Influence of low temperature on germination and endogenous growth regulator contents in Scots Pine (*Pinus silvestris* L.) seeds *

J. KOPCEWICZ and Z. PORAZIŃSKI

Department of Plant Physiology, Institute of Biology, Copernicus University, Toruń,
Poland

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Abstract:

Twenty days-long stratification of Scots pine seeds results in the seeds germinating in similar high percentage in darkness as in light. The period of stratification enough to overcome the "barrier" of light is connected with simultaneous increase of gibberellin contents, especially with the appearance of the new groups of gibberellins qualitatively similar to those occurring in pine tissues under the influence of red and white light. It was also found that seedlings grown from stratificated pine seeds produce the increased amounts of gibberellins.

INTRODUCTION

It is evident that germination of pine seeds is controlled by a reversible red — far-red mechanism (Nyman 1963). In the preceding paper (Kopcewicz 1970) it has been 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. It has been shown (Kopcewicz 1971, 1972) that the increase of both germination of seeds and gibberellins content and decrease of abscisic acid-like inhibitor amount occur in pine seeds and seedlings irradiated with continuous white, red, orange and blue light. The interrelations between synthesis of particular gibberellins and the quality of applied light has also been found. Above results suggest that there exist clear correlations between seeds germination capacity and gib-

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berellin and inhibitor contents in pine tissues treated with light of determined quality.

The literature of the subject also shows that keeping of swelling pine seeds at low temperature results in the seeds germinating in a similar high percentage both in darkness and in light (Nyman 1963). Since in the process of pine seeds germination gibberellins may react vicariously in relation to light, and the same physiological effect can be reached with low temperature there arose the problem of interrelations between low temperature and gibberellins. The question was whether, as in the case of light, there exist any causalities between low temperature, seed germination in darkness and metabolism of endogenous growth regulators.

MATERIAL AND METHODS

The pine cones have been collected after ordinary cuttings in normal stands of trees not specially selected for seed collection. Extraction of seeds from cones was performed in the laboratory in green safelight. The seeds were soaked for 48 hrs, in darkness at +22°C. Then they were placed in glass containers and kept at +3°C, in darkness, for 30 days, maintaining the degree of seed hydration within the range of 40%. The investigations of both seed germination capacity and endogenous growth regulators content were carried out after 5, 10, 15, 20, 25 and 30 days of stratification.

The pine seeds were germinated on Whatman No. 1 paper soaked in distilled water, in a Petri dish, at +22°C in darkness. The proportion of germinating seeds was determined after 7 and 14 days. Above experiments were repeated 10 times. A single Petri dish with a hundred pine seeds was taken for one repetition.

Gibberellins and inhibitors were extracted and fractionated according to the methods previously described (Kopcewicz 1971). The thin-layer chromatography in benzene - acetic acid (10 : 3) followed by lettuce hypocotyl (Frankland and Wareing 1960) and dwarf pea (McComb and Carr 1958) bioassays for gibberellins was used. 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, extract was streaked on Kieselgel G TLC plates and developed to 10 cm long strips in benzene - acetone - acetic acid 70 : 30 : 1 v/v. The abscisic acid zone (R_f 0.2 - 0.4) was removed, eluted with methanol and then rechromatographed on Whatman 3 paper in distilled water. Inhibitor was localized in the front of chromatograms (R_f 0.9 - 1.0). The content of inhibitor was estimated by the *Avena* section straight growth test (Bonner and Audus 1959). 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. 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. The results are recorded graphically only in regard to dry weight. Data referring to fresh weight were essentially identical.

The investigations concerning the changes in gibberellin contents during the germination of stratified pine seeds were also conducted. The seeds were germinated in sterile sawdust in darkness at $+22^{\circ}\text{C}$. The material was taken at the following stages: I. 24 hours after stratification, II. 48 hours, III. seeds with pierced coat, IV. seedlings at the primary stage of cotyledon development, V. seedlings with free cotyledons. All experiments were repeated four times. Significant differences in relation to control were defined by estimating LSD at $P = 0.01$. More methodical details are given in (Michniewicz and Kopcewicz 1966).

RESULTS AND DISCUSSION

The results of experiments on influence of low temperature on pine seed germination in darkness, show that only 15-days-long stratification causes the considerable increase in the number of germinated seeds (Tab. 1). Beginning with 20 days of stratification the seeds germinated to similarly high degree in darkness as in white or red light (Kopcewicz 1972).

Table 1

Effect of low temperature on germination of pine seeds in darkness

Days of stratification	p.c. of germinated seeds after	
	7 days	14 days
0	8	11
5	9	12
10	8	10
15	28	29
20	84	87
25	86	86
30	83	85

Both the quantitative and qualitative changes in the content of gibberellins in pine seeds depending on the time of low temperature action were also stated (Fig. 1). Gibberellin-like substances were localized on chromatograms in three active zones (Fig. 1). In dry non-stratified seeds the presence of gibberellins was not established. Gibberellins localized at R_f 0.0 - 0.2 were present in quite great amounts after 5 days of seed stratification, and after that their level rapidly decreased,

Fig. 1. Changes in the content of gibberellin-like substance during the stratification of pine seeds

Fig. 2. Total activity of gibberellins and inhibitors during the stratification of pine seeds

reaching afterwards the maximum after 20 days of stratification. Next period was characterized by the successive fall in the content of these substances. The second group of gibberellins localized at R_f 0.4 - 0.6 showed similar to above changes. The third group (R_f 0.8 - 1.0) appeared in great amounts only after 20 days of seed stratification. In later period the content of these gibberellins was decreased. It seems interesting that three similar groups of gibberellins were found also in seeds and seedlings exposed to white or red light (Kopcewicz 1971, 1972), which are highly active in the induction of pine seeds germination. A closer physico-chemical and physiological description of properties of the gibberellins occurring in Scots pine were presented in the previous paper (Kopcewicz 1968).

The total amount of gibberellins was high after 5 and 20 days of stratification (Fig. 2). It seems characteristic, however, that after 5 days of stratification the seeds contain only 2 groups of gibberellins. The third group of gibberellins (R_f 0.8 - 1.0) appeared only after 20 days of low temperature action, when the seeds began to germinate in darkness. It seems that first of all this group of gibberellins is causatively connected with overcoming the light "barrier" in the process of pine seed germination. This suggestion finds the confirmation in previously obtained results showing that pine seed germination in light is connected with increased content of similar gibberellins (Kopcewicz 1971, 1972) and that only some gibberellins may replace the inductive action of light in pine seed germination (Kopcewicz 1970). Generally it seems probable that the increased content of gibberellins in stratified for 20 days pine seeds may be one of the causes of the initiation of metabolic reaction whose consequence is the possibility of seed germinating in non-inductive conditions, i.e. without light energy.

The influence of low temperatures on the growth inhibitors content has also been investigated (Fig. 2). It seems interesting that when pine seeds begin to germinate in darkness and, at the same time, produce the increased amounts of gibberellins, the content of inhibitors decreases. These data suggest the participation of inhibitors in transmutations leading to pine seeds acquiring the capacity for germination in darkness. Previous experiments (Kopcewicz 1971) have shown that the inhibitor extracted from pine seeds may be abscisic acid.

Further investigations were carried out on pine seeds stratified for 30 days, which were afterwards germinated in darkness at $+22^{\circ}\text{C}$. The aim of these experiments was to compare the changes in the content of gibberellins during the germination of stratified seeds with previously obtained data concerning the changes in the content of gibberellin-like substances in non-stratified pine seeds germinated both in light and darkness. The results of those investigations (Kopcewicz 1971) showed that non-stratified pine seeds germinate in a very low

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

Fig. 4. Total activity of gibberellins in stratified pine seeds germinated in darkness

percentage in darkness, containing at the same time small amounts of gibberellins localized on chromatograms at R_F 0.4 - 0.6. The same seeds germinated in light contain three groups of gibberellins and their amount increases in the course of seed swelling, reaching its maximum in the period of pierced coat (Kopcewicz 1971). The results of presented here experiments (Fig. 3) show that stratificated pine seeds germinated afterwards in darkness contain, similarly to non-stratificated and germinated in light seeds, three identical groups of gibberellins. So under the influence of low temperature it comes to initiating the production of similar gibberellins in pine seeds as in the case of white and red light irradiation. As it is known the action of light in the plant morphogenesis and metabolism of biologically important endogenous organic compounds is connected with the presence and regulating role of phytochrome in plant tissues (Mohr 1969). A very interesting problem, although at present not known, is the mechanism of the influence of temperature on hormonal system in plants. It is difficult to decide how and through what regulating-control systems the temperature impulses are transmitted; it seems, however, that the connection between the endogenous systems absorbing the temperature effects and the phytochrome cannot be excluded.

Although both low temperature and light cause the production of qualitatively similar gibberellins, it seems interesting, however, that the changes in the contents of these compounds are not identical. In non-stratificated seeds germinated in light the maxima of gibberellin contents are in the period of swelling and pierced coat. In young seedlings the amounts of gibberellins is small (Michniewicz and Kopcewicz 1966, Kopcewicz 1971). The present experiments (Fig. 4) show, however, that in stratificated seeds in the period of pierced coat the level of gibberellins is low, whereas in young seedlings the intensive production of these compounds take place. It seems that this fact may explain the results of other authors (Schröck 1951; Simak and Gustafsson 1957; Zentsch and Jahnelt 1960) who established that stratification of pine seeds leads to the increase of growth and effectiveness of pine seedlings.

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*Wpływ niskiej temperatury na kielkowanie i zawartość
endogennych regulatorów wzrostu w nasionach sosny zwyczajnej (Pinus silvestris L.)*

Streszczenie

Dwudziestodniowa stratyfikacja powoduje, że nasiona sosny kielkują w ciemności w podobnie wysokim procencie jak i na świetle. Okres stratyfikacji, w którym dochodzi do przełamania „bariery” światła, jest związany z podwyższeniem się zawartości giberelin w nasionach sosny, a zwłaszcza z pojawieniem się nowej grupy giberelin jakościowo zbliżonej do giberelin tworzących się w tkankach sosny pod wpływem działania światła białego i czerwonego. Stwierdzono ponadto, że siewki wyrosłe ze stratyfikowanych nasion sosny produkują podwyższone ilości giberelin.