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Relationship between the concentration of sugars in the roots and VA mycorrhizal infection

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Summary The VA-infected wheat varieties showed an increase of total (Lozano var.) and reducing (Lozano and Pane vars.) sugars in their root extracts. However, no clear relationship between sugar concentration in the root and VA mycorrhizal infection level could be established.

In addition, the VA mycorrhizal hosts sorghum, alfalfa, sunflower and maize, and non-host radish and cabbage plants were tested for sugar content in their root extracts after fifteen days of growth. Sugars present in the root extracts of these plants did not seem to be a decisive factor in plant susceptibility to VA infection.

Introduction

A typical feature of vesicular-arbuscular (VA) mycorrhizal symbiosis is that the fungus depends upon the host for a supply of photosynthate from the root[9]. Owing to the heterotrophic nature of the VA endophyte, the carbohydrate status of the plants ought to change when roots sustain the fungus growth. In fact, mycorrhizal differ from uninfected plants in the significantly higher C translocation from shoot to the root system[15,22]. But carbon drain in mycorrhizal plants may be compensated by an increased C assimilation[22] and photosynthesis[1]. It has been suggested that Glomus does not stimulate movement of sugars to it, by providing a sink for photosynthate[16].

On the other hand, the amount of sugar present in the root tissue seems to affect the frequency of penetration by the VA endophyte[13,21]. This suggests that the sugar content of roots plays a crucial role in colonization of the host root cells by VA mycorrhizal fungi.

The possible relationship between sugar content of extracts of plant roots and the establishment and development of VA infection was examined in this study by using host plants of different mycorrhizal susceptibility and non-host plants.

Material and methods

The experiments were carried out in open pots of soil[3]. This soil was steam-sterilized and then mixed with sterile sand (5:2 v/v), reinoculated with a soil filtrate containing common soil microorganisms except for propagules of Endogonaceae. Ten seedlings (in accord with

the experiment) were placed into each pot containing 300 g of the soil/sand mixture. The VA mycorrhizal endophyte was a form of *Glomus mosseae* from a stock plant culture. Plants were grown in a glasshouse at 19–25°C, watered from below and given phosphate-free Long Ashton nutrient solution[12] at a rate of 5 ml week^{-1}. Five replicate pots of each treatment were prepared. At harvest, samples of the root systems were stained[18] and the mycorrhizal infection assessed by the gridline intersect method[7].

A known weight (2 g) of fresh root tissue was homogenized using the Bligh and Dyer[4] method modified by Donaire (pers. comm.). Aliquots of the supernatant were tested separately for their total and reducing sugar content by the standard colorimetric procedures using anthrone[23] and Cu tartrate[6] reagents, respectively. The concentration of total and reducing sugars was expressed as μg equivalents of glucose, per g fresh root wt.

Experiment 1

In order to study the possible changes in the content of root sugars during the VA infection development, three wheat (*Triticum vulgare* L.) varieties (Lozano, Pane and Negrillo), selected for their different degree of susceptibility to VA mycorrhizal infection[2], were tested. There were VA inoculated and uninoculated plants. Harvests were made after 2, 6, 10 and 16 weeks of growth, and shoot dry weights, mycorrhizal infection, and total and reducing sugar concentration in their roots were determined.

Experiment 2

We examined the possible relationship between root sugar (total and reducing) content in the root extracts and VA colonization at early stages of plant growth. The plants tested were the hosts sorghum (*Sorghum vulgare*), alfalfa (*Medicago sativa*), sunflower (*Heliantus annus*) and maize (*Zea mays*); and the non-host radish (*Raphanus raphanistrum*) and cabbage (*Brassica oleraceae*). After fifteen days of plant growth, VA infection and total and reducing sugar content in the root extracts of these plants were determined as previously described.

Results and discussion

A typical VA infection curve as well as a fungal effect on wheat growth were observed (Table 1). The VA infected wheat varieties showed an increase in total (Lozano, Table 2) and reducing (Lozano and Pane, Table 3) sugars of their roots after six weeks of growth. However, in previous experiments[2] with these wheat varieties, but with developed ears, mycorrhizal infection negatively affected the sugar content in their roots. There are indications that the amount of sugar in mycorrhizal roots seems to be determined by two processes The negative effect produced by fungal drain of host photosynthate[5,14] and the stimulating effects caused by the increase of P supply and other advantages from VA mycorrhizas[1,11,19]. The different results obtained on the influence of VAM on the carbohydrate status of infected roots suggest that carbohydrate level in root depends on the balance between C demand of the fungus and C status the host[8]. Our results (Tables 2 and 3) show that fungus demand for carbon did not significantly affect the quantity of sugar in the root during the first stage of fungus development. But when the endophyte was well established, an increase in the amount of sugar in the infected roots was found. Similar results

Table 1. Shoot dry weight and mycorrhizal infection of three wheat cultivars

Cultivars		After (weeks) 2 Shoot dry wt (g)	2 % root length infected	6 Shoot dry wt (g)	6 % root length infected	10 Shoot dry wt (g)	10 % root length infected	16 Shoot dry wt (g)	16 % root length infected
Pane	– M	0.19 a	0	0.51 d	0	1.19 f	0	2.69 i	0
	+ M	0.15 b	1.7 a	0.65 e	18.2 b	1.35 g	28 c	2.80 j	29 c
Lozano	– M	0.18 ab	0	0.60 e	0	1.32 g	0	2.35 k	0
	+ M	0.16a	0.16 a	0.64 e	29.8 c	1.40 h	42 e	2.50 l	45 e
Negrillo	+ M	0.22 c	0	0.7 e	0	1.21 f	0	1.86 m	0

Each figure is the mean for five pots. – M = minus mycorrhiza; + M with inoculum of vesicular-arbuscular mycorrhizas. Within shoot dry wt. and within % root length infected values followed by the same letter are not significantly different according to Duncan's multiple range test $P = 0.05$.

Table 2. Total sugar content of root extracts for three wheat cultivars

Cultivars		μg total sugars g^{-1} fresh root after (weeks)			
		2	6	10	16
Pane	−M	1,344 a	4,950 bi	8,145 c	15,500 d
	+M	1,979 eh	3,975 b	8,775 c	15,075 d
Lozano	−M	1,732 ca	3,325 bfg	2,550 fh	5,130 i
	+M	2,070 eh	3,300 g	3,540 g	8,760 c
Negrillo	+M	3,375 g	7,931 c	8,415 c	13,860 d

Values followed by the same letter are not significantly different according to Duncan s multiple range rest, $P = 0.05$. Rest of the legend as in Table 1.

Table 3. Reducing sugar content of root extracts for the three wheat cultivars

Cultivars		μg reducing sugars g^{-1} fresh root after (weeks)			
		2	6	10	16
Pane	−M	360 af	875 bh	922 b	1,996 c
	+M	365 af	810 bh	2,396 cd	3,093 d
Lozano	−M	382 af	645 e	472 f	1.012 g
	+M	303 a	525 e	1,057 g	2,212 cd
Negrillo	+M	765 h	915 b	1,980 c	2,356 cd

Legend as in Table 2.

Table 4. VA mycorrhizal infection and total and reducing sugars in the roots extracts of plants after fifteen days of growth

Plants	μg total sugars g^{-1} fresh root	μg reducing sugars g^{-1} fresh root	% root length infection
Sorghum	1,825 a	416 a	3 a
Alfalfa	1,592 ac	211 b	12 b
Sunflower	458 b	58 c	8 c
Maize	1,397 ac	976 d	6 d
Radish	1,717 ac	899 d	0
Cabbage	1,206 c	358 ab	0

Column means followed by the same letters are not significantly different according to Duncan's multiple range test, $P = 0.05$.

have been obtained by other authors[16,20]. However, in our experiment the increase of sugars was irrespective of the level of mycorrhizal infection of the wheat varieties obtained during the assay (Table 1). The absence of a close relationship between the fungal metabolic activity and the sugar content of plant roots has also suggested[17].

As Tables 2 and 3 show, the level of total and reducing sugars in the root extracts was higher in the non-mycothrophic Negrillo var. than in the Lozano and Pane vars. after fifteen days of plant growth (Experiment 1). This contrasts with some studies where it was found that hyphal penetration frequency was positively correlated with soluble carbohydrate status of roots[13]. Because of that plants of different mycorrhizal susceptibility as sorghum, alfalfa, sunflower and maize were tested (Experiment 2) at early stages of plant growth. The non-host radish and cabbage were also used in order to see if there is any relationship between the non-susceptibility of plant root to VA mycorrhizas and the sugar content in their roots. However, total and reducing sugar content in the root extracts of these host and non-host plants (Table 4) indicates that plant susceptibility to VA infection was independent of the level of sugar content in their roots.

It is known that external factors such as light, temperature, P supply and defoliation[10,21] can strongly affect the initiation and development of infection. In that way a correlation between mycorrhizal association and root sugar content in plant roots has been established[10]. However, these factors affect the vigour of the host, and hence nutrient availability to the fungus, not only root carbohydrate status, but also other elements such as hormones, vitamins and salts[8] which would have more direct relevance on the fungus performance.

Our results indicate that low infection level is not caused by insufficient C concentration in root. A determinate sugar not evaluated in this study or factors other than soluble sugars can possibly affect host susceptibility to mycorrhizal infection.

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