original root exudates and distilled water (as control) were used for a root colonisation experiment.

Inoculation and plant growth

Spores of the arbuscular mycorrhizal fungus *G. margarita* Becker & Hall were collected from a commercial inoculum (Central Glass, Tokyo, Japan) by wet sieving (Gerdemann and Nicolson 1963). Spores were cleaned, surface-sterilised (Tawaraya et al. 1996b) and inoculated into pots at the rate of 20 spores per pot by placing the spores 1 cm below the root tips of seedlings. Onion seeds were germinated on moist vermiculite and three 7-day old seedlings were transplanted to each pot containing 60 g of autoclaved sand with 12.2 mg P kg^{-1} available phosphorus (Truog 1930) and a pH(H_2O) of 5.8.

Pots were placed in a growth cabinet (14 h photoperiod, 150 μMOL m^{-2} s^{-1} PPFR, 25 °C) and allowed to grow 24 days. Root exudate or the EtOH soluble and water-soluble fractions were applied to pots every 2 days. Nutrient solution (8 mg P l^{-1}) as used for the solution culture was applied to each pot every 2 days. The total volume of root exudates, distilled water or nutrient solution applied to each pot was 45 ml. Pots were arranged in a completely randomized design with three replications of each treatment.

Plant analysis

Plants were harvested at 24 days after transplanting and shoots and roots were separated. All roots were boiled in 100 g l^{-1} KOH solution at 80 °C for 10 min and stained with 0.5 g l^{-1} aniline blue in 700 ml l^{-1} glycerol solution at 90 °C for 5 min. Total root length was determined (Newman 1966). The proportion of colonised root was determined by the grid-line intersect method (Giovannetti and Mosse 1980) and the number of appressoria formed on each root was determined at × 100 magnification after mounting each root on microscope slides.

Shoot and root dry matter were determined after drying at 70 °C for 72 h, and then ground and digested for P analysis with HNO_3-$HClO_4$-H_2SO_4 (Jones and Case 1990). The P content was determined colorimetrically using the vanado molybdate yellow assay (Olsen and Sommers 1982).

Statistical analysis

The data were statistically analyzed by an analysis of variance after arcsin transformation for percentages. When a significant ($P<0.05$) treatment effect was found, the mean values were compared using the Tukey test ($P<0.05$).

Results

Plant dry matter and P concentration of onion plants grown in solution culture at the P0 level were significantly lower than those at the P2 level (Table 1). P concentrations in onion tissues below 1.0 g P kg^{-1} are considered to be deficient whereas those higher than 3.0 g kg^{-1} are sufficient for normal growth (Piggott 1986; Jones et al. 1991). Therefore, root exudates were collected from P-deficient and P-sufficient 24-day-old onion plants.

Table 1 Dry weight and phosphorus concentrations of onions grown in solution culture at two P levels. Means followed by the same letter are not significantly different ($P<0.05$) by Tukey test

P level	Dry weight (mg/plant)		Shoot P
	Shoot	Root	(g P/kg)
P0	14.81 a	2.80 a	0.36 a
P2	28.85 b	3.66 b	4.67 b

Root length was significantly greater in onion plants which received whole root exudates and EtOH fractions than in controls, or those treated with the water-soluble fraction of root exudates (Fig. 1). No differences were found between any P0 and P2 fractions.

Arbuscular mycorrhiza levels were significantly greater in plants treated with either the whole root exudate or the EtOH fraction from low-P plants than those treated with fractions from P-sufficient plants or the untreated control (Fig. 2). The EtOH fraction of exudates from P-sufficient plants also significantly increased root colonisation over the control. This effect was not observed with whole root exudate or the water-soluble fraction from P-sufficient plants.

Treatment with whole root exudate and the EtOH fraction of P-deficient plants (P0 level) significantly increased the number of appressoria per unit root length but not those from P-sufficient plants (P2 level) (Fig. 3). Application of the water-soluble fraction of root exudate did not affect appressorium formation.

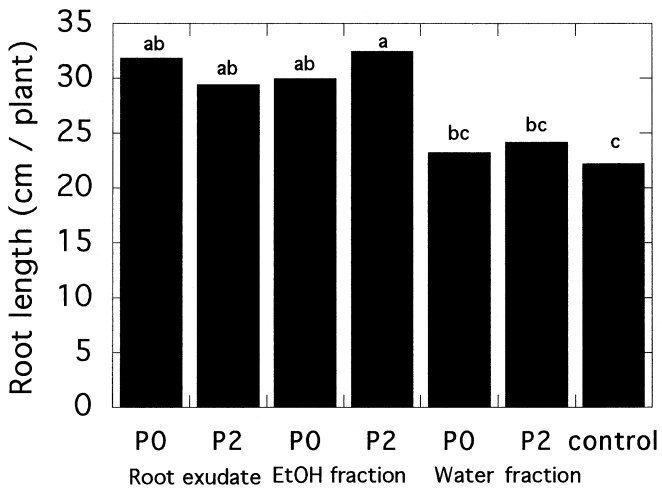

Fig. 1 Effect of whole root exudates, the EtOH fraction or the water fraction collected from P-deficient (P0), P-sufficient (P2) or control plants on root growth of onion. Means followed by the same letter are not significantly different ($P=0.05$)

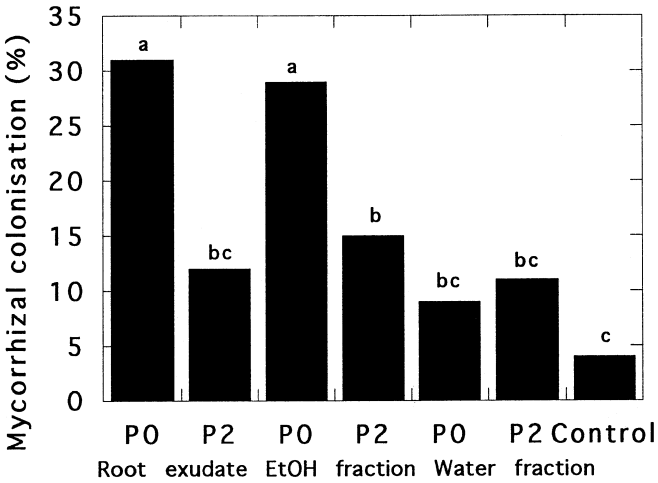

Fig. 2 Effect of whole root exudates, the EtOH fraction or the water fraction collected from P-deficient (P0), P-sufficient (P2) or control plants on arbuscular mycorrhiza development by *Gigaspora margarita* in onion roots. Means followed by the same letter are not significantly different ($P=0.05$)

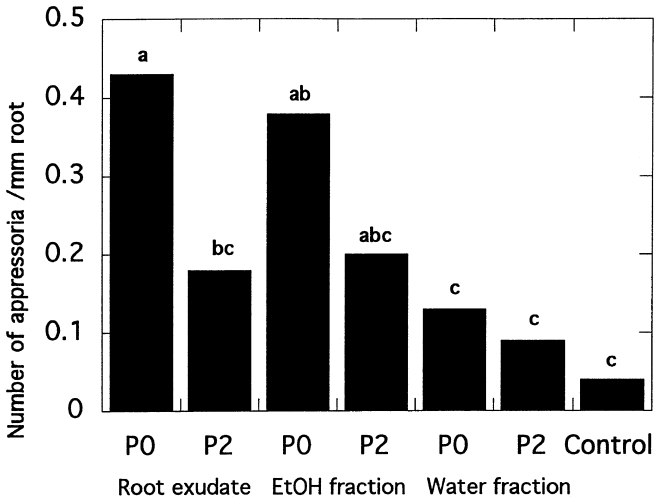

Fig. 3 Effect of whole root exudates, the EtOH fraction or the water fraction collected from P-deficient (P0), P-sufficient (P2) or control plants on appressorium formation by *G. margarita* on onion roots. Means followed by the same letter are not significantly different ($P=0.05$)

Discussion

Treatment with root exudates from P-deficient onions increased appressorium formation and root colonisation by the arbuscular mycorrhizal fungus *G. margarita*. Root exudates from P-deficient Troyer citrange (Graham 1982) and white clover (Elias and Safir 1987), and suspension-cultured cell exudates of *Pueraria phaseoloides* (Paula and Siqueira 1990) increased hyphal growth of arbuscular mycorrhizal fungi. In addition, root exudates produced by Ri T-DNA-transformed *Daucus carota* grown without P gave more hyphal growth from spores of *G. margarita* than exudates produced in the presence of P (Nagahashi et al. 1996). Root exudates from onion of clearly different P status also affected the hyphal growth of *G. margarita* (Tawaraya et al. 1996a) and the formation of secondary appressoria was inhibited by phosphate application to shoots, indicating the inhibitory effect of plant P status (Tawaraya et al. 1994). These results reinforce observations that the phosphorus nutrition of the host plant influences the extent of root colonisation by arbuscular mycorrhizal fungi through its effect on root exudates (Sanders 1975; Menge et al. 1978; Tawaraya et al. 1994).

However, factors other than P in root exudates may affect arbuscular mycorrhizal fungi. Hyphal growth of *G. margarita* was stimulated by root exudates of clover (Gianinazzi-Pearson et al. 1989) and carrot (Bécard and Piché 1990) but not of non-host lupin and sugar beet. Host root exudates can elicit hyphal morphogenesis which facilitates root contact and appressorium formation (Giovannetti et al. 1993a, 1993b; Suriyapperuma and Koske 1995), although recent observations on appressorium formation on isolated host cell walls indicate that contact recognition does not require a chemical signal (Nagahashi and Douds 1997).

The EtOH fraction of root exudates from P-deficient plants showed the same effect on appressorium development and root colonisation as the original root exudates. Since hydrophobic compounds in root exudates were adsorbed on XAD-4 resins (Tang and Young 1982) and extracted with EtOH, whilst hydrophilic compounds were collected in the inactive water fraction, it can be concluded that the effective molecules are hydrophobic. Among the many possible hydrophobic compounds are phenolics in root exudates, which are known to act as signal molecules in plant-microbe interactions (Siqueira et al. 1991). Isoflavone in clover leaves increased under phosphate deficiency (Rossiter and Beck 1966), whilst an additional unknown compound was detected in phosphate-deficient parsley (Franken and Gnädinger 1994). Two isoflavonoids were isolated from clover roots grown under phosphate stress (Nair et al. 1991) and it is thus possible that the hydrophobic compounds in the root exudates which affected colonisation are phenolics. The effects of other hydrophobic compounds, such as terpenoids, on mycorrhiza development should also be examined, since signal molecules in P-deficient root exudates may regulate the gene expression of hyphal growth and appressorium formation by arbuscular mycorrhizal fungi (Smith 1995).

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