Response of pine hypocotyl sections to growth regulators and related substances

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ABSTRACT

Growth response of *Pinus silvestris* hypocotyl sections to some synthetic growth regulators and related substances was studied. Elongation of hypocotyl sections was stimulated by naphthaleneacetic acid, indole-3-acetic acid, indole-3-propionic acid, indole-3-butyric acid, 2,4-dichlorophenoxyacetic acid, indoleacetic amide, indoleacetic nitrile and coumarin. Indole-3-acetic acid and naphthaleneacetic acid extended period of growth up to 16 and 24 hours, respectively. Growth was inhibited by kinetin, trans-cinnamic acid and 2,3,5-tri-iodobenzoic acid. No effect of gibberellic acid, tryptophan and biotin was observed.

INTRODUCTION

Even carefully purified by standard methods growth hormone extracts from coniferous plants contain admixtures of nonspecific compounds, particularly resins, which may interfere with the growth reaction in standard biological test, causing difficulties in the detection and identification of growth substances (Wodzicki, 1964, 1968). Therefore for investigation of growth regulators of these plants, highly sensitive and selective tests are necessary which at the same time would be unsensitive to resins. The tests based on the growth reaction of tissues or organs of the plant species from which the extract was isolated most probably fulfill this requirement as it was demonstrated by Kopcewicz (1968), and Dunberg (1974). These authors studied stem elongation of pine and spruce seedlings following still earlier tests with pine hypocotyl sections by Hashizume, 1965; Giertych and Forward, 1966, and later by Wodzicki et al. 1971. Extensive studies concerning natural growth regulators of *Pinus silvestris* L. so much needed and well advanced in Poland (Michnie-

wicz, 1967, 1970; Kopcewicz, 1968; Michalski 1967; Rogozińska, 1967) stimulated the work on biological test using tissue of this species (Witkowska-Żuk and Wodzicki, 1970). It has been already used for investigation of natural auxins extracted from the cambial zone of pine stem (Wodzicki, Wodzicki, 1973), however, its selectivity in respect to other substances than IAA and GA is practically not known. In the presented study the growth reaction of hypocotyl sections of *Pinus silvestris* L. to a number of known or potential growth regulators or to substances which may be cofactors in the process of growth was investigated. The influence of these substances on the growth and development of gymnosperms, and particularly coniferous trees is as yet little known.

MATERIAL AND METHODS

The growth reaction of Scots' pine hypocotyl sections to the following substances was tested:

- indole-3-acetic acid (IAA)
- naphthaleneacetic acid (NAA)
- indole-3-propionic acid (IPA)
- indole-3-butyric acid (IBA)
- 2,4-dichlorophenoxyacetic acid (2,4-D)
- indoleacetic amide (AIA)
- indoleacetic nitrile (IAN)
- L-tryptophan (L-TTP)
- gibberellic acid (GA)
- 6-furfurylaminopurine (FAP)
- 6-benzyladenine (BAP)
- biotin (B)
- coumarin (C)
- trans-cinnamic acid (t-CA)
- 2,3,5-tri-iodobenzoic acid (TIBA)

The experiments were performed according to the method of hypocotyl section growth test with *Pinus silvestris* L. (Witkowska-Żuk and Wodzicki, 1970).

Scots' pine seeds of high germinating power were soaked in tap water for 20 hours in darkness at 26° C and then, under the same conditions, seeded in boxes with moist river sand. After 4 days red light was switched on. Seedlings with hypocotyl of 25—30 mm length were taken after 8—9 days and 4.5 mm sections were cut from them at a distance of 2 mm below the cotyledone node. The sections were washed 4 times in the course of 1 hours in redistilled water. Ten sections were placed each in a 10-ml glass vial containing 2 ml of the solution to be tested and incu-

bated on a rotator in the dark for 20 hours. In investigations on the influence of auxins on growth dynamics, the sections were measured at 4 hours intervals, 7 times in the course of 24 hours. The length of the sections after incubation was measured under 4 fold magnification under a photographic enlarger. Owing to the high tolerance of the test to the influence of the environment, all the operations with the exception of seedling culture were carried out in room conditions under daylight. The tested substances were made up in 2 per cent sucrose solution and 0.5 M phosphate-citrate buffer, pH 5.0. Glass-redistilled water was used for the solutions. Growth substances were tested in various concentrations depending on their biological activity and anticipated concentration at which their maximum influence on growth would occur. Each substance was tested several times with three replications of each concentration. The results are given in terms of mean lengths of the sections in millimeters or as per cent of the growth of the control sections.

RESULTS

It was found that indole-3-acetic acid, naphtalene-acetic acid, indole-3-propionic acid, and indole-3-butyric acid at concentrations as low as $0.001 \text{ mg} \cdot \text{l}^{-1}$ significantly stimulated growth as compared with the control (Fig. 1). 2,4-dichlorophenoxyacetic acid and indoleacetic amide stimulated hypocotyl growth significantly only in concentrations as high as $0.01 \text{ mg} \cdot \text{l}^{-1}$ and indoleacetic nitrile at $1.0 \text{ mg} \cdot \text{l}^{-1}$. Maximal stimulation by various substances was noted at different concentrations: by NAA at $0.1 \text{ mg} \cdot \text{l}^{-1}$, by IAA, IBA and 2,4-D at $1.0 \text{ mg} \cdot \text{l}^{-1}$, by IPA, AIA and IAN at $10.0 \text{ mg} \cdot \text{l}^{-1}$.

None of the remaining substances evoked progressive stimulation and only minimal significant stimulation was observed in the case of coumarin.

In the presence of IAA or NAA the hypocotyl sections grew far a longer time than in the water control in which growth ended already after 8 hours of incubation (Fig. 2). The sections incubated with IAA grew for 16 hours at all auxin concentrations and those incubated with NAA grew for 16—24 hours (depending upon NAA concentration). A protraction of the period of growth was observed with increasing concentrations of NAA in interval 0.01 to $1.0 \text{ mg} \cdot \text{l}^{-1}$. On the other hand the rate of growth of the sections depended on IAA concentration. Growth was quickest at 1.0 and slowest at $0.01 \text{ mg} \cdot \text{l}^{-1}$ but no major differences were found in the duration of growth.

An increase in furfurylaminopurine concentration in the test solution from 0.001 to $1.0 \text{ mg} \cdot \text{l}^{-1}$ caused a progressive significant decrease of the length of the sections (Fig. 1).

Fig. 1. *Pinus silvestris* L. hypocotyl section growth test for particular growth regulators. Horizontal lines indicate water control level. Vertical lines denote standard error. For abbreviations see text

This reaction did not appear in the presence of 6-benzyladenine whereas in interaction with IAA both BAP and FAP distinctly inhibited section growth as compared with the control (Fig. 3A).

Fig. 2. Growth dynamics of *Pinus silvestris* hypocotyl sections in the presence of indole-3-acetic and naphthalenacetic acids. Section length was measured at 4 hours intervals from the beginning of incubation. Mean initial length was 4.8 mm. Vertical lines denote standard error.

Gibberellic acid only in a $0.01 \text{ mg} \cdot \text{l}^{-1}$ concentration and in interaction with IAA evoked a slight stimulation. At other concentrations significant influence of GA hypocotyl growth was noted (Fig. 3A).

In the presence of both trans-cinnamic and 2,3,5-tri-iodobenzoic acid in concentrations of 0.01 to $10.0 \text{ mg} \cdot \text{l}^{-1}$ inhibition of section growth was not noted (Fig. 1). The inhibitory effect of t-CA and TIBA was revealed in interaction with auxin with its rising concentration (Fig. 3B). TIBA in a $100 \text{ mg} \cdot \text{l}^{-1}$ concentration produced a change in the colour of the sections and loss of turgor. In this case complete inhibition of growth also occurred.

No significant growth response of hypocotyl sections was observed in the presence of biotin or L-tryptophan (Fig. 1).

Fig. 3. Influence of cytokinins and gibberellic acid in interaction with IAA (A) and of growth inhibitors in interaction with IAA (B) on *Pinus silvestris* hypocotyl section growth. Length of section expressed as per cent of length of section incubated with IAA alone in concentrations of 0.01, 0.1 and 10 mg.l⁻¹, respectively. For abbreviations see text

DISCUSSION AND CONCLUSIONS

IAA stimulated elongation growth of pine hypocotyl sections similarly as it did in the investigations of Hashisume (1965) and Witkowska-Żuk and Wodzicki (1970). Under the influence of other tested auxins the hypocotyl sections also responded by enhanced growth, however, the sensitivity of the test to the particular auxins differed. The duration of the period of growth was the same in the case of IAA at all concentrations used. The differences in the final length of the sections in dependence on the concentration occurred on account of the increased growth increment per time unit with rising IAA concentrations. Thus, it may be said that, at various IAA concentrations, the period of section growth is constant, only its intensity varies. The principle of NAA action seems to be different. In this case it was found that the period of growth

is protracted in proportion to the rise of concentration, whereas growth intensity is similar and even identical (cf. 0.1 and 1.0 ppm concentrations) for all the concentrations tested. This fact should be taken into account when establishing the incubation time which in the case of testing the stimulation with NAA should be considerably prolonged. In contrast to the pine hypocotyl section growth test, Burström (1969) while investigating the growth dynamics of cells in intact wheat seedlings found that a rise in NAA concentration was associated with more intensive cell growth and simultaneous reduction of the time of growth.

The lack of any noticeable influence of GA on hypocotyl sections growth or the slight synergism in the interaction of GA with IAA indicate that the pine hypocotyl section growth test in the form in which it is applied is, but little sensitive to this kind of gibberellin as has been demonstrated earlier (Witkowska-Żuk and Wodzicki, 1970; Kopećewicz, 1968). It is known from the literature that GA does not affect hypocotyl growth of *Pinus nigra*, *P. thunbergii*, *P. densiflora* (Hashizume, 1968) and *Picea abies* (Dunberg, 1974). Neither has any significant influence of GA on callus growth (Rogozińska, 1970) and cambial activity (Zajączkowski, 1973), of Scots' pine been noted.

Other investigations indicate, however, that Scots' pine hypocotyl growth (Kopećewicz, 1968) and that of Norway spruce (Dunberg, 1974) are strongly stimulated by extracts of natural gibberellins identified in specific bioassays for determination of endogenous gibberellins of these species. None of the gibberellin-like substances detected so far in Scots' pine (Kopećewicz, 1968; Michalski, 1967; Michniewicz, 1967, 1970) has been identified as gibberellic acid. It is also probable that in the tissues of conifers gibberellin is synthesized in great abundance, therefore it is not a factor inhibiting growth. A rise of its level due to exogenous application will, therefore, not produce the growth effect. GA does not practically affect healthy intact spruce seedlings but it abolishes the unfavourable influence of growth retardants (Dunberg and Eliasson, 1972), which are believed to depress gibberellin synthesis, and stimulate the growth of dwarf varieties (Hansen, 1970) probably deficient in endogenous gibberellins.

Cytokinins may also be treated as factors enhancing cell division and elongation growth produced by other growth substances. Their interaction with auxins is in fact of synergistic character (Skoog and Miller, 1957; Olszewska, 1958; Rogozińska, 1970). The influence of kinetin and benzyladenine on the growth of pine hypocotyl sections, particularly in interaction with IAA is in marked contrast with the observation of the above mentioned authors, although in investigations on cambium sensitivity in isolated sections of *Pinus silvestris* L. shoots kinetin stimulates cambial activity in the spring period (Hejnowicz, Tomaszewski, 1969; Zajączkowski, 1973). The latter author demon-

strated, however, that such an effect cannot be elicited in late summer or autumn. The pine hypocotyl section growth test may vary in sensitivity to IAA in dependence on the season (Wodzicki and Wodzicki, 1973). It is not excluded that the change in sensitivity involves also cytokinins.

L-tryptophan is considered to be the precursor of indole-3-acetic acid. Its transformation to auxin in the oat coleoptile has been demonstrated (Skog, 1937) as well as in the cells of various organs of other plants (Gordon, 1954). The weak reaction of hypocotyl pine sections to tryptophan did not confirm the supposition that this compound could, under the conditions of this test, be actively incorporated into pine tissue and transformed to auxin. Potential plant growth inhibitors such as t-CA, TIBA and coumarin which strongly inhibit wheat coleoptile growth in tests for inhibitors (Wodzicki and Witkowska-Żuk, 1964) practically did not inhibit the growth of pine hypocotyls as compared with the water control, while coumarin had even a slight stimulating effect. In interaction with IAA the influence of coumarin on pine hypocotyl sections growth was negligible. The effect of TIBA and t-CA was manifested as inhibition of growth stimulated by higher auxin concentrations.

Summary

Pinus silvestris L. hypocotyl sections straight growth test used for auxin determination was further investigated in order to establish its selectivity towards some synthetic plant growth stimulators and inhibitors. The resistance of the pine hypocotyl to resinous substances which normally inhibit growth in wheat and oat coleoptile section tests gives wide possibilities of application of this test for determination of natural growth regulators in pine. Growth of hypocotyl sections was stimulated by naphthaleneacetic acid, indole-3-propionic acid, indole-3-butyric acid in concentrations of 0.001 to 10 ppm; indoleacetic amide, 2,4-dichlorophenoxyacetic acid in concentrations of 0.01 to 10 ppm; indoleacetic nitrile in concentrations of 1 to 10 ppm; coumarin in concentrations of 1 to 100 ppm. Growth was inhibited by kinetin in concentrations of 0.01 to 1 ppm, 2,3,5-tri-iodobenzoic acid and trans-cinnamic acid in a concentration of 100 ppm. Kinetin and 6-benzyladenine in concentrations of 0.001 to 1 ppm with IAA in concentrations of 0.01 and 0.1 ppm, 2,3,5-tri-iodobenzoic acid and transcinnamic acid in concentrations of 0.1 to 100 ppm with IAA in concentrations of 0.1 to 1 ppm stimulated less elongation of hypocotyl sections than auxin controls. Gibberellic acid in concentration 0.01 ppm with IAA in concentration 0.01 ppm acted synergistically to elongation of pine hypocotyl sections. The growth of control hypocotyl sections placed in the medium composed of 2% sucrose and 0.5 M phosphate-citrate buffer pH 5.0 continued for 8 hrs. Addition of IAA and NAA extended these period up 16 and 24 hours respectively.

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*Reakcja wzrostowa hypocotyłu sosny zwyczajnej na działanie substancji
o charakterze regulatorów wzrostu*

Streszczenie

Test prostoliniowego wzrostu odcinków hypocotyłu sosny (*Pinus silvestris* L.) do oznaczania auksyn (Witkowska-Żuk i Wodzicki 1970) poddano dalszym badaniom w celu określenia jego selektywności w stosunku do niektórych syntetycznych stymulatorów i inhibitorów wzrostu roślin. Odporność hypocotyłu sosny na substancje żywiczne, które normalnie hamują wzrost w testach koleoptile pszenicy i owsa (Wodzicki i in. 1971), stwarza duże możliwości zastosowania tego testu do oznaczania naturalnych regulatorów wzrostu sosny.

Wzrost wydłużeniowy odcinków hypocotyłu stymulowały:

- NAA, IBA, IPA w stężeniu od 0.001 do 10 ppm
- AIA, 2, 4-D w stężeniu od 0.01 do 10 ppm
- IAN w stężeniu od 1 do 10 ppm
- kumaryna w stężeniu od 1 do 100 ppm

Wzrost hamowały:

- kinetyna w stężeniu od 0.01 do 1 ppm
- TIBA i t-CA w stężeniu 100 ppm

Antagonistycznie działały:

- kinetyna i BAP w stężeniu od 0.001 do 1 ppm z IAA w stężeniu 0.01 i 0.1 ppm
- TIBA w stężeniu od 0.1 ppm do 100 ppm z IAA w stężeniu 0.1 i 1 ppm
- t-CA w stężeniu od 0.1 do 100 ppm z IAA w stężeniu 1 i 0.1 ppm

Działanie synergistyczne wykazywał GA w stężeniu 0.01 ppm z IAA w stężeniu 0.01 ppm. Odcinki hypocotyłu rosły do 8 godzin w kontroli wodnej, do 16 godzin przy obecności IAA i do 24 godzin przy obecności NAA.