THE EFFECT OF CYTOKININS AND AUXINS ON THE GROWTH OF MYCORRHIZAL FUNGUS SUILLUS VARIEGATUS* 

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Introduction

It is known that pine tree root exudate promotes the growth of the mycelium of mycorrhizal fungi. Melin (1959) considered that there were probably several components that influenced growth. In our earlier work (Gogala 1971), naturally occurring cytokinins, found in the exudate of pine tree seedlings (Pinus sylvestris), showed an influence on the growth of Boletus edulis. Following the fact that kinetin displays a similar effect (Gogala 1973), we tried to clarify the influence of the individual cytokinins extracted from the exudate on mycorrhizal fungi, as well as the effect of zeatin and zeatinriboside. Auxins, which are among the other hormones present in the exudate, were of special interest for us because of their potential common effect on mycelium.

Materials and methods

Pine tree seeds (Pinus sylvestris) were obtained from “Semeadike” Menges (1973). The sterile tissue of Suillus variegatus was taken from fruit-bodies in 1974 and grown on potato agar-agar culture media (Difco). The test on cytokinins was made by using the seeds of radish (Raphanus sativus — 1973).

Exudate preparation for bioassay:

Three week old pine tree seedlings were transferred from vermiculite to Petri dishes in such a way that only their roots were in contact with distilled water. After thermostating them for 6 days at 6 °C, cytokinins were extracted by paper chromatography. As chromatographic solvent a mixture of isopropanol-ammonia-water (10:1:1) was used. The ex-* Reported at the Second Meeting of the Yugoslav Society for Plant Physiology, Stubicke Toplice (Croatia, Yugoslavia), 20th—23rd May 1975.
extracted cytokinins were tested with cotyledons of radish (Leatham 1971).

For cytokinin elution from paper chromatograms, 35% ethanol was used, which was then removed by evaporation. The extracted cytokinins were added to the substrate in two different concentrations — expressed in the equivalent of 2.5 and 5.0 units of “M” factor. The substrate with added cytokinins was inoculated with the tissue of S. variegatus and the weight of fungal mycelium determined the 12th day after application. In parallel experiments, kinetin (KIN), zeatin (Z) and zeatinriboside (ZR), prepared in concentrations from $10^{-9}$ to $3 \times 10^{-7}$ g/ml, were added to the substrate with the inoculated mycelia of S. variegatus and further treated in the same way as cytokinins.

Results

Three of the naturally occurring cytokinins in the exudate of pine tree seedlings were found before 1970, using various bioassays and the application of paper and thin-layer chromatography (Googala 1970, 1971). As solvent water saturated secondary butanol was mainly used. Repeated experiments and the use of paper chromatography with isopropanol-ammonia-water solvent mixture, showed another substance to be present in the exudate (Fig. 1). $R_f$ values for individual substances are following:

<table>
<thead>
<tr>
<th>Substance</th>
<th>$R_f$</th>
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<tbody>
<tr>
<td>Substance a</td>
<td>0.1 — 0.2</td>
</tr>
<tr>
<td>Substance b</td>
<td>0.2 — 0.5</td>
</tr>
<tr>
<td>Substance c</td>
<td>0.6 — 0.8</td>
</tr>
<tr>
<td>Substance d</td>
<td>0.8 — 1.0</td>
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$R_f$ value for cytokinin c is the same as for zeatin and zeatinriboside, while $R_f$ for cytokinin b corresponds to that of cytokinin ribotides.

From the data presented it is evident that naturally occurring cytokinins from the exudate of pine tree seedlings promote the growth of mycelium of S. variegatus (Fig. 2). Following the influence on the growth of mycelia of cytokinins a and c, which were added to the substrate in two different concentrations (2.5 and 5.0 units of “M” factor), it is evident that cytokinins a and c are more efficient at lower concentration (2.5). In the case of cytokinins b and d, higher concentrations (5.0) of “M” factor correspondingly higher effect which can be explained by content of cytokinins b and d in the exudate ($2.5 \times 10^{-8}$ g/ml) which is lower in comparison with the one of cytokinins a and c ($6 \times 10^{-8}$ g/ml).

The most effective growth of S. variegatus was observed after the addition of cytokinin d to the substrate.

The substrate was further inoculated with fungal mycelium with added zeatin and zeatinriboside in the concentration range from $10^{-9}$ to $3 \times 10^{-7}$ g/ml. Zeatin was more efficient than zeatinriboside. After incubation, 157% fresh weight of culture in comparison with the control was observed when adding optimal concentrations ($10^{-8}$ g/ml) of zeatin to the substrate, while in the case of zeatinriboside the growth was significantly increased at $3 \times 10^{-8}$ and $10^{-7}$ g/ml of zeatinriboside (Fig. 3). With kinetin the stimulative effect was still obtained at $10^{-7}$ g/ml, while the addition of IAcA ($\beta$-indolylacetic acid) had no effect on the growth, and even acted as an inhibitor at higher concentrations. A synergistic effect between KIN and IAcA was obtained by combining different concentrations of KIN with IAcA at the concentration of $10^{-8}$ g/ml (Fig. 4).
Fig. 1. Pine root exudate, bioassay on cytokinins with cotyledons of radish *Raphanus sativus*. Left butanol fraction, right water fraction and the control with KIN, an equivalent 10 units of "M" factor, Whatman 1, solvent isopropanol-ammonia-water 10:1:1. Abscissa: the position on the chromatogram; ordinate: the weight of cotyledons in per cent of the control.

Fig. 2. The growth of mycelium on natural cytokinins of root exudate (substance a, b, c, d). Each cytokinin was added in two concentrations: 5 and 2.5 units of "M" factor.
Fig. 3. The growth of *Suillus variegatus* on culture media with zeatin (Z — solid line) and zeatinriboside (ZR — dashed line) in various concentrations, fresh weight.

**Discussion**

The role of growth substances has recently become very important in the understanding of regulation of mycorrhiza in certain fungal species. Among the growth regulators cytokinins are probably most important because of their influence even on the growth of higher plants. As investigated by Crafts and Miller (1974), the content of naturally occurring cytokinins present in some of the mycorrhizal fungi was very low, while some other species were not able to produce them at all. Therefore it is very interesting that cytokinins present in large amounts in the pine tree root exudate, promote the growth of mycelium of mycorrhizal fungi. In the case of *S. variegatus*, cytokinins promote water uptake from the substrate to the mycelium. Similar effects on the growth of mycelium as for KIN was observed with zeatin and to a lesser extent with zeatinriboside as well, while common treatment of KIN and IAcA at low concentrations (to $10^{-8}$ g/ml) has shown a synergistic effect.

From the above results it can be concluded that the higher plant influences the growth of the mycorrhizal partner via cytokinin action, and thus indirectly, the uptake of minerals and water from the substrate to the mycelia.

**Conclusions**

Natural occurring cytokinins from pine tree root exudate, separated by paper chromatography and added to the substrate with the inoculated tissue of *S. variegatus*, promote the growth of fungal mycelia.
Fig. 4. The growth of *Suillus variegatus* on culture media with various concentrations of kinetin (KIN — dashed line) and KIN combining with $10^{-8}$ g IAcA/ml (solid line).

Judging from the results, cytokinin d seems to be the most efficient one. Zeatin promotes the growth of mycelia in a similar way to KIN, the optimal concentration being $10^{-8}$ g/ml, while zeatin riboside is about two times less effective.

A synergistic effect on the growth of mycelium was found by combining KIN and IAcA. The latter acted as an inhibitor at higher concentrations.

Growth substances from higher plants exudate influence the growth of the mycorrhizal fungus *S. variegatus*.

**References**

*Brown, N. A. C. and J. Van Staden, 1974: The effect of stratification on the endogenous cytokinin levels of seed of *Protea compacta* and *Leucadendron daphnoides*, Physiol. Plant. 28, 388—392.*


**SADRŽAJ**

**DJELOVANJE CITOKININA I AUKSINA NA RAST MIKORIZNE GLJIVE SUILLUS VERIEGATUS**

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Prirodni citokinini iz eksudata bora, koje smo rastavili papirnom kromatografijom i dodali podlozi na koju smo nasadili gljivu Suillus variegatus, utjecali su stimulativno na rast micelija. Kao što kinetin (KIN) pospešuje rast micelija i zeatin; optimalna koncentracija je $10^{-8}$ g/ml. Zeatinribozid ima gotovo za polovicu slabije djelovanje na rast kulture gljive S. variegatus. KIN sinergistično utječe na rast micelija u kombinaciji s β-indolocetnom kiselinom (IAcA, konc $10^{-8}$ g/ml). Sama IAcA nema utjecaja na rast te u većim koncentracijama djeluje inhibitorno (Gogala 1971).

Viša biljka utječe na rast mikorizne gljive S. variegatus svojim tvarima rastenja koje ona izlučuje u svoj okoliš.

**VSEBINA**

**UCINEK CITOKININOV IN AVKSINOV NA RAST MIKORIZNE GLJIVE SUILLUS VERIEGATUS**

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Naravni citokinini v eksudatu bora, ločeni s papirno kromatografijom in dodani v podlago, na katero smo cepili gljivo Suillus variegatus, vplivajo na rast micelija stimulativno. Podobno kot kinetin (KIN) pospešuje rast micelija tudi zeatin; optimalna koncentracija je $10^{-8}$ g/ml. Zeatinribozid ima skoraj za polovico manjši efekt na rast kulture S. variegatus. KIN vpliva na rast micelija v kombinaciji z β-indolocetno kisilino (IAcA, konc. $10^{-8}$ g/ml) sinergistično. Sama IAcA nima vpliva na rast ter je v višjih koncentracijah inhibitorna (Gogala 1971).

Viša rastlina v svojimi rastnimi substancami, ki jih eksudira v okolje, vpliva na rast mikorizne gljive S. variegatus.

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