Acta Bot. Croat. 35 (1976) 129—134

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

NADA GOGALA and FRANCI POHLEVEN

(Biological Department of the Biotechnical Faculty and Biological Institute of the University Ljubljana)

Received January 14, 1976

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 occuring 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 "Semesadike" 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 Jugoslav Society for Plant Physiology, Stubičke Toplice (Croatia, Jugoslavia), 20th—23rd May 1975.

tracted cytokinins were tested with cotyledons of radish (L e t h a m 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 innoculated 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 3×10^{-7} g/ml, were added to the substrate with the innoculated mycelia of *S. variegatus* and further treated in the same way as cytokinins.

Results

Three of the naturally occuring cytokinins in the exudate of pine tree seedlings were found before 1970, using various bioassays and the application of paper and thin-layer chromatography (G o g a l a 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:

Substance a	R_f	0.1 — 0.2
Substance b	R_f	0.2 — 0.5
Substance c	R_f	0.6 — 0.8
Substance d	R_f	0.8 — 1.0

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 occuring 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 wich can be explained by content of cytokinins b and d in the exudate (2.5×10^{-8} g/ml) which is lower in comparison with the one of citokinins a and c (6×10^{-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 innoculated with fungal mycelium with added zeatin and zeatinriboside in the concentration range from 10^{-9} to 3×10^{-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 3×10^{-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 (β-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).

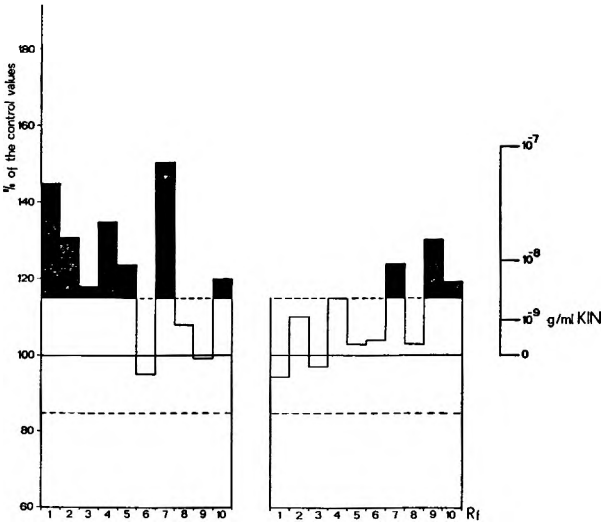

Fig. 1. Pine root exudate, bioassay on cytokinins with cotyledons of radish *Raphanus sativus*. Left buthanol fraction, right water fraction and the control with KIN, an equivalent 10 units of "M" factor, Whatman 1, solvent isopropanol-ammonia-water 10 : 1 : 1. Abscissa: the position on the chromatogram; ordinate: the weight of cotyledons in per cent of the control.

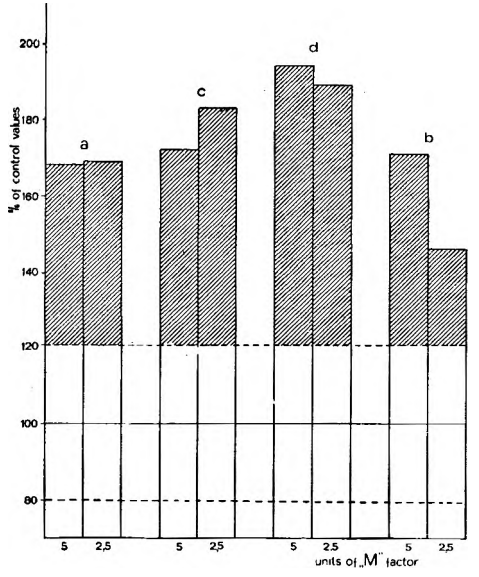

Fig. 2. The growth of mycelium on natural cytokinins of root exudate (substance a, b, c, d). Each cytokinin was added in two concentrations: 5 and 2.5 units of "M" factor.

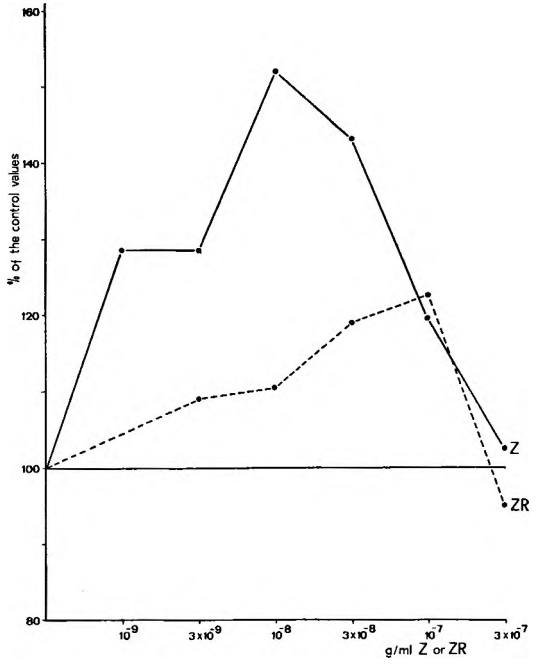

Fig. 3. The growth of *Suillus variegatus* on culture media with zeatin (Z — solid line) and zeatinriboside (ZR — dashed line) in various concentrations, fresh weight.

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 occuring 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 occuring cytokinins from pine tree root exudate, separated by paper chromatography and added to the substrate with the innoculated tissue of *S. variegatus,* promote the growth of fungal mycelia.

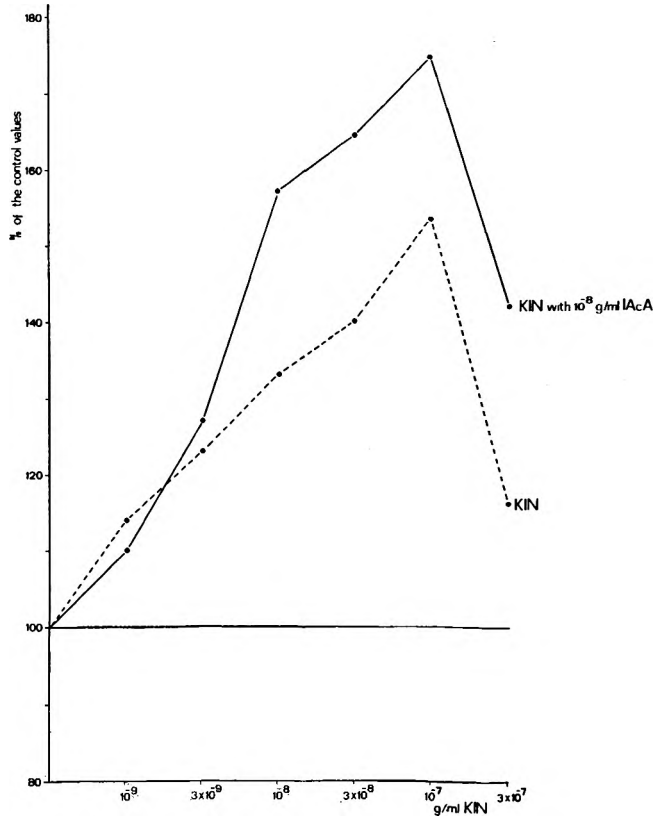

Fig. 4. The growth of *Suillus variegatus* on culture media with various concentrations of kinetin (KIN — dashed line) and KIN combining with 10^{-8} g IAcA/ml (solid line).

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.

Crafts, C. B. and *C. O. Miller*, 1974: Detection and identification of cytokinins produced by mycorrhizal fungi, Plant Physiol. *54*, 586—588.

Gogala, N., 1970: Einfluss der natürlichen Cytokinine von *Pinus sylvestris* L. und anderer Wuchsstoffe auf das Myzelwachstum von *Boletus edulis* var. *pinicolus* Vitt., Österr. Bot. Z. *118*, 321—333.

Gogala, N., 1971: Vloga rastnih substanc pri mikorizi med glivo *Boletus pinicola* Vitt. in borom *Pinus sylvestris* L., Razprave *14*, 123—202.

Letham, D. S., 1971: Regulators of cell division in plant tissues XII. A cytokinin bioassay using excised radish cotyledons, Physiol. Plant. 25, 391—396.

Melin, E., 1959: Mycorrhiza. *Ruhland, W.*, Handbuch der Pflanzenphysiologie, Bd. 11, Springer Verlag, Berlin, Göttingen, Heidelberg.

SADRŽAJ

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

Nada Gogala i *Franci Pohleven*
(Biološki oddelek Biotehniške fakultete i Biološki inštitut Univerze v Ljubljani)

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) pospješuje rast micelija i zeatin; optimalna koncentracija je 10^{-6} 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-indoloctenom kiselinom (IAcA, konc 10^{-8} g/ml). Sama IAcA nema utjecaja na rast te u većim koncentracijama djeluje inhibitorno (G o g a l a 1971).

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

VSEBINA

UČINEK CITOKININOV IN AVKSINOV NA RAST MIKORIZNE GLJIVE *SUILLUS VARIEGATUS*

Nada Gogala in *Franci Pohleven*
(Biološki oddelek Biotehniške fakultete in Biološki institut Univerze v Ljubljani)

Naravni citokinini v eksudatu bora, ločeni s papirno kromatografijo in dodani v podlago, na katero smo cepili glivo *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 kislino (IAcA, konc. 10^{-8} g/ml) sinergistično. Sama IAcA nima vpliva na rast ter je v višjih koncentracijah inhibitorna (G o g a l a 1971).

Višja rastlina v svojimi rastnimi substancami, ki jih eksudira v okolje, vpliva na rast mikorizne glive *S. variegatus*.

Dr Nada Gogala i
Franci Pohleven
Biološki inštitut Univerze
v Ljubljani, Aškerčeva 12
61000 Ljubljana (Jugoslavija)