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Effects of ionizing radiation on changes in the level of gibberellin-like substances during Scotch pine seeds germination*

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Abstract

The level of gibberellin-like substances in pine seeds exposed to ionizing radiation was investigated using extraction, fractionation, acid hydrolysis, partitioning on chromatographic columns and biological tests. It was found that the level of free and bound gibberellin-like substances is dependent on the applied gamma radiation dose. It is assumed that ionizing radiation may release bound gibberellins into free ones.

INTRODUCTION

Ionizing radiation causes deep physical and chemical changes in all the substances occurring in the plant (K u z i n 1962).

The data concerning the influence of ionizing radiation on endogenous growth regulators in plants are rather general. They are the result of some experiments carried out *in vitro:* this refers both to auxins (S k o o g 1935; G o r d o n 1957 et al.) and gibberellins (S i d e r i s et al. 1969, 1971). Studies on these substances *in vivo* are very scarce. Ionizing radiation decreases the level of gibberellins (S i d e r i s et al.) and especially the higher doses of radiation. At the same time the protective effect of these substances on post-radiation damages in plant has been observed (K r y u k o v a et al. 1967, K u m a r 1967, G a u r et al., 1969, M i c h a l s k i 1976, et al.).

There is lack of data concerning the effect of ionizing radiation on endogenous growth regulators in conifers.

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The aim of present investigation was a thorough study of the influence of different doses of gamma radiation on the level of gibberellin-like substances in germinating seeds of Scotch pine.

METHODS

Seeds of Scotch pine (*Pinus silvestris L.*), of lowland provenience from the 1970 collection (humidity — 7.6 p.c., germination capacity 93 p.c.), irradiated with gamma rays at doses of 0.5, 1.0, 2,0 and 5.0 kR from cobalt source (^{60}Co) mod. PXM-20 prod. USSR, were used as the experimental material.

For extraction 50 or 500 g samples of seeds were taken in the following variants of the experiment: I. — dry (0.0 hour), II. — soaked (7 hr), III. — swollen (24 hr), IV. and V. — germinating (72 and 144 hr), on Whatman's paper No 3 in moist chambers.

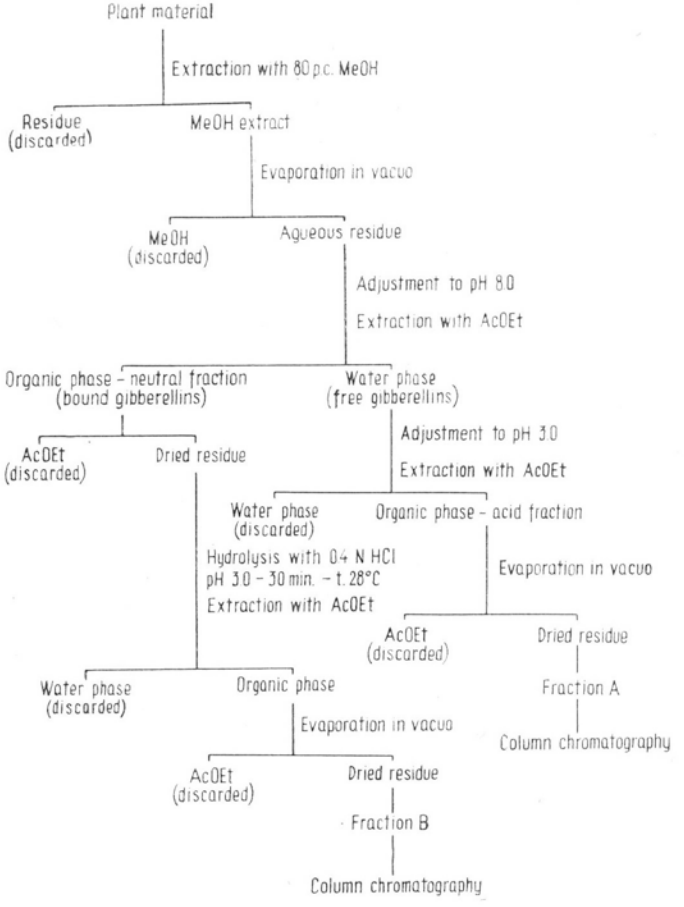

Fig. 1. Fractionation methods of free and bound gibberellin-like substances

The experimental seeds were homogenized with 80 p.c. methanol. Extracts were fractioned according to West et al. (1959) method and hydrolysed with HCl for the disengagement of bound gibberellins (Murakami 1961). Fig. 1 shows this procedure. Free and bound gibberellins were separated according to Reynolds (1970) on Sephadex columns (60 g: 3 × 30 cm) filled with gel G 15. Water was used as the solvent. The flow rate was 0.5 ml/min. 10 ml samples were gathered. The biological activity of partitioned substances was generally examined with the lettuce (cv. Böttner) hypocotyl test according to Frankland et al. (1960). Some samples were bioassayed with the dwarf pea (cv. Kelvedon Wonder) stem growth test after McComb et al. (1958) and barley (cv. S.W.H.N.) half-seed α-amylase release bioassay after Jones et al. (1967). All the experimental results were evaluated statistically.

RESULTS AND DISCUSSION

The preliminary analysis of the control material carried out with the lettuce hypocotyl test indicates considerable differences in biological activity of the individual fractions and exerimental variants. It is especially distinct in relation to fraction A, containing substances isolated from non-hydrolyzed extracts and thus containing free gibberellins. On the histograms (Fig. 2) two zones of outflow from Sephadex column can be distinguished: the first — including samples 1—28 and containing substances with distinct stimulatory effect and the second one in which growth inhibitors of lettuce hypocotyl were eluted. Substances with maximum growth activity of the test material were found in the zone of outflow of 16—21 samples. This zone is identical with that of the standard outflow of gibberellic acid. The character of the substance was confirmed with the lettuce test by the typical reactions to gibberellic acid — the lengthening of lettuce hypocotyl, increase of cotyledons surface and appearence of cotyledons chlorosis (Michalski 1968) as well as the characteristic lengthening of internodes in dwarf pea and also with the amylase test.

A considerable increase in gibberellin-like substances in the control material, during the experiment was noted. The occurrence of gibberellin-like substances in germinating pine seeds, was observed by Michniewicz et al. (1966). The above authors found, that under conditions of normal germination pine seeds contain two gibberellin-like substances with the changeable level.

The analysis of gibberellin-like substances in germinating, irradiated seeds shows sensitivity of these substances to ionizing radiation. A considerable drop in the level of gibberellins occurred when the dose of radiation was increased. This is in accordance with the data of Sideris et al. (1971) concerning the effects of higher doses of gamma radiation on the

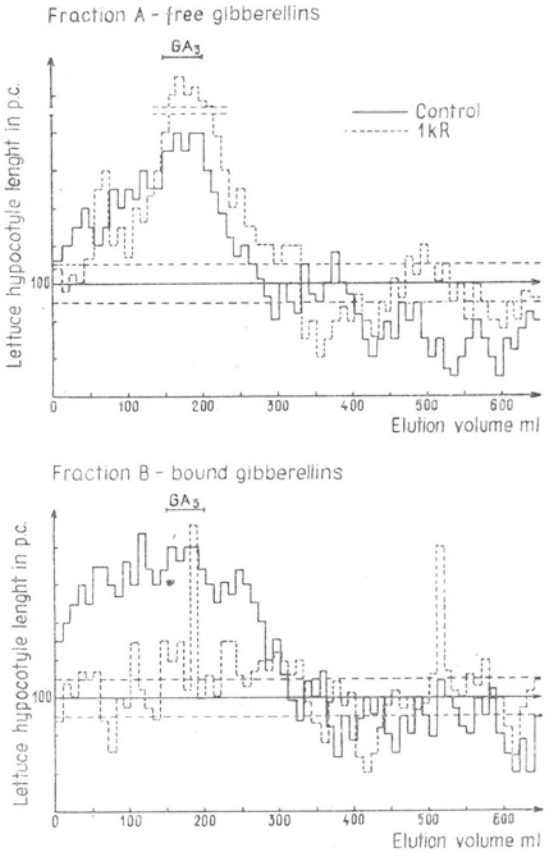

Fig. 2. Histograms of free and bound gibberellin-like substances from 24 hr germinating pine seeds exposed to radiation dose of 1.0 kR

level of gibberellin-like substances in barley seedlings. A surprising exception is the distinct increase of gibberellin-like substances in seeds exposed to the radiation dose of 1.0 kR was the presence of an active substance giving in the individual repetitions of the experiment an increase of lettuce hypocotyl growth even to 300%. The nature of this gibberellin-like substance was confirmed with dwarf pea and amylase tests. The level of this substance drops then so markedly that after 72 hr of germination it is equal with the level of controls. (Fig. 3 — Frac. A).

Fraction B covers bound gibberellin-like substances released from the extract during acid hydrolysis (Fig. 2 — Frac. B). It has been found that after the hydrolysis the level of gibberellin-like substances in controls increased considerably. This fact as well as the quantitative differences between free and bound gibberellins in the material exposed to ionizing radiation can be clearly seen while comparing Fig. 3. The biological analysis of hydrolyzed samples of seeds exposed to a 1.0 kR gamma radiation dose does not show, however a distinct difference in activity of these

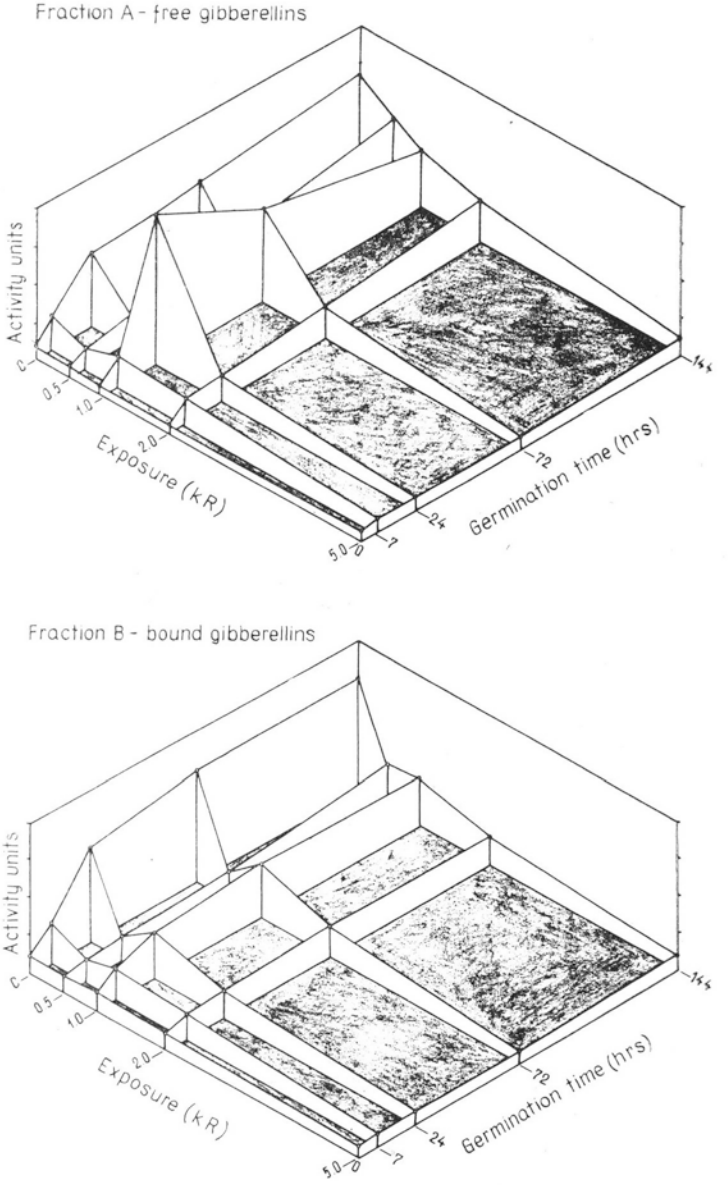

Fig. 3. Biological activity of free and bound gibberellin-like substances in pine seeds in dependence on germination time and radiation dose

gibberellin-like substances. On the other hand some other substances, stimulating considerably growth of lettuce hypocotyl and localized in single samples 19 and 52 have been found. They do not show, however, any growth effect in dwarf pea test and in amylase test.

Comparing the quantitative differences of free and bound giberellin--like substances in the particular experimental variants it may be said

that the total content of free gibberellins in irradiated seeds is higher than of the bound ones. Low level of bound substances in extracts from pine seeds iradiated with 1.0 kR dose and germinating during 24 hr in comparison with the level of free substances leads us to belive, that ionizing radiation exerting considerable influence on plant metabolism may also release the gibberellins from complex compounds. This hypothesis requires, however, more detailed studies.

CONCLUSIONS

1. A dependence of the level of gibberellin-like substances in germinating pine seeds on the applied dose of ionizing radiation was observed.

2. In all germination phases of irradiated seeds a higher level of free gibberellins in comparison to the bound ones was noted.

3. In seeds exposed to radiation dose of 1.0 kR, after 24 hr of germination, especially high level of free gibberellin-like substances was found. On the other hand an increase in the level of bound gibberellins has not been stated.

4. It is assumed that ionizing radiation may release bound gibberellins into free ones.

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Wpływ promieniowania jonizującego na zmiany poziomu substancji giberelinopodobnych podczas kiełkowania nasion sosny

Streszczenie

Metodami ekstrakcji, frakcjonowania, kwaśnej hydrolizy, rozdzielania na kolumnach chromatograficznych i testami biologicznymi badano poziom substancji giberelinopodobnych w nasionach sosny, poddanych promieniowaniu jonizującemu. Stwierdzono, że poziom wolnych i związanych substancji giberelinopodobnych jest zależny od stosowanej dawki promieni gamma. Przypuszcza się, że promieniowanie jonizujące może uwalniać gibereliny związane do wolnych.