AUXIN – INDUCED CHANGES IN THE GROWTH, PEROXIDASE – AND RIBONUCLEASE ACTIVITY OF MAIZE SEEDLINGS

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Received on November 19th, 1971

β-indol acectid acid (IAA) exerts many kinds of effects on the processes of plant metabolism. Numerous data refer to the circumstance that the growth stimulating effect of auxin is accompanied by stimulation of RNA- and protein metabolism: more intensive growth is concomitant with a more rapid synthesis of RNA and protein (N o o d e n, 1968). At the investigation of the effects of auxin, in recent years novel and interesting connections were found, which indicated that IAA was in interralation with the trend of the activity of nucleases: of the enzymes decomposing nucleic acids. Thus e.g. the ribonuclease (RNase) activity of cuts of wheat coleoptile and segments of pea stem decreased upon the effect of 10^{-5} M IAA, while auxin stimulated the elongation of the tissue cuts (C a l d o g n o et al, 1968; T r u e l s e n, 1967). Other findings go to prove a similar tendency in the endocarpium of the bean plant: treatment with auxin reduced the increase of RNase activity concomitant with the senescence of the tissue cuts (and, at the same time an increase of the RNA content was to be observed) (S a c h e r 1969). A connection to this effect was also found in lentil roots (P i l e t and B r a u n 1970). Regarding the senescence of Rhoeo leaves: by cutting off the leaf sections, RNase activity was increased to its 2 – 3fold (with a simultaneous inhibition of uridine- and leucine incorporation), and that effect could be completely "parried" by treatment with auxin (D e L e o and S a c h e r 1970). These results indicate that the effect of auxin is contrary to the processes of senescence, and point to the low RNase level accompanied therewith, as well as to the important part it has in the regulation of that level. The author's work aims at studying some parameters of the growth processes of maize, a plant of basic economic importance. The examinations fit in with the auxin investigations carried out in other plants. The author intendes to clear, how auxin in the nutritive solution acts on growth, on dry weight, on the chlorophyll content, on RNase activity of leaf discs from the seedlings, and how RN-ase

activity can be influenced by treatment with auxin *in vitro*. He also set himself the said aim, since there were few literary references known about the auxin treatment of maize. Among such belongs e.g. the finding, that an IAA treatment of maize coleoptiles caused, at a rise in RNA synthesis, following 6 hours of incubation, a decrease in RNase activity (K o b i l s k i and P o l e v o i 1969).

Material and method

Upon washing with tap water, the maize seeds were sterilized by means of a 3% solution of H_2O_2 for 6 minutes, then, rolled up in straps of humid filter-paper, pregerminated in dark for two days at room-temperature. Then the small seedlings were placed on boxes containing nutritive solution, covered with a plastic net. As culture medium K n o p solution was used (S z a l a y and F r e n y ó 1962), completed by various quantities of auxin, or without any. The applied concentrations of auxin were: 0.1; 1.0;2.0; 8.0; 32.0 and 128.0 mg/l. The vessels for cultivation were kept in a glass-house, at a temperature between 16 and 22°C, under the natural light conditions of day and night.

Of the plants cultivated on solutions of different auxin contents (control solution, ones containing 1.0 and 32.0 mg/l of auxin) leaf discs were cut, and floated on distilled water or on auxin solution under different conditions (dark and 9000 lux) for different periods (6 and 24 hours). By way of control material, plants were also cultivated in soil in a glass-house and with these latter auxin incubation was performed similarly in the way described above.

The growth of the shoots and roots was determined by measuring their length. For determining the dry weight of the variants, 6 samples were taken from the leaves of the seedlings, each between 100 – 150 mg, exactly weighed. These were dried for 2 hours at 110°C, then for 24 hours at 60°C. The weight of the material so treated was determined, and expressed in per cent of the fresh weight.

For the determination of RNase, samples were taken from the fresh material, each weighing 300 mg. Having been cut up, the samples were disintegrated in 2 ml of 0.1 M, pH = 5 acetate buffer solution, in a Potter-Elvehjem homogenizer. The enzyme preparations were then processed by means of a Janetski K – 24 type centrifuge at 6000 rpm for 10, then at 10000 rpm for 25 minutes at 0°C. The leaf discs used in the incubation experiments were rubbed off in 1 ml of the above buffer solution, then centrifuged in an LH – 412-type centrifuge at 0°C.

The determination of RNase activity – whether the material was obtained directly from the plant or from the leaf discs – was done practically according to Truelsen's method, slightly modified (T r u e l s e n 1967). The measure of activity, of the decomposition of the enzyme was expressed by the optical density (OD) unit to be measured at 260 nm.

The chlorophyll content was measured relying on the extinction to be measured in a given volume (663 nm), following methanol trituration of 200 mg samples (O s b o r n e 1962).

For measuring peroxidase activity, samples of 100 mg each were rubbed off, each in 1 ml phosphate buffer of 0.0017 M, pH = 6, and then centrifuged at 0°C for 15 minutes at 15000 rpm. Measuring was carried out by chronologically following the change of extinction to be observed at 420 nm. The composition of the mixture for the reaction: 1.9 ml buffer, 1.0 ml guayacol (0.1%), 1.0 ml H_2O_2 (0.2%) and 0.1. ml enzyme. The data were related to minutes and grames of fresh weight.

Results and their discussion

Maize seedlings kept on KNOP nutritive solution showed varying intensity of growth upon the effect of treatment with auxin of various concentration (0.1 – 128.0 mg/l). Instead of the effect to be expected most, when comparing the cultivation vessels, the author found that the plants grown on cultivation media not containing auxin were greatests in size while, with a rise in the auxin concentration, the inhibition of growth became more and more conspicuous. When evaluating the data, the lengths of the root and shoot were also measured separately (Table and Figure 1). As to be seen from the numerical data, the length of the shoots continuously decreases with the approach towards the higher concentration; at a value of 128.0 mg/l it is but 46% of the length of the control shoot. A similar connection – of inhibitory character – was also revealed when the length of the roots was measured, as it decreased to 70% at 2.0 mg/l and to as little as 35% at 32.0 mg/l. Thus it could be stated that treatment with auxin exerted an inhibitory effect on the length of the roots and shoots of the seedlings, and that the said effect became more and more intensive with increasing concentrations, and attained its maximum at a concentration of 128.0 mg/l; to be considered rather high with auxins.

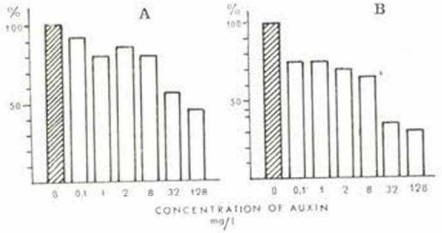

Figure 1. The trends of the length of the shoots (A) and roots (B) of the maize seedlings, expressed as a function of auxin concentration (in per cent of the control)

Table I.

Data of the length of shoots and roots, dry weight, peroxidase and ribonuclease activity, as well as chlorophyll content of maize seedlings, grown in nutritive solutions of various (0.1 mg/l – 128.0 mg/l) concentrations. (In the lines marked with %, the data are expressed at all times in per cent of the control. E_{260} indicates the extinction to be measured at 260 nm, E_{663} that at 663 nm)

IAA (mg/l)	C	0.1	1.0	2.0	8.0	32.0	128.0
LENGTH OF PLANT (cm)	35	32	28	30	28	20	17
standard deviation	±3.0	±2.6	±4.1	±2.0	±3.7	±3.0	±2.2
%	100	92	80	86	80	57	46
LENGTH OF ROOT (cm)	20	15	15	14	13	7	6
standard deviation	±1.4	±1.9	±1.4	±1.2	±1.5	±0.8	±1.5
%	100	75	75	70	65	35	30
DRY WEIGHT (in % of fresh weight)	8.45	8.79	8.31	8.62	9.58	10.57	9.9
standard deviation	±0.58	±0.85	±0.63	±0.68	±0.90	±0.92	±0.3
%	100	104	98	102	113	125	118
RN-ASE ACTIVITY (E_{260} nm)	0.66	0.81	0.77	0.66	0.41	0.83	0.8
standard deviation	±0.04	±0.03	±0.04	±0.08	±0.11	±0.04	±0.03
%	100	120	116	93	62	128	131
PEROXIDASE ACTIVITY (change in extinction) g/min	10.9	18.3	17.7	13.9	17.9	18.4	15.8
standard deviation	±1.41	±1.16	±1.14	±0.69	±1.63	±0.63	±0.51
%	100	175	171	128	174	175	154
CHLOROPHYLL CONTENT (E_{263} nm)	0.33	0.46	0.90	0.75	0.70	0.86	0.90
standard deviation	±0.05	±0.06	±0.05	±0.04	±0.05	±0.07	±0.05
%	100	139	272	227	208	261	270

The trend of the data on dry weight shows a connection of different character (Table and Figure 2). While the variability of the parallels was high enough (although from each of the variants 6 samples were taken), an auxin-induced inhibiting effect failed to appear, since, up to the con-

centration of 2.0 mg/l, it was practically unchanged (the difference related to the control was 4% at most), while from 8.0 mg/l on the dry-weight values were higher than the control (by 13, 25 and 18%). The inhibitory effect ensuing in longitudinal growth did not appear in the data of the dry weight, because at the lower concentration they remained unchanged, and at the 3 highest concentrations they were higher than those of the control. In other words this also means that in these instances water content slightly decraesed, and dry-substance synthesis became relatively more intensive.

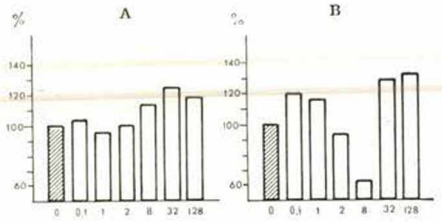

Figure 2. Changes of the dry weight (A) and ribonuclease activity (B) of the maize seedlings expressed in the function of the applied auxin concentrations (in per cent of the control)

The data of the RNase activity of the maize leaf (Table and Figure 2) display no significant differences among the variants. However, it can be stated that in case of the 2 least growing variants – the 32.0 and 128.0 mg/l ones – the activity was higher than 28 and/or 31% as compared to the control. In other words: the inhibition of growth is concomitant with a rise in the RNase level, although the latter is not too high.

The data on peroxidase activity present a different picture (Table and Figure 3). There is a conspicuous divergence between the control and the variants: peroiydase activity is, namely, markedly higher with each variant than with the control seedlings. The increase in activity

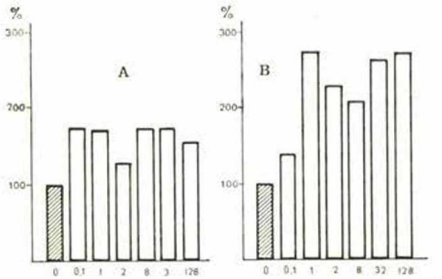

Figure 3. Data on the peroxidase activity (A) and chlorophyll content (B) of the maize seedlings at various auxin concentrations (in per cent of the control)

observed was between 28 – 75%, and that in a way, that with four variants it was 70 – 75%. Auxin concentration seems to have had no particular significance here, the only important circumstance was, whether there was auxin in the nutritive solution or not.

A conspicuous change was found at the valuation of the data on chlorophyll condent. It appeared that the chlorophyll content of seedlings, of which the growth had been inhibited, was nearly three times as high as that of the control plants (Figure 3). The chlorophyll content of the seedlings of inhibited growth seems to increase in an almost direct proportion with the rise of auxin concentration. Curiously, the difference to be observed in the chlorophyll content was clearly visible already while the plants were growing.

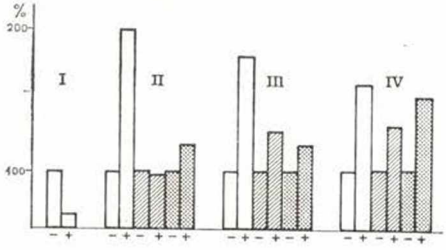

Figure 4a. Changes in the RNase activity of maize leaf discs upon the effect of treatment with 16.0 mg/l auxin. The variants of the treatment: 6 hours in dark (I), 24 hours in dark (II), 6 hours at 9000 lux (III), 24 hours at 9000 lux (IV)

white: leaf discs of plants grown on auxin-free nutritive solution,
striped: on a nutritive solution of 1.0 mg/l auxin content,
dotted: on one of 32.0 mg/l auxin content.
– (minus) = untreated variants
+ (plus) = treated variants

The data are expressed in per cent of the untreated control

Part of the author's experiments on auxin incubation were carried out in the course of the series described above, with a plant material cultivated on nutrivite solutions of various auxin concentration. Besides the control, he worked with two variants, cultivated on other concentrations (of 1.0, respectively of 32.0 mg/l). Basically, the author intended to find out, on the one hand, how the RNase activity of the leaf discs was affected by *in vitro* auxin treatment, and how auxin activity depended on the conditions of the treatment, on the other. It was also interesting to find out further, whether there would appear a difference between the behaviour of the plants not yet treated with auxin in the nutritive solution and of those which had received auxin pretreatment (the plant material grown on solutions of 1.0 and 32.0) mg/l concentrations represents these). Incubation was carried out in a solution containing 16.0 mg/l of IAA, for 6 and/or 24 hours, in dark and/or in a light of 9000 lux.

The result of the series of experiments are summarized in Figure 4a. A 6 hours treatment in dark decreased the RNase activity of the discs cut from the control plants by 30%, while, following upon the other treatments (24 hours dark, 6 hours light, 24 hours light) activity rose, in turn, by 91, 80 and 60%; consequently, an auxin concentration of 16.0 mg/l brought about a significant rise in RNase activity. If leaf discs of plants grown on nutritive solutions of 1.0 mg/l and/or 32.0 mg/l concentration were exposed to the same incubation – which thus means that the treatment with auxin affected the already "pretreated" pieces of plant tissue – much less change in RNase activity was observed. With the plant pretreated with 1.0 mg/l, following a 24 hours' treatment in dark, activity remained unchanged, treatment of 6 hours in light increased activity by 27, that of 24 hours in light by 31%. When the leaf discs of plants grown in a 32.0 mg/l auxin concentration were treated, growths of 19, 18 and 52% were observed.

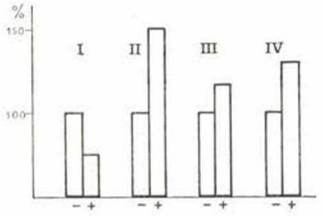

Figure 4b. Trend of RNase activity of leaf discs of maize plant, grown in soil, without pretreatment with auxin, upon the effect of 16.0 mg/l auxin incubation (expressed in per cent of the untreated control)

From the series of experiments it appeared that the RNase activity of the leaves of seedlings grown on nutritive solution was increased to various degrees by 6 or 24 hours of auxin treatment taken place in dark or in a light of 9000 lux. It was the control plant which proved to be the most sensitive, on the other hand, the increase in activity to be observed with the plants cultivated on solutions containing auxin, was less. Incubation in light proved, in general, to be more effective. In connection with the results of the experiments, the question came up, whether, following auxin incubation, the plants grown on nutritive solutions not containing auxin but cultivated in soil in glass-houses, did behave in the same way. For this reason the author counducted experiments identical with the ones described above, with leaves of plants grown in soil and not treated with auxin (Figure 4b). As to their tendency, the obtained results were concordant with those of the preceding series of experiments. A treatment of 6 hours in dark caused though a 25% decrease, however, the other variants showed increases of 52, 16 and 30% in RNase activity.

As a summary of the author's results it can be said, that auxin treatment raised the RNase activity of the leaf discs of the plants in nearly all instances; wether the plant had grown on a nutritive solution or in soil. The conditions (duration, light) did not essentially influence the effect.

The said results seem interesting because in some other subjects just the opposite effects were demonstrated (Caldogno et al., 1968; Pilet and Braun, 1970; Truelsen, 1967); that is, attention was called to the effect of auxin decreasing RNase activity, and the latter was brought into connection with other metabolic processes and/or growth processes induced by auxin. On the other hand, in case of maize seedlings – according to the data of the author – the presence of auxin in the nutritive solution had an inhibitory effect on growth, it did not lower the RNase level; at the same time some increase in dry weight, a marked rise in chlorophyll content and a high peroxidase level as compared with the control could be demonstrated. Regarding the explanation of the inhibition of growth, the author also had to take the possibility into consideration that in case of maize, the optimal interval with auxin – consequently the one stimulating growth – would be entirely different from intervals of concentration recognized in other instances and found to be surprisingly accordant with one another up to now. Further experiments conducted with plant material did bring the result discussed above: i.e. in this case treatment with auxin could not parry the increase in RNase activity indicative of senescence. Consequently, it can be stated that in the author's series of experiments the effects and consequences of auxin treatment differ from the data to be found in the literature in many respects. On the one hand, the inhibition of longitudinal growth and the rise in peroxidase activity, on the other hand the marked accumulation of chlorophyll denote that – under the conditions of the applied concentrations – auxin had an antagonistic effect in the various spheres of metabolism. Another curious phenomenon of this kind: the aging effect of the auxin incubation of the leaf discs was more intensive than the control with distilled water. The fact may supply with new data for the examinations on the senescence of plants. The experimental findings also point out that the effects of the plant hormones extend to several metabolic processes, and are composed of intricate – often seemingly contradictory elements.

Summary

Maize seedlings were cultivated on nutritive solutions containing various concentrations of auxin (IAA). The lenght of the shoots and roots, the dry weight of the leaf, the RNase and peroxidase activity of the leaf homogenate, as well as its chlorophyll content were assessed. The RNase activity of the discs cut from the leaves of the plants grown in auxin – containing and in control nutritive solutions was examined, too, upon a 16.0 mg/l auxin incubation lasting for different periods (6, 24 hours), and under different conditions of light (dark and 9000 lux). The

same examination was carried out with leaf discs from seedlings grown in soil and not pretreated with auxin.

Regarding plants cultivated on nutritive solutions of 0.1 – 128.0. mg/l auxin concentrations, the following could be stated:

a) On the length of the shoots and roots of the plants, the growing concentration of auxin exerted an increasing inhibition.

b) The dry weight of the leaves changed only in the variants, of which the growth was inhibited most (32.0, 128.0 mg/l): it showed a slight increase.

c) The RNase level remained unchanged with practically all variants, no decrease in RNase was found.

d) As compared with the control, peroxidase activity was significantly higher with each variant, and this increase was seemingly independent of the applied concentration of auxin.

From the data supplied by the auxin incubation of the leaf discs it appears that:

Auxin incubation (16.0 mg/l) increased RNase activity, the increase was greatest with the control plant – i.e. with one cultivated in auxin-free nutritive solution –, while at the incubation of the discs of plants grown on auxin, the rise was less.

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