Pinus silvestris L. hypocotyl section straight growth test for auxin *

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Several authors discussed recently the usefulness of the bioassay techniques basing on the tissues of the same species from which the bioassayed natural growth substances are extracted and studied (Wareing, Eagles, and Robinson 1964).

The auxins from various species of pine have been already studied by the specially developed pine hypocotyl section straight growth test (Hashizume 1965 a, b, Giertych and Forward 1966). The present experiments aimed at finding an analogous test for substances extracted from tissues of *Pinus silvestris* L. Extensive studies upon natural growth substances of this valuable timber species carried on by Michniewicz and his coworkers (Michniewicz and Kopcewicz 1966, 1968; Kopcewicz, Michniewicz, and Kriesel 1967; Michalski 1967) make this investigation well grounded.

The effects of following conditions upon the response of pine hypocotyl sections to IAA were tested:

- a) conditions of illumination and temperature in which the seedlings are grown before sectioning, and during sectioning
- b) distance of the section from cotyledonary node
- c) phase of seedling growth
- d) duration of incubation period
- e) pH of incubation solution.

Final experiments concerned the response of pine hypocotyl sections to natural auxin extracted from cortical tissues of adult trees of *Pinus silvestris*. Occasionally the effect of gibberellic acid was also tested.

METHOD

Seedlings of *Pinus silvestris* L. used for bioassay originated from seeds ** germinated in moist sand in darkness after 20 hrs of soaking in

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tap water at 26°C. When the seedlings were grown under additional red-light, the illumination was provided constantly starting with the 3-rd day of growth in darkness. Sectioning of seedlings was performed in laboratory day-light (unless otherwise indicated). The sections were 4.5 mm long. After cutting the sections were washed in distilled water for 1 hr. They were incubated by tens in 2 ml of the solution tested while rotated in complete darkness at 26°C on most occasions during 20 hrs. The results are given as average length of sections in millimeters or in percentages of growth of controls.

RESULTS

Effect of conditions in which seedlings grew, and the position of the hypocotyl section in respect to the cotyledonary node

Seedlings were grown in darkness, or in darkness followed by red-light at 26°C, or under day-light at room-temperature (about 20°C). The sections of hypocotyl cut at various distance from the cotyledonary node of plants grown in red-light showed a different response to three concentrations of IAA dissolved in 2% sucrose (Table 1). The most responsive were those closest to the node (2 mm distant). There was no difference practically between the response of sections cut from seedlings grown in darkness or in two different light and temperature conditions. However, the seedlings under red-light were more uniformly grown. No effect of light conditions during the sectioning of hypocotyl was observed.

Effect of seedling age

The seedlings grown under red-light were sectioned 2 mm below the cotyledonary node after 8, 9, 10 or 11 days. Before sectioning they were additionally distributed into 6 classes in respect to the hypocotyl length. In most series, the sections were incubated during 20 hrs, however, two classes of hypocotyl length (8—12 mm nad 28—32 mm) were incubated also for three different periods: 12, 24 and 48 hrs in the solution containing 2% sucrose.

Taking into account the variability of the results obtained, it is seen from Table 2 that the total growth of sections stimulated by auxin did not differ significantly. A somewhat greater stimulation expressed as the percentage of length of control sections, observed in sections cut from a longer hypocotyl (19—21, 24—26, 29—31, 34—36 mm) was due to the

Table 2

Effect of seedling age and incubation time upon the growth response of hypocotyl sections to IAA

Age of seedlings (days)	Hypocotyl length (mm)	Incubation (hrs)	No. of replicate tests	IAA mg/l					
				0 (controls)		0.01		0.1	
				mm	%*	mm	%*	mm	%*
9	14-16	20	3	6.0±0.3	105	6.3±0.4	105	7.1±0.5	118
	19-21		3	6.3±0.4	106	6.7±0.3	106	7.4±0.5	117
	24-26		3	6.1±0.3	106	6.5±0.4	106	7.3±0.5	120
10	29-31		3	6.0-0.4	107	6.4±0.4	107	6.9±0.5	115
	34-36		3	5.9±0.5	108	6.4±0.5	108	7.1±0.5	120
	39-41		2	5.8±0.3	102	5.9±0.4	102	7.0±0.6	121
10	24-26	20	3	6.1±0.3	113	6.9±0.5	113	7.6±0.5	124
	29-31		3	6.1±0.3	110	6.7±0.5	110	7.4±0.5	121
	34-36		3	6.4±0.4	105	6.7±0.6	105	7.6±0.7	119
8	8-12	18	2	5.6±0.4	102	5.7±0.3	102	6.4±0.3	114
		24	2	5.7±0.4	107	6.1±0.5	107	6.5±0.4	114
		48	2	5.9±0.4	107	6.3±0.3	107	6.4±0.6	108
10	28-32	18	2	6.0±0.1	108	6.5±0.1	108	6.8±0.3	113
		24	2	5.8±0.3	114	6.6±0.4	114	7.1±0.4	122
		48	2	6.4±0.6	105	6.7±0.5	105	8.0±0.5	125

* per cent of controls

Table 3
Effect of pH of tested solution upon the growth response of hypocotyl sections to IAA**

Medium	pH	IAA mg/l					
		0 (controls)		0.01		0.1	
		mm	%*	mm	%*	mm	%*
2% sucrose in citrate-phosphate buffer	4.4	5.7 ± 0.4	119	6.8 ± 0.6	119	7.4 ± 0.5	130
	5.4	5.7 ± 0.5	112	6.4 ± 0.6	112	7.2 ± 0.6	126
	6.4	5.6 ± 0.4	112	6.3 ± 0.4	112	7.3 ± 0.5	130
2% aqueous sucrose	6.4—4.0***	5.8 ± 0.4	110	6.4 ± 0.4	110	7.3 ± 0.5	127
						7.5 ± 0.6	132
						7.3 ± 0.5	128
						7.6 ± 0.3	136
						7.3 ± 0.6	127

* per cent of controls.

** means from three replicate tests.

*** pH of this solution changed from pH 6.4 to 4.0 during of 20 hrs incubation period.

lesser growth of controls. There was practically no visible effect of duration of the incubation period within the range of 18—48 hrs upon the growth of sections cut from a hypocotyl 28—32 mm long.

Effect of pH of tested solution

The tested solution of 2% sucrose in the citrate-phosphate buffer was adjusted to one of the three pH values: 4.4, 5.4, 6.4. One series of sections was incubated in 2% sucrose dissolved in fresh redistilled water of pH 6.4. The pH of this solution slowly changed to pH 4.0 at the end of the 20 hrs incubation period.

The results presented in Table 3 showed practically no effect of the pH of the solution tested within the range of pH from 4.4 to 6.4.

Response to IAA, pine auxin and GA

Pine hypocotyl sections incubated in the mixture of 2% sucrose and citrate-phosphate buffer pH 5.0 were found to be sensitive to IAA in concentration as low as 0.001 ppm (Table 4). Concentrations higher than 10.0 ppm stimulated growth of sections progressively less.

Table 4

Effect of IAA and natural auxin of *Pinus silvestris* upon the growth of pine hypocotyl and oat mesocotyl sections

Substances tested		Pine hypocotyl test			Oat mesocotyl test		
		No. of replicate tests	mm	%	No. of replicate tests	mm	%
H ₂ O (controls)		12	5.6±0.3	100	4	5.3±0.3	100
IAA mg/l	0.001	8	5.8±0.4	104	4	5.4±0.2	102
	0.005	8	5.9±0.4	105	—	—	—
	0.01	12	6.0±0.4	107	4	6.4±0.7	121
	0.1	12	6.8±0.5	121	4	7.9±1.1	149
	1.0	12	7.1±0.6	127	4	8.8±1.1	166
	10.0	12	7.3±0.6	130	4	8.8±0.9	166
	50.0	4	6.6±0.5	118	—	—	—
	100.0	4	6.0±0.4	107	—	—	—
Natural pine auxin eqv. gfw/ml	0.025	4	6.0±0.4	107	4	6.4±0.7	121
	0.05	8	6.2±0.4	111	4	6.8±0.8	128
	0.125	8	6.7±0.5	120	4	7.4±1.0	140
	0.25	8	6.9±0.5	123	4	7.9±1.3	149
	0.5	8	7.1±0.5	127	4	8.2±1.2	155
	1.0	4	6.8±0.6	121	—	—	—

Table 5
Effect of GA upon the growth of hypocotyl sections**

Conditions of seedling growth	Light conditions during sectioning	GA mg/l					
		0 (controls)		0.1		1.0	
		mm	%*	mm	%*	mm	%*
Darkness and red-light	day-light	5.8±0.3	98	5.7±0.3	98	5.8±0.3	100
	red-light	6.0±0.3	100	6.0±0.4	100	6.2±0.3	103
	red-light	5.6±0.3	100	5.6±0.3	100	5.7±0.3	102
Darkness	green-light	5.5±0.3	100	5.5±0.4	100	5.6±0.4	102
						5.4±0.3	98

* per cent of controls
** means from three replicate tests

Sections responded to the pine auxin extracted from the inner bark and the cambial zone of pine and purified as described elsewhere (Wodzicki 1968). The amount of pine auxin present in the extract from 125 mg of tissue either in March or in June 1969 and given in 1 ml of the tested solution gave a stimulation equivalent to 0.1 ppm of authentic IAA. Significant stimulation was observed in the extract from 25 mg of tissue, given in 1 ml of the tested solution, but the effect of still lower concentration could be probably detected.

There was no effect of gibberellic acid upon the growth of pine hypocotyl sections (Table 5).

DISCUSSION AND CONCLUSIONS

The results obtained agree with those reported by Hashizume (1965 a) for hypocotyl section of three other pine species. Section of *Pinus silvestris* hypocotyl appeared nearly as sensitive to IAA as the sections of oat mesocotyl (Nitsch and Nitsch 1956). The most sensitive to auxin were sections cut 2 mm below the cotyledonary node. This test is not sensitive to gibberellic acid. It is sensitive to pine auxin extracted from tissues of *Pinus silvestris*, but not in a greater degree than the oat mesocotyl section straight growth test. The convenience of the pine hypocotyl section test lies in its great tolerance in respect to the conditions of seedling growth and to the acidity of the tested solutions.

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SUMMARY

The growth response of pine (*Pinus silvestris* L.) hypocotyl sections was investigated. The most sensitive to auxin were found sections of 4.5 mm long cut 2 mm below the cotyledonary node. The response of sections cut from seedlings grown in darkness followed by red-light treatment at constant temperature 26°C was not different from that of seedlings grown in day-light at room temperature. There was also no difference in respect to the age of seedlings grown 8 to 11 days. The best results were obtained with hypocotyl length 19–32 mm. There was also no effect of pH of solution tested within the range: pH 4.4 to pH 6.4. Pine hypocotyl sections responded to as low concentration of IAA as 0.001 ppm, and to pine auxin extracted from cortical tissue of pine equivalent to 25 mg of fresh wt per ml of tested solution. They did not respond to gibberellic acid.

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*Test wydłużeniowy odcinków hypocotyłu Pinus silvestris L.
do oznaczania auksyn*

Streszczenie

Badane odcinki hypocotyłu pochodziły z 8—11 dniowych siewek *Pinus silvestris* L. Siewki hodowano z nasion, które moczone w wodzie wodociągowej przez 20 godzin, a następnie wysiewano w wilgotnym piasku. Rośliny rosły w stałej temperaturze 26°C, początkowo w ciemności, a od trzeciego dnia po wysiewie przy świetle czerwonym. Przygotowanie odcinków o długości 4.5 mm przeprowadzano w warunkach oświetlenia dziennego przy temperaturze pokojowej (około 20°C). Pobrane odcinki płukano w wodzie destylowanej w ciągu 1 godziny, a następnie inkubowano w ciemności przy temperaturze 26°C po 10 sztuk w 2 ml badanego roztworu przez okres 20 godzin.

Badano wpływ następujących czynników na wrażliwość odcinków hypocotyłu względem IAA:

- warunki oświetlenia i temperatury w okresie wzrostu siewek oraz w czasie krojenia hypocotyłu
- odległość odcinka od węzła liścieniowego
- faza wzrostu siewki
- długość okresu inkubacji
- pH badanego roztworu.

Uzyskane wyniki wykazały, że najbardziej wrażliwe na działanie IAA były odcinki cięte w odległości 2 mm poniżej węzła liścieniowego. Wrażliwość na IAA odcinków hypokotyłu uzyskanych z siewek hodowanych przy stałej temperaturze 26°C w ciemności lub w ciemności z dodatkowym czerwonym światłem nie różniła się od czułości odcinków ciętych z siewek rosnących w warunkach temperatury pokojowej przy świetle dziennym. Nie było także różnic we wzroście odcinków pozyskanych z siewek w wieku od 8 do 11 dni. Najlepsze wyniki uzyskano gdy badane odcinki cięto z siewek o długości hypokotyłu 19–32 mm. Nie stwierdzono wpływu pH badanego roztworu (w zakresie pH 4.4 do pH 6.4) na reakcję wzrostową odcinków w obecności IAA.

Odcinki hypokotyłu były wrażliwe na IAA przy koncentracji 0.001 ppm i na auksynę ekstrahowaną z tkanki sosnowej w ekwiwalencie 25 mg świeżej tkanki w 1 ml badanego roztworu.

Odcinki hypokotyłu sosny nie wykazywały wrażliwości na kwas gibberelinowy.

Test hypokotyłu sosny pospolitej jest tylko nieco mniej czuły względem auksyn niż test mezokotyłu owsa, natomiast jest wygodniejszy w pracy ze względu na jego dużą tolerancję w stosunku do warunków hodowli siewek i pH badanego roztworu.