Soluble Carbohydrates in Mycorrhized and Non-Mycorrhized Fine Roots of Spruce Seedlings

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Z. Naturforsch. **47 c,** 201–204 (1992); received November 21, 1991

Amanita muscaria, Ectomycorrhiza, *Picea abies,* Plant Sugars, Tissue-Specific Compartmentation

We present results on the compartmentation of carbohydrates such as sucrose, glucose, fructose, and mannose in different parts of an ectomycorrhiza established between *Picea abies* and *Amanita muscaria* and compare it with non-mycorrhized fine roots. Lyophilized mycorrhizas and fine roots (< 2 mm length) were dissected into about 0.5 mm thick slices which represent 4 zones of different physiological functions. The total amount of the analyzed carbohydrates was about 30% higher in non-mycorrhized (n-myc) compared to mycorrhized (myc) fine roots, with sucrose being the dominating sugar in both root types. A longitudinal distinction of sucrose pools showed lowest levels in the middle parts of a mycorrhiza, which represent areas of most intense symbiotic interaction. Fine roots without fungal infection did not show longitudinal variations in sugar content.

Introduction

In 1942, Björkman formulated the "carbohydrate theory" for mycorrhizal development [1]. His conclusion was that "mycorrhizae develop characteristically if the roots of the host plant contain a surplus of soluble carbohydrates" [2]. Although this theory is widely accepted there are only a few attempts reported in literature which deal with a more detailed quantitative analysis of carbohydrate levels in either unmycorrhized or mycorrhized fine roots [*e.g.* 3]. Applying methods of quantitative histochemistry it was thus the aim of this study to get an idea about the longitudinal variation of pool sizes of soluble carbohydrates along individual mycorrhized or non-mycorrhized fine roots.

Materials and Methods

A strain (MG2 [5]) of the fly agaric (*Amanita muscaria* [L. ex Fr.] *Hooker*) was isolated from a fruiting body in 1985 and maintained as a stock on MMNC-agar (MMN, supplemented with casein hydrolysate, glucose monohydrate and malt extract, 0.2%, 2%, 1% (w/v), resp. [5]) at 8 °C. Seedlings of Norway spruce (*Picea abies* (L) *Karst*) were grown aseptically from surface sterilized seeds. For germination the seeds were kept on MMNC-agar (0.8%) at 20 °C. After 4 weeks the seedlings were transferred into covered jars (0.5 l), filled with perlite/charcoal and 125 ml MMNS-medium (MMN containing 1% (w/v) sucrose), and kept under controlled conditions for additional 4 weeks (16 h light, about 90 µmol photons $\cdot m^{-2} \cdot sec^{-1}$, 20 °C). In parallel, 2 ml of a 3-week-old (20 °C, darkness) liquid culture of *Amanita muscaria* (liquid MMNC-medium) was dispersed on charcoal filters, which were placed in petri dishes filled with MMNC-agar (2%) and incubated for one week. Seedling transfer and synthesis of ectomycorrhizas was according to [4] but modified as follows: the roots of individual spruce seedlings were placed on a nutrient agar (MMN-agar, 2%) covered with a sheet of cellophane instead into a nutrient solution [5]. The petri dishes containing seedling with or without suspended mycelia (root parts darkened, shoots exposed to light (same conditions as above)) were harvested after 8 weeks. Finally, we arrived at three different types of "fine roots", (I) mycorrhized (myc) and (II) non-mycorrhized fine roots (n-myc) from inoculated petri dishes, and (III) fine roots which were not inoculated with the fungal partner (FR). After a freeze-stop by submerging root-system plus charcoal filter in liquid N_2 the

Abbreviations: CR, carrier roots; FR, fine roots without fungal partner; myc, mycorrhized fine roots; n-myc, non-mycorrhized fine roots; MMN, modified Melin-Norkrans.

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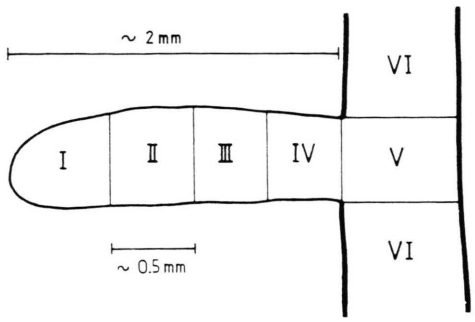

Fig. 1. Dissection of a mycorrhiza or a fine root into slices.

samples were lyophilized at −35 °C and stored under vacuum at −30 °C.

Using a stereo-microscope, the freeze-dried root material was dissected into six different parts (Fig. 1) using fine forceps and microknives (for detailed information about microdissection see [6]). Four to 5 specimen of the same zone from different fine roots were pooled in order to obtain about 20 to 40 µg dry weight (DW). The samples were extracted with 65% EtOH (100 µl, 1 h, 60 °C) and pelleted (5 min, 10,000 × *g*). The carbohydrates contained in a 5-µl-aliquot of the supernatant were assayed enzymatically in a buffer (500 µl) containing 100 mM imidazole (pH 6.9), 0.5 mM ATP, 0.5 mM NADP, 5.0 mM $MgCl_2$, 1 mM EDTA, 0.5 mM DTT and glucose-6-phosphate dehydrogenase (0.35 U/ml). The assay was performed by sequential addition of 0.35 U/ml hexokinase (glucose), 0.4 U/ml phosphoglucoseisomerase (fructose), 14 U/ml β-fructosidase (sucrose), and 25 U/ml α-mannosidase (mannose). The respective formation of NADPH was followed by fluorescence (SFM 25, Kontron, Neufahrn, F.R.G.). Each determination was carried out with 3 parallels.

Results

The data given in Fig. 2 show for all preparations of fine roots that sucrose was the most abundant of the carbohydrates analyzed.

The sum of the carbohydrates was about 30% lower in myc compared to n-myc roots. This difference was mainly due to the decreased level of sucrose (45, myc; 85, n-myc; nmol/mg DW). The level of sucrose in the carrier roots (CR) was always higher compared to fine roots (FR, Fig. 2). Glucose, in contrast, was higher in FR, with largest amounts in n-myc (inoculated) and fungus-free fine roots (not inoculated). Fructose and mannose exhibited only small amounts in all analyzed samples. In n-myc, mannose was below the detection limit (40 pmol/ml).

In Fig. 3 variations in soluble sugar content from tip to base of fine roots are presented. Only sucrose showed pronounced changes in pool sizes along the analyzed zones. In the tip of all three root systems (zone I) the sucrose content was on a comparable level. In contrast, sucrose levels in zones II to IV largely depended on sample type, with a most expressed decrease in myc, no change in FR, and a kind of intermediate state in n-myc. Levels of glucose and fructose were more variable, with fructose running to some degree in parallel to sucrose in myc samples. The glucose content in n-myc samples (zone II to IV) showed a strong difference between the two cultivations (high standard deviations, Fig. 3).

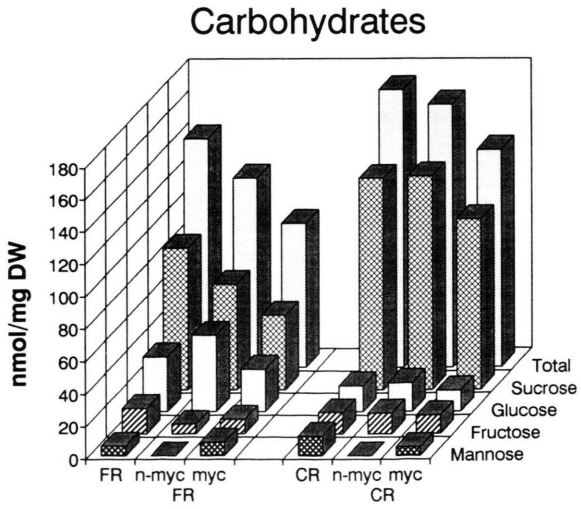

Fig. 2. Content of soluble carbohydrates in whole fine roots (left) and adjacent carrier roots (right). The CR part of the Fig. (right) represents pools in carrier roots connected to the respective n-myc/myc fine roots.

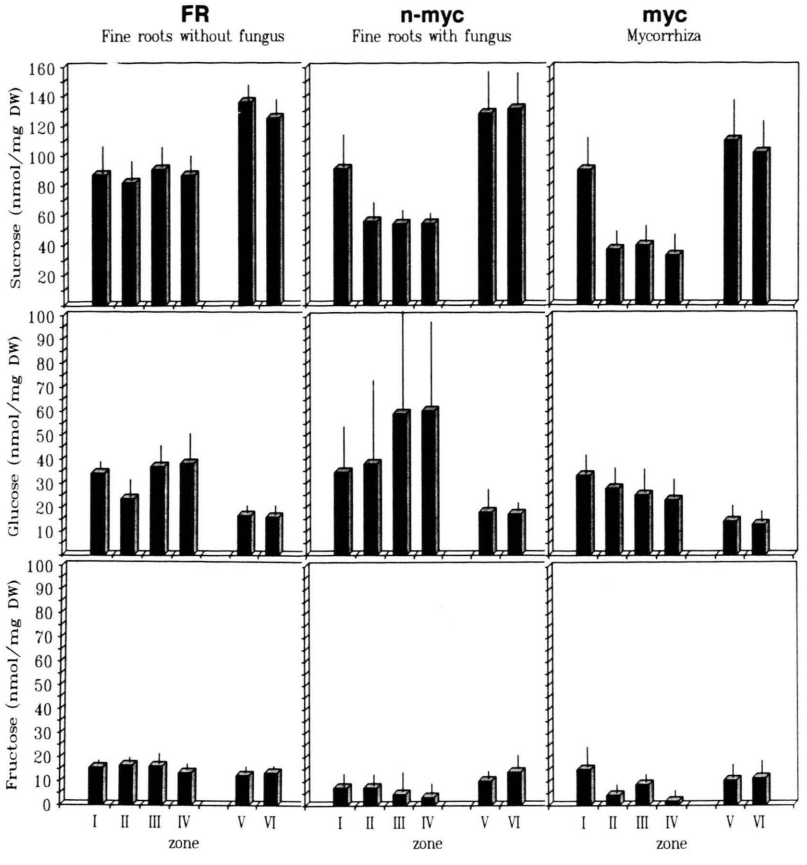

Fig. 3. Content of soluble carbohydrates in six different zones of the fine roots (see Fig. 1 for zone definition). The data represent mean values and standard deviations of four different preparations (out of two different cultivations) with 3 parallels each.

Discussion

Atkinson [7] described four different zones in mycorrhizas, a root cap region (about 0.4 mm in length corresponding to our zone I, Fig. 3), a pre-Hartig-net region (about 0.3 mm long, zone II), a Hartig-net-region which can make up to 25 mm in length dependent on species (our zones III and IV), and a late-Hartig-net region (> 100 mm from the root-cap). The most interesting part of our data is the compartmentation of sucrose along these different zones. Sucrose showed in general a decrease from carrier roots towards fine roots. This is in accordance with its transport from source to sink. In FR samples the sink strength appeared rather homogeneous from base to tip. In contrast, mycorrhized fine roots exhibited a pronounced decrease of sucrose levels in areas with possibly most intense interaction between host tissue and fungus (zones II to IV). Here, obviously sucrose is delivered to the fungus. This coincides nicely with increased ATP/ADP ratios reported recently [8], indicating an increased energy demand (transport, differentiation) in this region.

The intermediate state of sucrose compartmentation in n-myc samples from inoculated dishes could indicate biochemical interaction of both partners at an early stage were yet no visible fungal mantle can be located.

With regards to the sink concept of assimilate flow the increase of sucrose levels toward the very tip is surprising. This could mean that its sink activity (meristematic tissue) is higher compared to the zone of symbiotic interaction. As meristematic tissues lack vacuoles which could be used for sucrose storage, apparent sucrose accumulation could simply be due to the reference (DW; tip cells

are thin-walled). In this case, however, the other sugars should behave similar which is not the case. Alternatively, sucrose accumulation could be the result of specific biochemical regulation in the tip zone. In ongoing work we will focus on this regulatory aspect (compare [9]).

Acknowledgements

The authors are indebted to H. Thelen for help in preparing and harvesting the different cultures, and to the Deutsche Forschungsgemeinschaft for financial support.

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