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Content of plant growth regulators in the developing seeds of oak (*Quercus robur* L.)*

III. Kinetin-like substances

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Abstract

Kinetin-like substances in developing oak seeds have been investigated. The methods of extraction, fractionation, paper and column chromatography and bioassays, demonstrated that an active cell division factor appears in various phases of seeds development. A high level of this substance has been found mainly in developing acorns. In the course of thier ripening this level decreases considerably.

INTRODUCTION

The occurrence of cytokinins in unripe seeds of many plants is common enough. The investigations to data have dealt mostly with harbaceous plants. Much less is known about the occurrence of cytokinins in seeds of woody plants, especially of forest trees (Letham 1967, Skoog and Amstrong 1970).

Information concerning the content of this kind of growth substances during seed development is very scarce. Some data concerning this problem deal with a few species of herbaceous plants (Witham and Miller 1963, 1965; Burrows and Carr 1970), but not with forest trees. Taking these facts into consideration we decided to study changes in the level of kinetin-like substances in developing oak acorns.

METHODS

Oak seeds (*Quercus robur* L.) from the 1969-1971 harvests were analysed at 7 stages of development (see explanation to Fig. 1) just as in the investigations on gibberellin-like substances and auxins (Michalski 1968, 1969). For extraction in

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aqueous methanol (70 p. c.), samples of seeds were taken with progressively increasing weight, beginning with 25 g for stage I of development up to 100 g in the sample of ripe seeds. For the analysis whole germs in stages I-III (variant I) or only isolated embryos taken from germs in phase IV to VII (variant II) were used.

The active substances, after elementary fractionation, were absorbed by activated charcoal and eluted with n-butanol after Nitsch and Nitsch (1965).

In the first variant of the experiment the eluted substances were partitioned by chromatography on Whatman's paper no. 3 (solvent system: n-butanol-acetic acid-water (2:1:1 v/v) and bioassayed by the *Amaranthus* test, according to the procedure described by Köhler and Conrad, modified by Bigot (1968).

In the second variant, cytokinins were partitioned by column chromatography on Sephadex gel LH-20. The extracts were introduced on a column (bed dimensions: 12 g, 1.2 $\times$26 cm) and developed with methanol as solvent at a flow rate of 0.35 ml/min. One milliliter fractions were investigated spectrophotometrically in the UV at 220-280 and 268 nm wave-length (Spectromom 201-MOM Hung.), and bioassayed by the tobacco pith test (Wis. 38) after Murashigi and Skoog, modified by Rogozinska (1966).

RESULTS AND DISCUSSION

The active kinetin-like substance was determined by paper chromatography. It was localized in the Rf 0.6 — 0.7 zone. The Rf value for the kinetin standard was 0.87. Thus, the chromatographic characteristics of active kinetin-like substance extracted from oak acorn germs show that it is not 6-furfurylaminopurine.

The level of active substance in the earliest stages of development (I-II) determined in the first variant of the experiment was high (Fig. 1). This high level of cytokinins at the beginning of acorn development is probably connected with the stimulation of cell division. In the course of further acorn development stages (III-IV) this level decreases. This phenomenon finds confirmation in the observations of other investigators (Witham and Miller), who report a decrease of the level of these substances at the time of passing from milk to the dry phase of maturity of seeds. The ripening acorns (stages V-VII) contain only small amounts of kinetin-like substances. This fact is supported by the statement that unripe seeds are generally the main source of cytokinins (Letham, Skoog and Amstrong).

In further investigations, the method of partitioning on a Sephadex LH-20 column, yielded some active kinetin-like substances. The diagram of elution from the column shows two peaks of the extinction value at 268 nm wave-length, corresponding to samples 22 (substance B) and 29 (substance A) (Fig. 2). For identification of λ_{max} of absorption of these samples the whole spectrum in the range of 220-280 nm was analyzed (Fig. 3). λ_{max} (268 nm) for substance A (sample 29) is similar to that for standard kinetin (K). Whereas λ_{max} for substance B (sample 22) is considerably shifted in relation to standard kinetin and substance A.

The use, for identification of the biological activity of substances isolated with the help of the Sephadex column, of the method of tabacco pith bioassays showed that

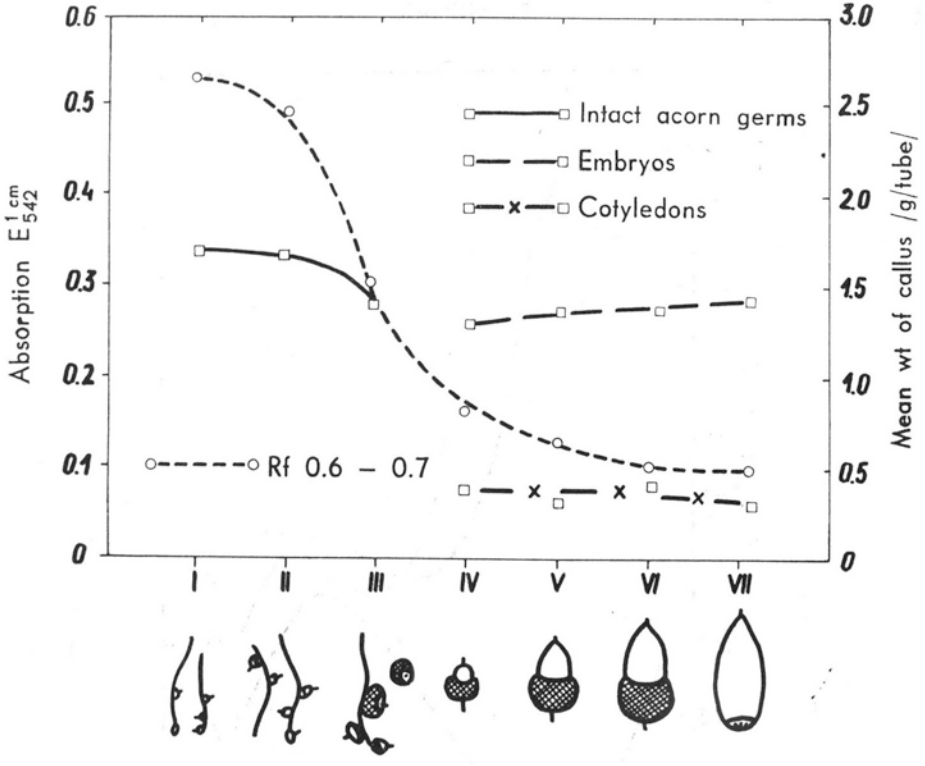

Fig. 1. Dynamics of kinetin-like substances during oak seed development from stages I to VII bioassayed by the *Amaranthus* and tobacco pith test.

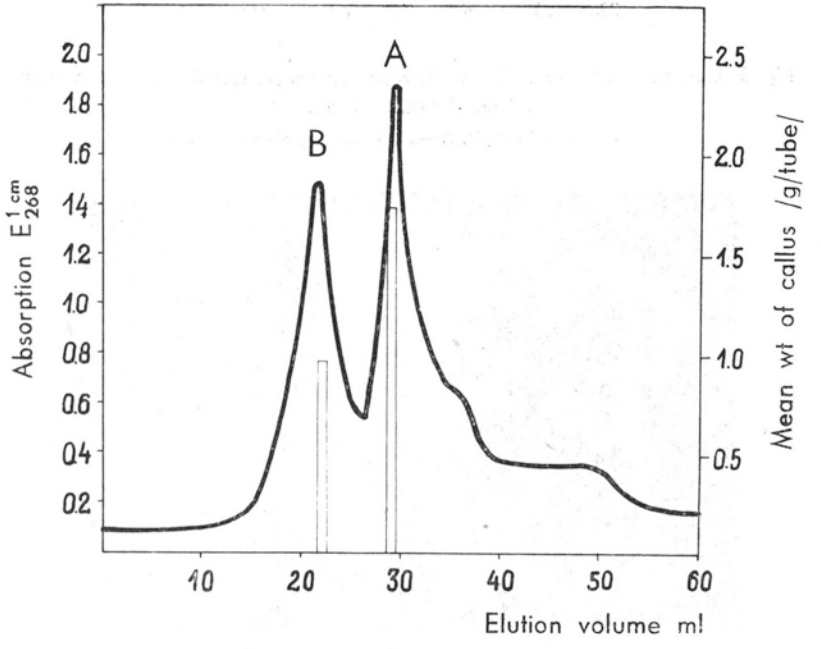

Fig. 2 Elution diagram of kinetin-like substances from acorn germs (II development stage) on Sephadex LH-20.

Solvent: methanol. Bed dimensions: 12 g, 1.2×26 cm. Flow rate: 0.35 ml/min. Sample: 1 ml.

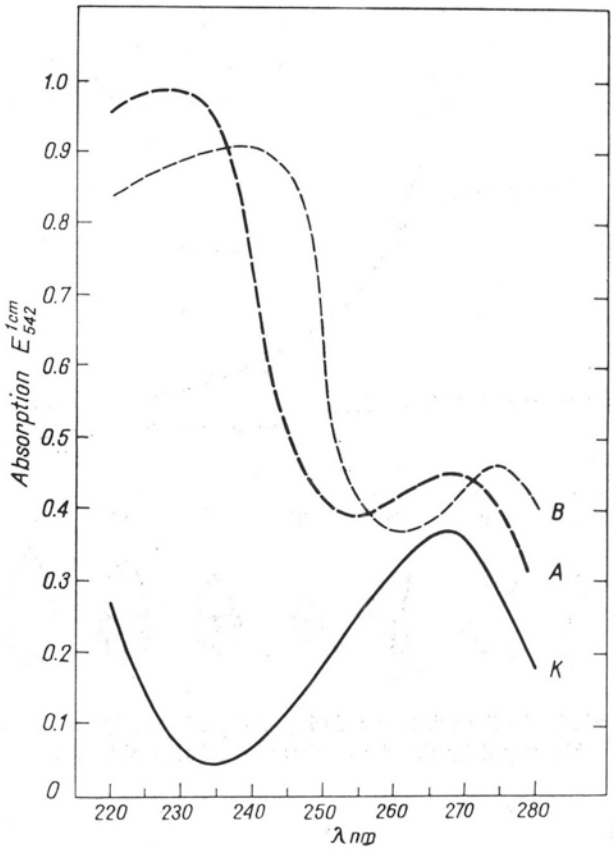

Fig. 3 Absorption spectra of kinetin-like substances separated from acorn germs (II development stage).

A — 29 ml sample, B — 22 ml sample, K — kinetin.

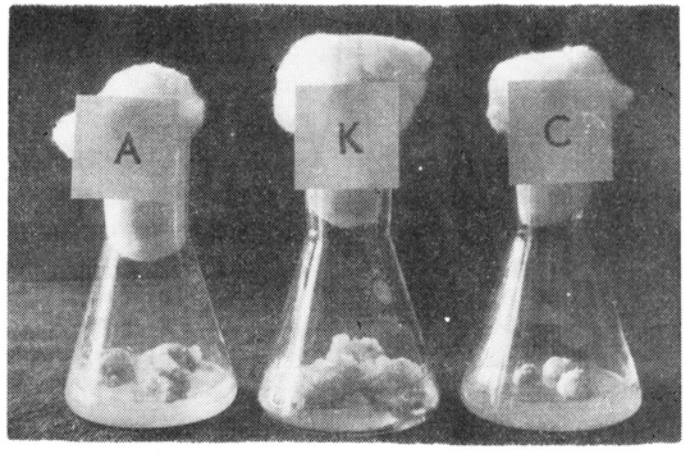

Fig. 4 Growth of tobacco callus as affected by acorn germs extract and kinetin.

A — acorn germs extract (29 ml sample); K — kinetin 0.2 mg/l; C — control (kinetinless)

the mean fresh weight of tobacco callus grown for 4 weeks on a basic medium containing the individual substances, differed considerably between themselves (Fig. 4). For substance A (sample 29) this weight was — 1.750 g. On the other hand, tissue from the control series — K which contained standard kinetin in the amount of 0.2 mg/l. weighed 8.500 g, whereas in the control series C (kinetinless basic medium) it weighed — 0.630 g. The considerable growth of tobacco tissue in the basic medium with the addition of substance A shows distinctly the presence of a cell division factor.

Differences in the level of kinetin-like substances isolated from meristematic tissues were observed, in the second variant of experiment. The level of this substance decreased with advancing growth of seeds.

Plumules and *radicules* isolated from seeds investigated from stage IV on, contain a slightly lower level of kinetin-like substances than the whole germs in stages I — III. This level did not change fundamentally in the embryos in the course of seeds growth. The cell division factor was not found in the cotyledons after application of the investigation methods.

The occurrence of kinetin-like substances in unripe seeds is common enough. They were found in *Zea mays* as zeatin (Miller 1961). In many seeds they were initially determined as ribosylzeatin (Miller 1965, 1967, Letham 1966), or its 5′monophosphate (Miller, Letham). It is also thought that cytokinin isolated from plums is similar to zeatin (Letham), whereas that separated from seeds of yellow lupin is identified with dihydrozeatin (Matsubara et al. 1966, Koshimizu et al. 1967). Many new reports inform about the occurrence in plants of cytokinins of unknown structure (Carr and Burrows 1966, Maheshwari and Gupta 1967, Abou-Mandour et al. 1968), giving, however, the typical growth responses for tobacco tissue. The nature of the substances isolated from oak seeds, however, remains an often question, because the presence of some other, new types of the cell division factor have been revealed (Wood 1964, Wood and Braun 1967, Wood et al. 1969).

CONCLUSIONS

1. Two kinetin-like substances have been found in oak seed germs, analysed in seven development stages.
2. Differentiation in the level of these substances, depending upon the stage of seed development, has been observed.
3. The largest amount of the active cell division factor was found in developing acorn germs. In the course of seed ripening the level of this factor decreases.
4. These substances are located mainly in meristematic tissues and here their level does not undergo any considerable changes. Their presence, however, has not been established in acorn cotyledons.

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Zawartość regulatorów wzrostu roślin w rozwijających się nasionach dębu (*Quercus robur* L.)

III. *Substancje kinetynopodobne.*

Streszczenie

Badano substancje kinetynopodobne w rozwijających się nasionach dębu. Przy pomocy metod ekstracji, frakcjonowania, chromatografii bibułowej, kolumnowej i testów biologicznych stwierdzono występowanie w różnych fazach rozwoju nasion, aktywnego czynnika podziału komórek. Wysoki poziom tego czynnika stwierdzono głównie w rozwijających się żołędziach. W miarę ich dojrzewania poziom ten znacznie się obniżał.