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Mycorrhiza

Short note

Ectomycorrhiza and nitrogen effects on root IAA: results contrary to current theory

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Summary. Indole-3-acetic acid (IAA) concentrations of mycorrhizal and non-mycorrhizal Scots pine roots under moderate and high-nitrogen nutrition were assayed using mass spectrometry with an internal standard. Contrary to current theory, IAA was lower in mycorrhizal roots than in the controls, and higher during high-nitrogen nutrition.

Key words: IAA assay - Hormone theory

Introduction

It has been well established that the development of ectomycorrhiza is influenced by the mineral nutrition of the host but the underlying mechanisms have never been satisfactorily determined. Slankis (1974) proposed that IAA (indole-3-acetic acid) production by the fungus is the main regulatory factor, acting on the carbohydrate supply from the host to the fungus, but responding itself to the nitrogen availability in the soil. The theory clashed with an earlier one of Björkman (1942), which assumed a direct interaction between nitrogen uptake and carbohydrate availability. Despite lack of proof, Slankis' theory gained much attention because of circumstantial evidence and its general plausibility (review by Nylund 1988), however not without reservations (Harley and Smith 1983). Measuring IAA by gas chromatography-selected ion monitoring-mass spectrometry (GC-SIM-MS), we have obtained results suggesting that the relationships between nitrogen availability, mycorrhiza development and IAA concentration in the roots are not in line with that predicted by Slankis' theory and previous observations (Mitchell et al. 1986): mycorrhiza formation caused a decrease in the IAA concentration of almost 50%, while nitrogen addition tended towards an increase.

The issue is of both theoretical and practical interest: other symbioses between microorganisms and plants, such as *Rhizobium* with legumes and *Frankia* with alder and other woody plants, also show a reduction in development or function with high nitrogen availability. Ectomycorrhizal tree seedlings are raised in nurseries and subject to forest fertilization, conditions which influence their mycorrhiza. Without the symbioses, the trees do not thrive; in fact damage to mycorrhiza is thought to be a major contributing cause (as well as an effect) of *Waldsterben*, forest decline in Europe (Jansen and Dighton 1990).

The research community generally accepted the fundamental importance of a sufficient supply of carbohydrate from the host to the fungus for ectomycorrhiza development, as stated by Björkman (1942). Yet, the morphogenic effects of IAA on pine roots observed in the early work of Slankis, and the effect of IAA in creating carbon sinks caused Meyer (1968), on re-examining Björkman's results, to propose a regulatory role for IAA; at about the same time Slankis (1974 and references cited therein) was thinking along similar lines. However, to date only one report has been published in which reliable physiochemical methods were used to measure IAA in mycorrhizal roots. In this study, Mitchell et al. (1986) found elevated IAA concentrations in mycorrhizal roots, unfortunately only with a single measurement. Slankis based his assumptions of elevated nitrogen levels leading to reduced IAA concentrations, and hence to reduced mycorrhiza development, on a single study of pure-culture fungi (Subba-Rao and Slankis 1959), using the highly unreliable techniques then available. In 1980, Sherwood and Klarman, using colorimetry of doubtful methodological validity, reported that IAA levels in whole seedlings were higher in the presence oif *Amanita rubescens* mycorrhiza. Three years later, Stenström (in Ek et al. 1983) using GC-MS assay, showed that tryptophan-containing culture medium filtrates of several fungi known to be vigorous mycorrhiza formers contained considerable amounts of IAA while other, less tenacious symbionts contained an order of magnitude less. Whilst absence of IAA in culture filtrates does not necessarily prove low IAA production, since other reactions could have

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led to its degradation, high concentrations certainly indicate that production is taking place, although the rate may not be proportional to the filtrate concentration.

Materials and methods

We measured IAA in roots of *Pinus sylvestris* L. seedlings, some 4 months old, either free of mycorrhiza or inoculated with *Laccaria bicolor* (Scop ex Fries) Bk & Br, strain 238, which has high concentrations of IAA when cultivated in the presence of tryptophan (Ek et al. 1983), and is a vigorous mycorrhiza former (Nylund and Wallander 1989). The seedlings were grown in a climate chamber using a semi-hydroponic system (brick pellets flooded with liquid medium for 1 min every hour) at 350 μmol $m^{-2} s^{-1}$ continuous light. The "normal" nitrogen treatment was optimal for mycorrhiza development and consisted of a balanced nutrient supply allowing a 4% daily relative growth rate and resulting in about 10 ppm nitrogen in the medium. For the excess nitrogen treatment, 200 ppm ammonium was added to the basic medium and the concentration was kept constant for 8 weeks (details in Nylund and Wallander 1989). The whole root systems of three seedlings were pooled into one sample, with three samples per treatment. In order to examine within-root differences, we also prepared a few samples of short roots only; because of the minimal quantity available, no replications could be made.

Quantitative analysis of IAA was performed by GC-SIM-MS, using [$^{13}C_6$]IAA as an internal standard according to Sundberg (1990). The M+/base peak ratios were used to check peak homogeneity, while the $^{13}C/^{12}C$ base peak ratio was used to quantify the amount of endogenous IAA by reference to a standard curve. The whole-root data were analysed statistically using two-way ANOVA.

Results and discussion

The results, shown in Table 1, were surprising in the light of previous findings and Slankis' theory. Contrary to Slankis' and Meyer's predictions, mycorrhiza certainly did not enhance the IAA concentration of roots, but lowered it by almost 50%. The excess nitrogen treatment had the effect of increasing IAA (albeit the statistical analysis showed the increase to be significant only within the mycorrhiza subset), whereas Slankis' (speculative) prediction was a substantial reduction; mycorrhiza biomass was reduced in line with previous results (cf. data in Wallander and Nylund 1991). There was no significant interaction between the treatments. The IAA concentration of the short roots was only half of that of the whole root system. Mycorrhiza again reduced the concentration by about 50% (data not shown).

Regarding the mycorrhiza effect, there is no doubt about the validity of the result. We do not wish to speculate about the reasons for the contrasting results of previous workers. With regard to the predicted nitrogen effect, there has never been any evidence in favour of it, apart from a brief report by Subba-Rao and Slankis (1959). In spite of the lack of statistical significance of the evidence for an enhancement, our results certainly do not indicate the predicted depression. Thus, in our opinion, that part of the theory should definitely be disregarded.

This is not to imply that IAA has no role at all in mycorrhiza formation. The notable stimulation of infection and development of *Suillus* on Scots pine in the vicinity of indole-3-butyric acid-containing agar plugs in non-sterile culture observed by Unestam and Stenström (1989) is just one item of evidence. Work is now in progress using hyperauxinic *Hebeloma* mutants to examine the effects of enhanced IAA supply on root IAA content, carbohydrate flow and mycorrhizal development. The simple correlations and effects proposed by Slankis, however, appear most likely to be illusory.

Table 1. IAA concentration of mycorrhizal and non-mycorrhizal roots under low and high nitrogen nutrition. LSD=45.4. *P*: Mycorrhiza: 0.0003; nitrogen: 0.1177; interaction: 0.4173

Mycorrhiza	IAA concentration (ng/g root dry wt.)		
	Nitrogen 10 ppm	Nitrogen 200 ppm	Treatment mean
Yes	135 ± 5[a]	153 ± 4	144 ± 5
No	241 ± 29	292 ± 25	267 ± 21
Treatment mean	188 ± 27	223 ± 33	

[a] Results are given as mean of 3 samples ± standard error of the mean

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