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Studies of the mechanism of light and low temperature action on germination in seeds of Scots pine (*Pinus silvestris* L.)*

I. *Effect of some growth regulators on the germination of pine seeds in darkness*

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It is evident that germination of pine seeds is controlled by a reversible red-far-red mechanism (Sarvas 1951; Richtar 1958; Nyman 1963; Kantor and Simančik 1966). It is also known that pine seeds in darkness germinate very weakly (Richtar 1958; Nyman 1963). In the course of preliminary investigations it was also proved that keeping of swelling pine seeds during 30 days at low temperature (+3—4°C) results in the seeds germinating in a similar high percentage both in darkness and in light. Such a dependence has been also found by Nyman (1963).

These data were the basis for investigations, the purpose of which is a better knowledge of the mechanisms of transformations leading to the possibility of substituting the action of light by the influence of low temperature. In the first stage of this work the influence of various growth regulators on the germination of pine seeds in darkness was investigated.

MATERIAL AND METHODS

Seeds decorticated in complete darkness were used for the experiments and all the operations were conducted only in green light. The pine seeds were treated with auxins: indolyl-3-acetic acid (IAA), β-indolyl-butyric acid (IBA), indolyl-pyruvic acid (IPyA), gibberellins: (A_3, A_{4+7}, A_{13}), steroids: (cholesterol, estrone, estradiol-17β, β-sitosterol, testosterone), 2-chloroethyl-trimethylammonium chloride (CCC) and kinetin in 0.1, 1, 10 and 100 mg/l concentrations.

All the substances used except steroids were applied in the form of water solutions. The steroids were dissolved in a small amounts of Tween 80 before adding the water. All steroid treatments including

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controls received a similar amount of Tween 80 whose final concentration was 0.1%.

The pine seeds were germinated on Whatman No. 1 paper soaked in solutions of the substances applied, in a Petri dish, at +22°C in darkness. The proportion of germinating seeds was determined after two weeks. The experiments were repeated 10 times. A single Petri dish with a hundred pine seeds was taken for one repetition.

RESULTS AND DISCUSSION

It is known that light is a very important factor necessary for the correct course of pine and some other seeds germination. Studies on the effects of irradiation with coloured light have shown that the wavelengths most effective for promotion of germination were to be found within the red (around 6600 Å) and that the red promoting could be reversed by light of longer wavelengths with maximal effects in the far-red (around 7300 Å). There was a repeatedly reversible red-far-red mechanism controlling the germination, indicating the existence of phytochrome as the regulating pigment (Evenari 1956; Borthwick 1961; Borthwick and Hendricks 1961; Mayer and Poljakoff-Mayber 1963; Nyman 1963).

It is also evident that light requirements in seeds of many plant species may be satisfied by the action of gibberellins and cytokinins. These substance may partly or fully substitute the promoting action of red light in some plants (Miller 1956; Miller 1958; Ikuma and Thimann 1960; Nikołajewa 1962; Czopek 1963; Grzesiuk and Rejowski 1963). Up till now there only a few investigations were conducted concerning the influence of growth regulators on the Scots pine germination. The results of these experiments are varied and only in few cases was it possible to show the positive influence of gibberellins on the ratio of germinating pine seeds (Michniewicz 1967; Suszka 1967). In the accessible literature no data could be found concerning the influence of growth substances on pine seeds germination in darkness. So it seemed interesting to investigate this problem, since the results obtained my throw some light on the lines of further investigations, the purpose of which is a better knowledge of the mechanism of transformations leading to the possibility of substituting the action of light by the influence of low temperature.

As seen from the results achiewed (Table 1) only gibberellins A_{4+7} and A_{13}, estrone, estradiol-17β and kinetin increase considerably proportion of pine seeds germinating in darkness. The results confirm the data referring to the possibilities of substituting the influence of light by the action of kinetin and gibberellins also in the pine seeds. The lack of

reaction in the case of gibberellin A_3 can be explained by the previous suggestions concerning the causes of the weak influence of gibberellic acid on the growth and development of conifers (K o p c e w i c z 1968).

Table 1

Influence of some growth regulators on the germination of pine seeds in darkness

Substances	Percentage of germinated seeds			
	Concentration (mg/l.)			
	0.1	1	10	100
IAA	13	12	9	5
IPyA	14	11	10	4
IBA	15	13	8	3
GA_3	18	15	16	15
GA_{4+7}	33	56	54	48
GA_{13}	76	81	69	59
CCC	12	10	9	10
Kinetin	85	79	72	45
Estrone	48	46	59	50
Estradiol-17β	51	56	68	49
Testosterone	10	11	6	3
Cholesterol	9	15	12	8
β-sitosterol	13	14	10	9
Control in darkness	0.1% Tween 80		H_2O	
	9		11	
Control on light	92		96	

The possibility of substituting the influence of light by the action of steroidal hormones seemed interesting. At present it is known that estrogens are found in small amounts in plant tissues (H e f t m a n n 1968) and that they influence the growth and development processes in plants (C z y g a n 1962; B o n n e r et al. 1963; M o o r e and A n d e r s o n 1966; L e s h e m 1967; K o p c e w i c z 1969a). In previous reports (K o p c e w i c z 1969 b, c, d) it was also shown that estrone and estradiol-17β increase the gibberellins and auxins content in the tissues of pea and pine. Thus there is a possibility that the positive influence of estrogens on pine seeds germination in darkness is the result of an increase in the content of endogenous growth regulators in them. However, since the available information concerning the mechanism of steroidal hormones action in plants is scant, this problem remains opened for further investigations.

SUMMARY

Seeds decorticated in complete darkness were used for experiments and all the operations were conducted only in green light. The pine seeds were treated with auxins (IAA, IPyA, IBA), gibberellins (A_3, A_{4+7}, A_{13}), steroids (cholesterol, estrone, estradiol-17β, β-sitosterol, testosterone), CCC and kinetin in 0.1, 1, 10 and 100 mg/l concentrations.

It was found that only gibberellins A_{4+7} and A_{13}, estradiol-17β, estrone and kinetin produced considerable increase of the proportion of pine seeds germinating in darkness. The mechanism of steroidal hormones action in the process of pine seeds germination in darkness is also discussed.

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Badania nad mechanizmem wpływu światła i niskiej temperatury na kiełkowanie nasion sosny zwyczajnej (Pinus silvestris L.)

I. *Wpływ substancji wzrostowych na kiełkowanie nasion sosny w ciemności*

Streszczenie

Do doświadczeń użyto nasiona sosny wyłuszczone w kompletnej ciemności, wykonując następnie wszystkie czynności wyłącznie przy świetle zielonym. Nasiona sosny traktowano auksynami (IAA, IPyA, IBA), giberelinami (A_3, A_{4+7}, A_{13}), steroidami (cholesterol, estron, estradiol-17β, β-sitosterol, testosteron), CCC oraz kinetyną w stężeniach 0.1, 1, 10 i 100 mg/l. Wyniki doświadczeń wykazują że tylko gibereliny A_{4+7} i A_{13}, estradiol-17β, estron oraz kinetyna wpływały na podwyższenie ilości kiełkujących nasion sosny w ciemności. W pracy dyskutuje się problem mechanizmu działania hormonów sterydowych w procesie kiełkowania nasion sosny.