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Effect of root exudation on VA mycorrhizal infection at early stages of plant growth

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Summary No relationship between the degree of VA mycorrhizal infection and total sugar content in root exudates of several plant species of different degree of mycorrhizal susceptibility were observed during the early stages of plant growth. Even more, the non host plants tested showed higher sugar exudation ability, when expressed as the amount exuded per g of root, at these early periods of their growth, than plants susceptible to mycorrhizal infection.

Root exudates from host and non host plants influenced similarly the percentage of spore germination and number of secondary spores under controlled conditions.

Introduction

It is widely recognized in the VA mycorrhizal symbiosis the carbon and energy requirement of the fungus are supplied by the photosynthate of the host plant.

Root exudation, specially of sugars, can be considered an important factor affecting mycorrhizal development. Thus any factor which affects the rate of root exudation such as light intensity and soil temperature, stage of plant development or application of certain herbicides[5, 9, 14] might consequently affect VA mycorrhizal infection. Moreover, during the first stages of the VA fungus development, root exudates must be specially important because the metabolites they contain are thought to sustain the growth of the fungus during the pre-infection phases. Hence, it would be interesting to know whether the net leakage of sugars from roots at these stages is related to the extent of mycorrhizal infection, and to the mycotrophic condition of the plant species.

Materials and methods

Experiment 1

The experiment was carried out using two different systems simultaneously: Open pots with soil, and test tubes with nutrient solution in sterile conditions.

The host plants alfalfa (*Medicago sativa* cv. Aragon), sorghum (*Sorghum vulgare*), sunflower (*Heliantus annum*), the three wheat cultivars of *Triticum vulgare viz*, Pane Lozano and

Negrillo[2], and the non-host plants radish (*Rhaphanum raphanistrum* var. "Aplastada de Egipto") and cabbage (*Brassica oleracea* cv. Brunswick) were used as the test plants.

The test soil[4] was steam sterilized and then mixed with sterile sand (5 : 2 mixture).Seedlings were transplanted into pots containing 300 g of the soil-sand mixture. Five replicates for each treatment were prepared. The VA mycorrhizal endophyte assayed was an isotype of *Glomus mosseae.* Plants were inoculated with soil from stock plant culture which contained spores, mycelium and infected root fragments. Five g of such inoculum were added per pot. Plants were watered from below and given Long Ashton nutrient solution[8] (5 ml wks^{-1}) without phosphate.

For the test tube experiment, surface sterilized seeds were previously germinated and transferred to sterilized glass tubes (5 replicates containing a strip of filter paper[1] and 10 ml of a diluted (1/5) Hewitt's nutrient solution. After two weeks of growth, 10 ml of sterilized full strength Hewitt's nutrient solution were added every fortnight.

Plants were grown simultaneously in pots and tubes in a glasshouse at 19–25°C. After fifteen and thirty days of growth the plants were harvested and the exudation abilities in the different plant species was evaluated. Root exudates were obtained as described by Ratnayake *et al.* (1978)[13]. Plants grown in pots were carefully removed from soil and washed. Once lifted out of debris plants were placed in beakers with their root systems completely covered with aerated distilled water for a period of 17 h in a controlled temperature (26–27°C) under continuous low light. The plants were removed at the end of this period and their root weight recorded. The content of the beakers were concentrated and aliquotes of one ml were tested separately for its total sugars content by the standard colorimetric procedure using anthrone[15]. The concentration of total sugars were expressed as μg equivalents of glucose g^{-1} fresh root weight. Root exudates from plants grown in tubes were those accumulated in the nutrient solution over all the period of plant growth. Contaminated tubes were discarded.

Only fifteen day old plants growing in pots were used since in 30 day old plants the rate of root exudation might have changed drastically with mycorrhization.

Roots samples of each replicate pots were cleared and stained[12] and the root length infection evaluated by the gridline intersect method[5].

Experiment 2

Spores of *Glomus geosporus* were isolated by wet sieving from a plant stock culture. Ten surface sterilized spores[10] were placed in a sterilized petri dish (35 mm diameter) with water-agar (1% Difco Bacto Agar), covered with a Millipore membrane (45 mm, and 0,45 μm pore size) and fixed with paraffin. Pots were first covered with 2 cm of steamed soil or sterilized sand. Petri dishes were placed horizontally inside the pots and then filled with the same steamed soil or sterilized sand up to 1 cm of the rim. Afterwards the seedlings were transplanted into the pots. The soil used was the same as in Experiment 1.

Several mycorrhizal host and non host plants were tested. These were: Maize (*Zea mays*), lavender (*Lavandula spica* var. vera L) and alfalfa (*Medicago sativa* cv. Aragón) as mycorrhizal hosts, and radish (*Raphanum raphanistrum* var. "Aplastada de Egipto") and cabbage (*Brassica oleracea* cv. Brunswick) as non hosts. Hewitt's nutrient solution were added every fortnight as before and pots without plants were used as control.

The experiment was also carried out in sterile conditions and the petri dish containing spores were placed in sand tubes. Surface sterilized seeds of alfalfa and cabbage were used and tubes without plants were the controls.

Plants were grown in a glasshouse as in Experiment 1 and after 8 weeks the mycelia from VA spores were stained[1] and the percentage of spore germination and the number of secondary spores recorded.

Results

The plant species tested showed great differences in their mycorrhizal infection levels. After fifteen days of inoculation all the susceptible

Table 1. VA mycorrhizal infection and total sugar content in the root exudates of plants grown in sterile soil pots inoculated with *Glomus mosseae*

Test plant	Root length infected (%)		μg total sugar exudates (g^{-1} fresh root)
	15 days	30 days	15 days
Alfalfa	12	62	54 ± 3
Sorghum	3	29	45 ± 4
Sunflower	8	56	54 ± 8
Pane	2	3	88 ± 5
Lozano	2	8	60 ± 4
Negrillo	0	0	124 ± 5
Cabbage	0	0	154 ± 3
Radish	0	0	122 ± 2

Each figure is the mean for five replicates.
Standard errors of mean are given.

Table 2. Total sugar content in the root exudates of plants grown in test tubes on sterile culture solution

	Total sugar exudates, μg					
	g^{-1} fresh root			Total fresh $root^{-1}$		
	15 days	30 days	Diff. (%)* 15 *vs* 30 days	15 days	30 days	Diff. (%)* 15 *vs* 30 days
Alfalfa	1,768 ± 87.4	3,954 ± 316.3	+ 55	34 ± 1.7	106 ± 8.5	+ 67
Sorghum	795 ± 46.2	1,307 ± 280	+ 39	140 ± 8.1	234 ± 50	+ 40
Sunflower	450 ± 22.5	1,384 ± 240	+ 66	150 ± 7.4	349 ± 70.6	+ 56
Pane	1,169 ± 10	1,403 ± 222	+ 16	112 ± 1	199 ± 31.6	+ 43
Lozano	2,677 ± 220	2,700 ± 75.6	+ 1	200 ± 16.5	215 ± 6	+ 6
Negrillo	8,293 ± 291	3,736 ± 812	– 121	422 ± 14.8	476 ± 103.5	+ 11
Cabbage	4,038 ± 107.7	2,419 ± 600	– 66	105 ± 2.8	115 ± 27	+ 8
Radish	8,750 ± 350	3,924 ± 200	– 122	105 ± 4.2	110 ± 5.6	+ 4

Each figure is the mean for five replicates. Standard errors of mean are given.
* Difference (%) of root exudation between plants of 15 and 30 days of growth.

species became mycorrhizal and their VA infection level increased with time (Table 1). The non host cabbage and radish plants remained uninfected. No relationship between the amount of root length infected and the amount of total sugars in the root exudates was found. Moreover the non-mycorrhizal plants wheat cv. Negrillo, cabbage and radish, exuded more sugars per g of root than the mycorrhizal plants tested (Table 1 and 2).

The amount of sugars exuded in the second fifteen days period of growth, (as expressed as percent difference in exudation at 15 and 30 days growth, (Table 2) was lower in cabbage, radish and wheat cv.

Table 3. Percentage of spore germination and number of secondary spores in sterilized soil and sand pots and in sand tubes

	Soil pots		Sand pots		Sterilized sand tubes	
	Spore germination (%)	No. of secondary spores	Spore germination (%)	No. of secondary spores	Spore germination (%)	No. of secondary spores
Control	98	5 ± 2	80	3.2 ± 1.6	80	3.5 ± 1
Maize	94	12 ± 1.5	77	8.5 ± 2.7		
Lavender	96	10 ± 4.9	85	7.3 ± 1.4		
Alfalfa	92	16 ± 6.3	88	9.5 ± 3.2	84	4.3 ± 2.7
Radish	97	13 ± 4.6	72	8.9 ± 2.6		
Cabbage	96	11 ± 3.2	75	9.0 ± 3.1	90	8.4 ± 3.2

Each figure is the mean for three plants and 30 spores. Standard errors of mean are given.

Negrillo and Lozano than in alfalfa, sorghum, sunflower and wheat cv. Pane and was negative when expressed on per g fresh root basis.

Table 3 shows the effect of root exudation on the percentage of spore germination and number of secondary spores under the different experimental condition tested. No differences between the mycorrhizal hosts maize, lavender and alfalfa and the non hosts cabbage and radish in the percentage of spore germination and number of secondary spores were found. The number of secondary spores was higher in pots containing plants, irrespective of their mycotropic characteristics, than in the controls without plants.

Discussion

The importance of sugar concentration in root exudates on VA mycorrhizal infection have been pointed out by several authors[6,9] and a direct correlation between the mycorrhizal infection level and quantity of sugars in such plant product have been found. In fact, the lack of mycorrhizal infection in non host plants have been associated with the scarcity of sugars in the root exudates of these plants[14] and not with any detrimental effects on the fungus[11]. This suggests that root exudation is a critical factor controlling VA mycorrhizal formation. However, our results indicate that, though accepting the importance of root exudation, the amount of sugar exuded in itself is not a decisive factor for initiation of VA mycorrhizal infection. It appears that the reason why the non host plants do not become infected is not because the quantity of sugars they exude is insufficient to sustain initial fungal development.

Root exudation is lower in susceptible plants than in non-susceptible ones, when expressed per g of root weight (Tables 1 and 2). This indicates a relatively higher membrane permeability of their root cells at the initial stages of plant growth. But such exudation rates decrease with time in non host plants and increase in host plant (Table 2). This fact might explain why other authors[2] found higher sugar exudation rates in the mycorrhizal susceptible than in non-susceptible plants when measurements were made after several weeks of plant development and expressed per g of root weight.

According to these results plants susceptibility to VA mycorrhiza is unrelated to of the amount of sugar it exudates. Perhaps such susceptibility could be related to root membrane constitution and/or the presence or absence of a certain metabolite in the root exudates not detected in this study[7].

The effect of root exudates on spore germination and mycelial development here described are well documented in the literature[3].

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