

Effect of white light irradiation on the endogenous growth regulators content in seeds and seedlings of pine (*Pinus silvestris* L.)*

J. KOPCEWICZ

Department of Plant Physiology, Institute of Biology, Copernicus University, Toruń, Sienkiewicza 30/32, Poland

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Abstract:

The increase of gibberellin content and decrease of amount of inhibitors in light irradiated pine seedlings were found. It was also stated that stimulating effect of light on pine seeds germination is correlated with the increase of gibberellin and simultaneous decrease of the inhibitor content. The inhibitor isolated from pine seeds proved to be a compound similar in some properties to abscisic acid.

INTRODUCTION

The first reports about effect of light on the Scots pine seeds germination was published by Atterberg (1906) and Haack (1906). After that time several authors repeatedly have shown stimulating effects of light on the germination of seeds from *Pinus silvestris* as well as from other pine species (Nyman 1963). It is also evident that light requirements in seeds of many plant species may be satisfied by the action of growth regulators (Ikuma and Thimann 1960, Czopek 1963, Grzesiuk and Rejowski 1963). Until now only a few investigations were conducted concerning the influence of the growth regulators on the Scots pine germination (Michniewicz 1967; Suszka 1967). In the preceding paper (Kopcewicz 1970a) it was shown that among various growth regulators only kinetine, estrogens and some of gibberellins can substitute the inductive action of light in the pine seeds germination. This fact aroused the interest in the physiological role of growth substances in the germination and in the early stages of development of pine seedlings. In the first stage of this work the influence of light on the endogenous gibberellins and inhibitors content in seeds and seedlings of pine was investigated.

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MATERIAL AND METHODS

The cones have been collected after ordinary cuttings in normal stands of trees not specially selected for seed collection. Because the unimbibed seeds have been shown to be sensitive to light (Nordström 1953) all cones with opened cone scales were discarded before the start of the seeds extractions in order to obtain as uniform seed material as possible with regard to eventual effects of irradiations before the start of the experiments. For that reason all handlings of the seeds were done in green safelight (Withrow and Price 1957). All extractions were performed in the laboratory. The cones were placed in darkness at $+40^{\circ}\text{C}$ for 96 hours, whereupon all seeds were separated from the opened cones. Directly after the extraction the seeds were transferred to dark glass bottles and placed in a cold storage room at $+4-6^{\circ}\text{C}$. Stored under such conditions the seeds retain their germinative capacities unchanged for years. The seeds obtained in this way germinated in 91 per cent in light and 9 per cent in darkness.

The pine seeds were germinated in sterile sawdust in darkness at 22°C . After seven days, one part of the seedlings was exposed to light (fluorescent tubes daylight, intensity about 3000 lx) while the other was further kept in darkness. The investigations were performed after 12, 24 and 48 hours (Fig. 1, 2).

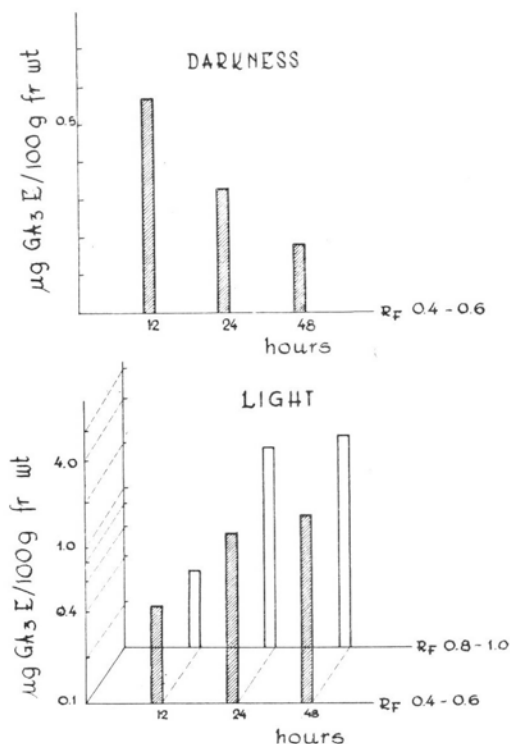


Fig. 1 Gibberellin-like substances in pine seedlings cultivated in darkness and in light

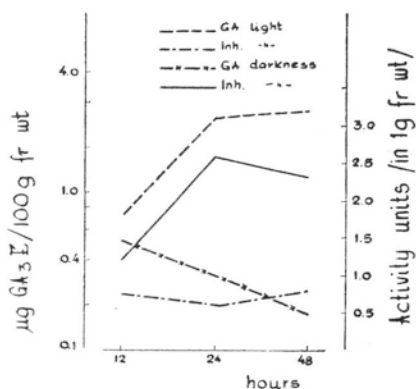


Fig. 2. Total activity of gibberellins and inhibitors in pine seedlings cultivated in darkness and in light

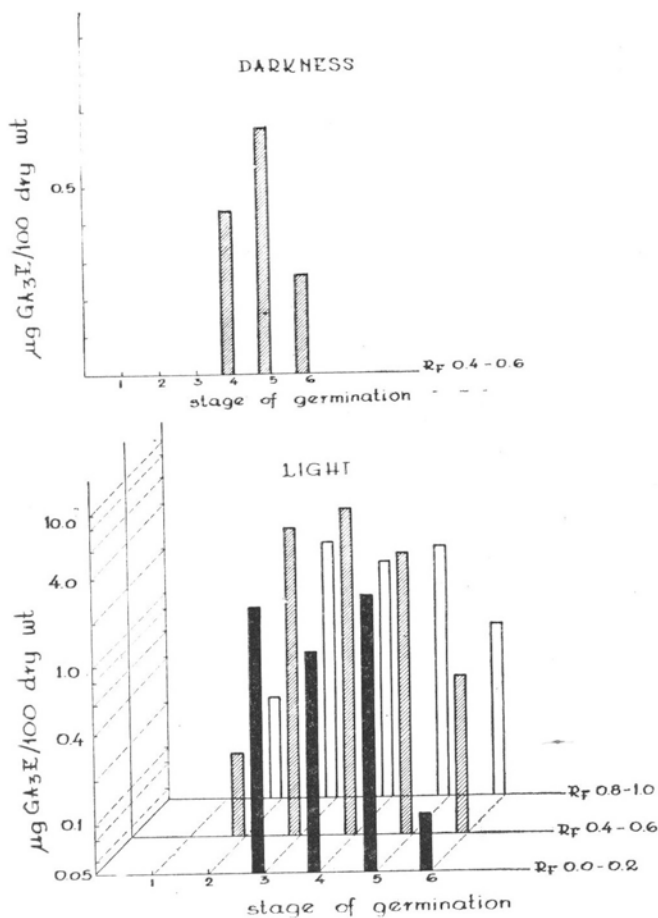


Fig. 3. Gibberellin-like substances in pine seeds germinated in darkness and in light

In further experiments (Fig. 3, 4) the seeds were germinated on Whatman 3 paper soaked in distilled water in darkness and in light. In darkness seeds were kept in a thermostat at 22°C. In light the same was done in a continuously lit chamber

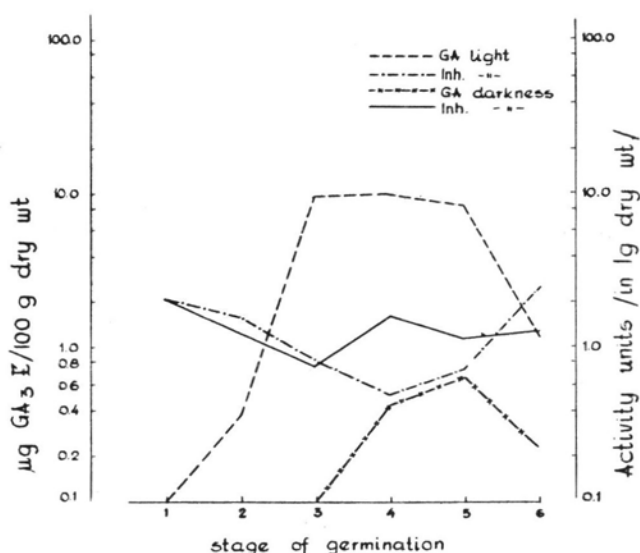


Fig. 4. Total activity of gibberellins and inhibitors in pine seeds germinated in darkness and in light

(fluorescent tubes daylight, intensity about 3000 lx) at 22°C. The material was taken at the following stages of germination: 1. dry seeds, 2. seeds germinated for 24 hrs, 3. 48, 4. 72, 5. 96 and 6. 120 hrs. The material for analysis was taken at random.

Gibberellins and inhibitors were determined in 20 g samples. Frozen material was extracted with 80% methanol for 48 hrs at 20°C. Evaporation at 30°C removed the methanol leaving the aqueous residue. This concentrate was adjusted to pH 8.0 with saturated NaHCO_3 solution and extracted with ethyl acetate. The aqueous phase was re-adjusted to pH 3.0 with 10% HCl and extracted again with ethyl acetate and then with ethyl ether. Ethyl ether was purified from peroxides immediately before analysis. The combined ethyl acetate-ethyl ether fraction was evaporated to dryness. The residue was dissolved in a small volume of absolute methanol. Thin-layer chromatography in benzene-acetic acid (10:3 v/v) as solvent was applied. The gibberellins were bioassayed by the lettuce hypocotyl (Frankland and Wareing 1960) and dwarf pea (Mc Comb and Carr 1958) tests. The position of gibberellin-like substances was examined in UV light and compared with the position of standard gibberellins in the control chromatograms. In the case of inhibitors, paper chromatography on Whatman 3 with redistilled water as solvent was used. The content of inhibitors was estimated by the Avena section straight growth test (Bonner and Audus 1959). The results were calculated per 100 g of fresh and dry weight in the case of gibberellins and per 1 g of fresh and dry weight in the case of growth inhibitors. Growth inhibition was expressed in activity units. As activity

unit 10 per cent growth inhibition of the test plants in relation to control was taken. Significant differences in relation to control were defined by estimating LSD at $P=0.01$ (Student test). All experiments were repeated four times. More methodical details are given in (Michniewicz and Kopcewicz 1966; Kopcewicz 1968a).

In order to learn more about the physico-chemical and biological properties of inhibitors several hundred gramm samples of seeds were extracted, then multiple paper chromatography was applied, and the sections of chromatograms containing inhibitors (R_f 0.8–1.0) were pooled together, eluted and investigated. In these experiments the following bioassays were used: first oat leaf (Michniewicz 1961), wheat coleoptile (Bentley and Housley 1954) and lettuce seed germination (Irving 1969).

RESULTS AND DISCUSSION

The experiments, the purpose of which was the investigation of the influence of light on gibberellin and inhibitors content, showed the considerable increase in gibberellins biosynthesis in pine seedlings irradiated with light. These seedlings contained two groups of gibberellins (Fig. 1), the content of which was increasing in the course of time (Fig. 2). In seedlings kept in darkness there was stated the presence of one group of gibberellins (Fig. 1) the amount of which was decreasing (Fig. 2). In irradiated with light seedlings the decrease of inhibitors content was also established (Fig. 2).

There are controversial data concerning the effect of light on gibberellins content in plants (Wheeler 1962; Kende and Lang 1964; Köhler 1966). The obtained results confirm data which show an increase in the gibberellins content under the influence of light. It seems also that inhibitory influence of light on the biosynthesis of inhibitors is worth noticing.

The experiments the aim of which was to learn about metabolism of gibberellins and inhibitors in pine seeds germinated on light and kept in darkness were also conducted. Both the quantitative and qualitative differences in the content of gibberellins were determined. In the case of seeds kept in darkness, gibberellins appeared as late as after 72 hrs. The presence of small amounts of these substances was established also after 96 and 120 hrs. These seeds contained only one group of gibberellins localized at R_f 0.4–0.6 (Fig. 3). In seeds germinated in light gibberellins appeared after 24 hours of germination, then their level increased reaching high values after 48, 72 and 96 hrs. These seeds contained three groups of gibberellins (Fig. 3). The inhibitors extracted from pine seeds and seedlings were localized near the front of the chromatograms at R_f 0.8–1.0. In seeds kept in darkness the high level of inhibitors during the whole period of investigation was observed (Fig. 4). In seeds germinated in light the high level of inhibitors was also noticed; however at the time of coat piercing and initial periods of root growth (72 and 96 hrs of germination) the level of inhibitors decreased (Fig. 4). This fact seems to indicate the participation of inhibitors in the regulation of pine seeds germination.

These results show that under the action of light the intensive biosynthesis of

gibberellins occurs also in pine seeds. Seeds kept in darkness contain only small amounts of gibberellins localized at R_f 0.4–0.6, which probably do not play any important role in the process of germination. In seeds germinated in light two additional groups of gibberellins are found and it seems that these gibberellins participate in the regulation of pine seeds germination. These data, together with the possibility of substituting the action of light by gibberellins (Kopcewicz 1970a), lead to the hypothesis that, in order for the germination to take place, a "suitable" level of gibberellins is necessary.

In light germinated pine seeds similarly as in seedlings there is intensive biosynthesis of gibberellins, from which only those localized at R_f 0.4–0.6 occur in darkness. It seems possible that the influence of light on gibberellins biosynthesis is indirect through general regulation both of cell permeability and phytochrome action. The effect of light on gibberellins released from non-active precursors and conversions of some gibberellins into others cannot be also excluded.

Table 1

Inhibitor localization on chromatograms in various solvents

Kind of chromatography	Solvent	R_f of inhibitor from pine	R_f of abscisic acid
PC	Water	0.8–1.0	0.9–1.0
	Isopropanol-ammonia-water (10:1:1)	0.6–0.8	0.75–0.85
TLC	Isopropanol-butanol-ammonia-water (6:2:1:2)	0.5–0.7	0.65–0.75
	Benzene-chloroform-formic acid (2:10:1)	0.1–0.2	0.1–0.2
	Benzene-ethyl acetate-formic acid (70:30:5)	0.3–0.5	0.25–0.35

In seeds germinated in light and seedlings, three groups of gibberellins have been found. Similar gibberellins were also found in the meristems, needles, shoots and cones of Scots pine (Kopcewicz 1968b; Kopcewicz 1970b; Kopcewicz et al. 1967). A closer physico-chemical and physiological description of properties of the gibberellins occurring in pine were presented in the previous paper (Kopcewicz 1968a).

The problem of quality of the inhibitor extracted from pine was also considered. The results show that the inhibitors greatly decreased the growth of wheat and oat coleoptile as well as lettuce seed germination. The first oat leaf reacted weakly and the lettuce hypocotyl was not influenced by this inhibitor (Tab. 1). The experiments undertaken to find the inhibitor localization on the chromatograms, showed that the inhibitor was localized in similar zones as abscisic acid in all the five solvents applied (Tab. 2). The UV spectrum of the inhibitor was also taken. It was evident

Table 2

Influence of inhibitor on growth of various test plants

Bioteest	Value in	Control	Influence of inhibitor isolated from seeds taken in the amount of:		
			10 g	5 g	1 g
Oat coleptile	mm	99.31	73.08	82.33	97.98
	%	100.00	73.58	82.90	98.66
Wheat coleoptile	mm	112.53	68.33	81.85	102.20
	%	100.00	60.73	72.73	90.82
First leaf of oat	mm	10.45	8.85	9.01	9.68
	%	100.00	84.68	86.22	92.65
Lettuce hypocotyl	mm	4.00	4.2	4.1	4.0
	%	100.00	105.00	102.50	100.00
Lettuce seeds germination	number	75.6	0	19.6	48.3
	%	100.00	0	25.92	63.88

from it that in extracts from pine a compound is present which similarly as abscisic acid has two peaks of absorption at 245 and 260 nm wavelength. Though further physico-chemical investigation are necessary in the light of the obtained results, however, it seems possible that the inhibitor is a compound with a similar structure as abscisic acid.

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Wpływ światła białego na zawartość giberelin i inhibitorów w nasionach i siewkach sosny (Pinus silvestris L.).

Streszczenie

Stwierdzono podwyższenie się zawartości giberelin i obniżenie ilości inhibitorów w siewkach sosny poddanych działaniu światła. Wykazano również że stymulacja kiełkowania nasion sosny przez światło jest skorelowana z podwyższeniem się ilości giberelin i jednoczesnym obniżeniem zawartości inhibitorów w nasionach. Wyodrębniony z nasion sosny inhibitor jest związkiem zbliżonym szeregiem właściwości do kwasu abscysynowego.