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# The influence of gibberellin on the level of nucleic acids and other phosphate fractions in maize seedlings

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Abstract:

Treatment of maize seedes with $GA_3$ increased the content of water, the phosphate acid soluble fraction, the phospholipid fraction and the low molecular weight r-RNA, s-RNA, DNA-RNA fractions in 5-days-old seedlings. On the other hand, the contents of RNA and DNA in such seedlings decreased.

## INTRODUCTION

In recent years there appeared many publications concerning the role of gibberellins in the activation of biosynthetic processes of nucleic acids and proteins in isolated parts of plants (Paleg 1960; Varner and Chandra 1964; Varner et al. 1965; Filner and Varner 1967; Jarris et al. 1968).

According to some authors, the mechanism of action of this hormone occurs at the gene level, it stimulates the DNA-dependent RNA synthesis, and consequently it influences the formation of specific enzymes (Chandra and Varner 1965; Overbeek 1966; Jarris et al. 1968, Johri and Varner 1967; Pearson and Wareing 1969). Johri and Varner (1967) reported recently that the influence of gibberellin on m-RNA synthesis in the nucleus is not direct but dependent on some cytoplasmatic factor.

Zeevaart (1966) and Overbeek et al. (1967), suggest that gibberellins may act through the cytoplasm as allosteric effectors of repressive proteins. It cannot be excluded, that plant hormones influence directly the activity of some enzymes, causing changes in metabolic processes (Briggs 1963; Fouly and Jung 1966, Jacobson and Varner 1967; Sarkissian and Schmalstieg 1969; Kretowicz et al. 1970).

In spite of many hypotheses explaining various biological effects of this hormone, none of these has found any final experimental confirmation.

The aim of the present study was the investigation of the influence of gibberellin on the level and composition of nucleic acids and other phosphate fractions in maize seedlings.

## MATERIAL AND METHODS

### 1. Material

Maize seed var. 'Wigor' obtained in 1969 from the Scientific Station IHAR in Bąkow were used in these investigations. A part of the seeds was soaked in water for 24 h, the other in 10 mg % water solution of $GA_3$. The seeds were germinated under red light at 25° for 4 days. From the moment of seedlings appearence, they were sprayed twice daily with the solution of $GA_3$.

### 2. Extraction and separation of phosphate fractions

Extraction and separation of phosphate fractions was performed according to the Schmidt-Thannhauser method in the modification for plant material (Urbanek 1963). Phosphate was determined by the Krajewski and Urbanek (1961) method.

### 3. Isolation of RNA and fractionation of RNA on a methylated albumin column (MAK)

Nucleic acids were isolated and purified by the Ralph and Bellamy (1964) method. Isolated and purified nucleic acids showed maximal absorption at 260 nm, minimal absorption at 230 nm. Ratio $E_{max}/E_{min}=0.25$, N=13.8%, P=8.2%, molar ratio N/P=3.37.

Purified RNA samples were separated on a MAK column according to Mandell and Hershey (1960) in the modification of Altmann et al. (1967). The modification consisted in using highly granulated kieselguhr; it allows a good regular and constant flow rate without application of any additional pressure. In our experiments Silica gel 60–80 mesh (L. Light Co. LTD. Colnbrook England) was used.

The solution containing 2 mg of RNA in 0.05 M Tris-HCl buffer pH 6.8 was applied to 2x21 cm MAK column equilibrated with 0,05 Mris-HCL buffer pH 6.8. Various RNA fractions were eluted with a linear gradient of NaCl (0.02–1.3 M) in 0.05 M Tris-HCl buffer, 4-ml fractions were collected. In every fraction the absorption at 260 nm was determined in a 1 cm long light path on a MOM spectrophotometer.

## RESULTS AND DISCUSSION

The results of our studies are presented in Table 1. It appeares from the data presented in this table, that gibberellin-treated plants were in general higher (by about 45%) but the dry weight of these plants decreased by about 22%.

This intensive growth of plant tissue could be according to Broughton (1969), due to cell elongation, because this process is not accompanied by a simultaneous increase in DNA content (Table 2). This author showed that the application of hormone stimulates the synthesis of DNA only in strongly proliferating plant tissues;

Table 1

Influence of $GA_3$ on seedlings growth and their dry weight

| | $GA_3$-treated | Control | Increase of growth % | Loss of weight % |
|---|---|---|---|---|
| Mean growth of seedlings, cm | 7.57 | 5.20 | 45.5 | — |
| Weight of dry substances obtained from 1 g of fresh tissue | 0.099 | 0.081 | — | 22.2 |

Three series of experiments were runn. The date express the mean length from 100 seedlings and the mean value of dry weight from four determinations.

Table 2

Influence of $GA_3$ on total phosphate, acid-soluble phosphate, phospholipid, RNA and DNA fraction contents in maize seedlings (μg P/1 g d.w.)

| | P acid-soluble fraction | P-lipid fraction | P-RNA fraction | P-DNA fraction | Total P |
|---|---|---|---|---|---|
| Control plants | 7625.87 | 677.77 | 1427.99 | 306.39 | 10084.00 |
| $GA_3$-treated plants | 11640.90 | 1007.25 | 1760.38 | 290.06 | 14722.6 |
| Relation of $GA_3$--treated to control % | 152.6 | 148.5 | 123.0 | 93.5 | 145.9 |

it does not influence, however, the amount of this acid in not dividing cells. The elongation of cells and absorption of water in them may be explained by the influence of $GA_3$ on α-amylase activity. The osmotically active compounds which are the products of α-amylase activity increase the suction power of the cell (Overbeek 1966).

The results of our investigations showed a distinct influence of $GA_3$ on the quantitative composition of various phosphate fractions. Total phosphate increased as compared with the control by about 46 %. This influence is particularly pronounced in the case of the acid-soluble phosphate fraction and phospholipid fraction. Although quantitative analysis of the acid-soluble phosphate fraction was not carried out, it is to be expected that its composition includes ceratain phospholabile substances, probably nucleotides, which take part as coenzymes in the intensification of the metabolic processes.

The content of the RNA fraction increased of about 23 % in relation to dry weight of the plant tissue, and the content of DNA decreased by about 6.5 %

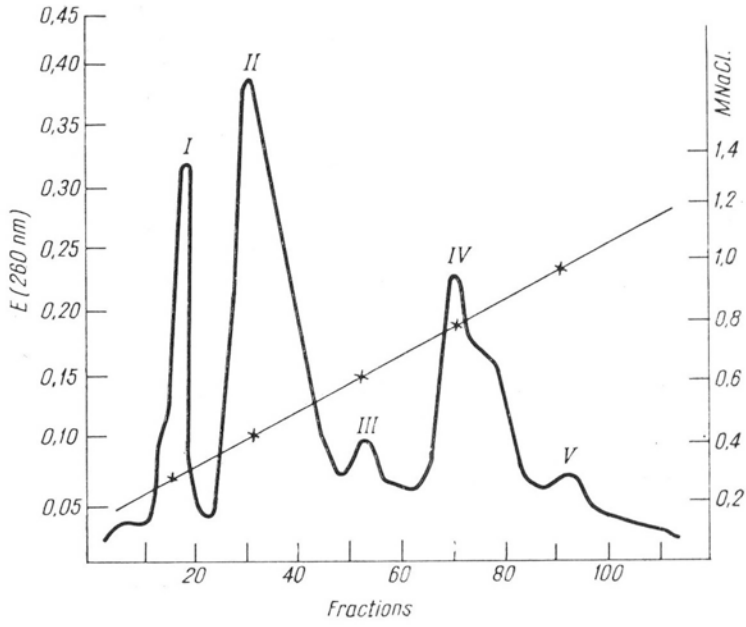


Fig. 1. Chromatographic separation of RNA from control maize seedlings on MAK column
2 mg of purified RNA preprations was applied on a 1×21 cm MAK column, equlibrated wiht 0.05 M Tris-HCl buffer, pH 6.8. Elution of the particular fractions was achieved with a linear gradient of NaCl in 0.05 M Tris-HCl buffer, pH 6.8.

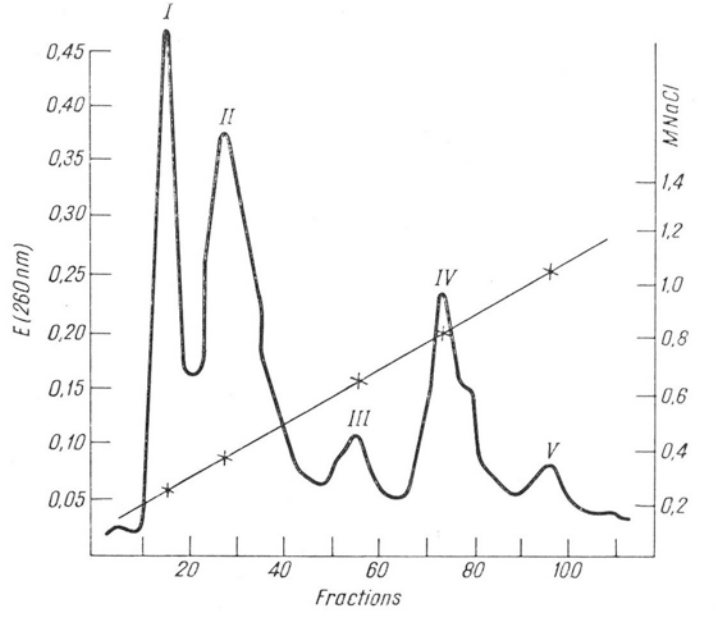


Fig. 2. Chromatographic separation of RNA from $GA_3$-treated maize seedlings on a MAK column
2 mg of purified RNA preparation was applied on a 1×21 cm MAK column, equilibrated with 0.05 M Tris-HCl buffer, pH 6.8. Elution of the particular fractions was achieved with a linear gradient of NaCl in 0.05 M Tris-HCl buffer, pH 6.8.

(Table 2). As seen from the above given results, gibberellin intensifies the biosynthesis of RNA in agreement with the results of other authors (Broughton 1968; Giles and Myers 1966; Nitson and Lang 1966).

Opinions differ, however, as regards the influence of this hormone on the particular RNA fractions. According Sugeura et al. (1962) and Salunkhe et al. (1962), plant hormones primarily activate the synthesis of s-RNA and r-RNA. Inglee et al. (1965) showed that $GA_3$ in intact and isolated tissues of soybean hypocotyls intensifies the synthesis of the complex DNA-RNA and decreases the r-RNA content. Nitson and Lang (1966) sugest on the basis of the experiments on lentil hypocotyls, that gibberellin influences the synthesis of DNA and r-RNA.

These controversial results inclined us to undertake further investigations concerning the influence of $GA_3$ on the qualitative and quantitative composition of RNA in 5-day-old maize seedlings.

In figure 1 the results of fractionation on a MAK column of purified RNA preparations from control samples and in Fig. 2 from gibberellin treated samples are shown.

Table 3

RNA fractions after MAK column chromatography — quantitative comparison of control and gibberellin-treated plants

| | I | II | III | IV | |
|---|---|---|---|---|---|
| | Low molecular weight RNA fraction | s-RNA fraction | Hybrid DNA-RNA fraction | r-RNA fraction | Amount of RNA recovered |
| Fraction no. | 10—22 | 22—48 | 48—65 | 65—88 | 1—120 |
| RNA from control plants | 0.1563 | 0.6729 | 0.1648 | 0.5193 | 1.697 |
| RNA from $GA_3$-treated plants | 0.4057 | 0.5820 | 0.1502 | 0.3684 | 1.700 |
| Relation of $GA_3$-treated to control % | 259.6 | 86.5 | 91.2 | 69.8 | |

RNA coccentration was estimated assuming that the value of 20 extinction unites ad 260 nm (1 cm light path) corresponds 1 mg/ml concentration of RNA.

The data expressed in milligrams of RNA shown in table 3, illustrate the differences between control and gibberellin-treated samples.

Five fractions of nucleic acids were obtained. The first of these resemble those described by Cherry (1967) and correspond to: I — low-molecular weight RNA, II — soluble RNA fraction (s-RNA), III — complex DNA-RNA, IV — ribosomal RNA (r-RNA). The fifth fraction has no parallel in the experiments of the above

mentioned author. The fourth fractions shows distinct malformation which could correspond to the m-RNA fraction, established in Cherry's experiments where $^{32}P$ was used and not discovered by him in the UV.

The quantitative composition of the particular RNA fractions after separation on a MAK column in control preparations and those obtained from gibberellin-treated seedlings show essential differences, namely a decrease of participation of s-RNA, the DNA-RNA complex and r-RNA expressed in percents in relation to appropriate RNA fractions from control plants and amounting to about 13.5%, 8.8% and 30% respectively. On the other hand, the increase in the low-molecular weight RNA fraction was found to be 159%. Taking into consideration the absolute content of the particular fractions, an increase in the s-RNA fraction (about 6%) and the complex of DNA-RNA (about 13%) is observed. The content of the r-RNA fraction decreased, however, by about 15%.

The increase of the absolute content of the DNA-RNA fraction suggests that gibberellin influences the process of transcription of genetic information. Probably the intensive increase in the low molecular weight RNA fraction induced by $GA_3$ treatment is the result of intensive degradation of m-RNA under the influence of RNA-se activated by gibberellin.

## SUMMARY

It was found that $GA_3$ (100 mg/l.) in 5-day-old maize seedlings induces:

1. Intensive elongation of seedlings, increases the water content and decreases dry weight.

2. An intensive increase of phosphate acid-soluble fraction, phospholipid fraction and RNA--fraction. The DNA-fraction content was, however, lower both in dry and wet weight.

3. Chromatographic analysis of RNA from control and $GA_3$-treated plants on a MAK column showed that the increase of total RNA content (123%) was mainly the result of synthesis of the low molecular weight RNA fraction. The content of the DNA-RNA complex increase by about 13% and that of s-RNA by about 8%, The r-RNA content was lower by about 15%.

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## *Wpływ giberelliny na poziom kwasów nukleinowych i innych frakcji fosforanowych w kiełkach kukurydzy*

Streszczenie

Stwierdzono, że $GA_3$ (100 mg/l) indukuje w pięciodniowych kiełkach kukurydzy:

1. Intensywne wydłużanie się kiełków oraz wzrost zawartości w nich wody.

2. Silny wzrost kwasorozpuszczalnej frakcji fosforanowej, mniejszy frakcji lipidowej i RNA przy jednoczesnym spadku zawartości DNA.

Analiza chromatograficzna na kolumnie MAK preparatów RNA uzyskanych z materiału kontrolnego i giberelinowanego wykazała, że wzrost zawartości ogólnego RNA pod wpływem $GA_3$ (123%) był głównie wynikiem syntezy frakcji niskocząsteczkowego RNA. Zawartość hybrydu DNA-RNA wzrastała ok. 13% a s-RNA ok. 8%, przy jednoczesnym spadku zawartości r-RNA o ok. 13%.