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Ann. Sci. For. (1989) 46 suppl., 721s-723s
Forest Tree Physiology, E. Dreyer *et al.*, eds.

Changes in pathways for carbon and nitrogen assimilation in spruce roots under mycorrhization

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Introduction

The absorption and assimilation of nitrogen by tree roots are modified by the establishment of an ectomycorrhizal association (France and Reid, 1983). Assimilation of inorganic nitrogen occurs in the sheath of the fungus and amino acids are furnished to the host plant roots. A part of photosynthates is diverted to the fungus to be stored or respired and metabolized to provide carbon for amino acid biosynthesis. Some enzyme markers associated with the pathways of nitrogen and carbon metabolism were examined in spruce ectomycorrhizae and in each partner (uninfected root and fungus) to detect the changes occurring during symbiosis.

Materials and Methods

The fungus (*Hebeloma* sp.) was grown in Pachlewski's medium. Spruce roots (*Picea abies* L. Karsten) and mycorrhizae, infected with *Hebeloma* sp., were collected from 4 yr old trees in a tree nursery (Merten, France). Washed mitochondria were isolated following the method of Gerard and Dizengremel (1988). Respiration rates of tissues and mitochondria were measured with a Clark type oxygen electrode. KCN and SHAM (salicylhydroxamic acid) were used to inhibit the electron flow through, respectively, the cytochrome and the alternative cyanide-resistant pathways. Enzymes were extracted in a medium containing protective agents. Activities were assayed spectrophotometrically at 340 nm.

Results

The respiration of spruce roots was severely restricted by KCN and a further addition of SHAM increased the inhibition (Fig. 1A). SHAM used alone highly inhibited oxygen consumption (data not shown). By contrast, the respiration of ectomycorrhizae, although of similar magnitude to that of uninfected roots, was found to be

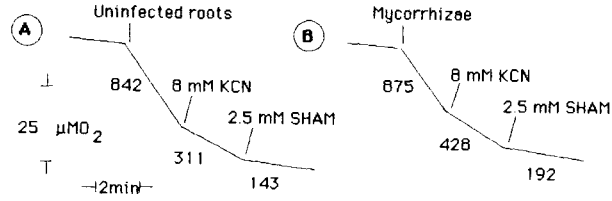

Fig. 1. Effects of KCN and SHAM on the oxygen uptake of uninfected roots (**A**) and mycorrhizae (**B**). Values along the tracings are in nmol $O_2 \cdot min^{-1} \cdot g^{-1}$ DW.

rather cyanide-resistant (Fig. 1B). SHAM was able to severely inhibit oxygen uptake (Fig. 1B), whereas an increased respiratory rate occurred when SHAM was added before KCN (data not shown). A similar action of inhibitors was observed during respiration of fungal tissues, although higher rates of respiration were obtained (data not shown). Mitochondria isolated from uninfected roots were highly cyanide-sensitive, whereas cyanide resistance was present in mycorrhizal mitochondria (data not shown), confirming the probable operation of the alternative pathway in mycorrhizal tissues.

The measurements, carried out on some enzyme markers of the two cytosolic carbohydrate degradation pathways (glycolysis and pentose phosphate pathway) and the mitochondrial Krebs cycle, also showed profound changes (Table I). The capacity of glucose-6-phosphate dehydrogenase was increased in mycorrhizae, whereas the opposite was true for the capacities of the glycolytic enzymes. Moreover, fumarase capacity was lower in mycorrhizae than in uninfected roots (Table I). In the fungus, the pentose phosphate pathway appeared to be predominant, since the capacity of G6PDH was higher than the capacities of enzymes from the glycolysis–Krebs cycle route (Table I).

As for enzymes involved in nitrogen assimilation, the rather high NADP-dependent GDH activity found both in the fungus and the mycorrhizal roots did not appear to be present in uninfected roots (Table I). Short-term labeling experiments also showed that spruce mycorrhizae were able to assimilate ammonium through the GS (glutamine synthetase) pathway (data

Table I. Capacities of enzymes of carbon and nitrogen metabolism in roots, ectomycorrhizae and the associated fungus.

Tissue	*Glycolysis*			*Krebs cycle fumarase*	*PPP G6PDH*	*Nitrogen assimilation*		
	PFK	*Ald*	*Gal3PDH*			*NADP-GDH*	*AAT*	*GPT*
Uninfected roots	0.34	0.24	0.37	0.19	0.25	ND	0.92	0.14
Ectomycorrhizae	0.26	0.13	0.31	0.13	0.34	0.69	0.91	0.45
Hebeloma sp.	0.15	0.04	0.32	0.11	0.68	1.18	1.11	0.62

The capacities are expressed in $\mu mol \cdot min^{-1} \cdot mg^{-1}$ protein. PPP: pentose phosphate pathway; PFK: phosphofructokinase; Ald: aldolase; Gal3PDH: glyceraldehyde-3-phosphate dehydrogenase; G6PDH: glucose-6-phosphate dehydrogenase; NADP-GDH: NADP-dependent glutamate dehydrogenase; AAT: aspartate aminotransferase; GPT: glutamate pyruvate transaminase.

not shown). However, aminotransferases (AAT and GPT) showed high capacity levels in ectomycorrhizae (Table I).

Discussion

The metabolism of carbohydrate breakdown appeared to be deeply perturbed during mycorrhization. Mitochondrial respiration became cyanide-resistant, whereas only the cytochrome pathway existed in uninfected roots. Moreover, the changes in the enzymatic capacities of glycolysis, the Krebs cycle and the pentose phosphate pathway indicated that mycorrhization caused a rearrangement of the carbohydrate metabolic sequences. If an increased respiration rate due to mycorrhization were to be confirmed, the functioning of the alternative pathway could allow both sufficient ATP synthesis and carbon skeletons needed for the production of compounds by NADPH-using pathways. Nitrogen metabolism appeared to be classical in both mycorrhizal fungus, where GDH predominates (Marzluf, 1981), and roots, where GS is the major route of ammonium assimilation (Oaks and Hirel, 1985). Our findings also show that both pathways might be operative in mycorrhizal tissues. The further transfer to an amino group or to other carbon skeletons might occur through aminotransferases, since both AAT and GPT were detected in the mycorrhizal tissues.

Acknowledgments

C. Namysl and P. Dizengremel gratefully acknowledge the EEC for financial support (DEFORPA Programme).

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