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Changes in some nitrogen fractions, protease and RNA depolymerase activity during induction by auxin of adventitious roots in tomato leaves cuttings

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Abstract

On tomato leaf cuttings treated with 10^{-4} M IAA for 24 h and then placed for 24 h in an incubation solution induces after 4 days the appearance of adventitious roots. Experiments with actinomycin D and chloramfenicol indicate that a period of 2—3 days is essential for the synthesis of protein specific for rooting. It was found that in this period a number of characteristic changes in metabolism occurs in cuttings treated in this way. A decrease of insoluble nitrogen (N_p) in the leaf blades was noted with simultaneous synthesis of this fraction and an increase of the soluble nitrogen fraction amount (N_s) in the basal part of the petiole. In this period an enhanced activity of proteolytic enzymes is observed in the blades of leaf cuttings treated with IAA, and a decrease of activity of these enzymes and of RNA depolymerases in the basal part of the petiole. It is suggested that treatment of the cuttings with IAA solution elicits in them a number of changes in metabolic processes, leading as consequence to initiation of adventitious roots.

INTRODUCTION

It is known that the appearance of adventitious roots on plant cuttings is regulated by the presence of auxin. There are, however, but few data concerning the mechanism of auxin action in regeneration of this kind.

According to Gregory and Samantari (1950), auxin stimulates rooting by increasing the activity of hydrolytic enzymes which mobilize the nutritional reserves of the cuttings. Similar observations were reported by Nanda et al. (1968). Basu and Pal (1966) and Basu et al. (1968) could not reveal a direct correlation between the stimulating action of auxin and the role of these enzymes in initiation of hydrolysis of nutrition reserves. On the other hand, they observed synthesis of proteins and

nucleic acids. Stimulation of amino acids incorporation into proteins in cuttings of *Justicia gendorussa* forming roots under the influence of auxin was observed by Ghos (1971). Overbeek et al. (1946) in experiments on rooting of *Hibiscus rosa sinensis* L. branches demonstrated marked protein synthesis in the basal part of the branches at the site of development of adventitious roots.

There is no doubt, therefore, that transformation of nitrogen compounds in cuttings during root formation by them is essential for processes of regeneration of adventitious roots primordia. It also seems that auxin plays an important role in the regulation of transformation of nitrogen compounds in rooting cuttings.

In this connection changes in the content of certain nitrogen fractions and in proteolytic enzymes and RNA depolymerase activity were studied in tomato seedlings stimulated with auxin in the period of adventitious roots development.

As the object of investigations served tomato leaf cuttings.

MATERIAL AND METHODS

Preparation of material. Several-day tomato seedlings (*Lycopersicum esculentum*, variety Stonor Wyniosły) were placed in pots with compost soil in a glasshouse under good illumination. After about 30 days the 3 oldest leaves were cut from each plant at the base of the petiole. They were placed for 24 h in beakers with 10^{-4} M indolylacetic acid solution (IAA) so that the petioles were immersed to a depth of 2 cm. After 24 h incubation with auxin the cuttings were taken out, the petioles were washed with distilled water and placed in dark glass jars of 50 ml capacity. In each jar 3 cuttings were placed with the petioles immersed up to about 2 cm in tap water. During the 9 days of experiment the jars with the leaves were kept in a chamber with high relative moisture (95%) under dispersed light. In the course of root formation the adventitious roots were counted and analyses were performed.

Analyses of the nitrogen fractions. The nitrogen fractions were determined in dry material, in the leaf blades and the basal part of the petioles (zone of adventitious roots formation). In the course of root formation 10 leaf cuttings were taken from each combination every day (always at the same hour) and the blades were detached from the petioles and dried at 105° C for 15 min to interrupt the enzymatic reactions and then at 65° C up to constant weight. Before drying the adventitious roots were cut off with a scalpel. After drying the material was ground in a mortar and stored in weighing bottles in an exsiccator.

For extractions 20—30-mg portions of dry material were taken. Soluble nitrogen fractions were extracted 3 times in 3 ml of 70 per cent et-

hanol on a shaker at 30°C (Puritch, 1967). The sediment was centrifuged off each time for 20 min at 3 000 r.p.m. and the supernatant was decanted. The combined supernatants constituted the soluble nitrogen fraction (N_s), whereas the nitrogen remaining in the sediment was considered as insoluble nitrogen (N_p). The soluble fraction consisted of inorganic ammonium nitrogen, amino acid and amide nitrogen, nitrogen of peptides and prolamine nitrogen. The insoluble fraction comprised mainly protein and nucleic acid nitrogen.

Both nitrogen fractions were determined by Kjeldahl's micromethod (Mejbaum-Katzenellenbogen Mochnacka, 1969).

Proteolytic activity. Enzyme extract from tissues was prepared from fresh material according to the method of Beevers (1968). Two grams of fresh leaf blades or of the basal part of petioles were homogenized in 7 ml of 0.05 M Tris with 0.005 M cystein hydrochloride at pH 7.5. The homogenate was filtered through 4-fold cheesecloth and centrifuged at 12 000 g for 20 min. The supernatant was dialysed for 24 h, the dialysing solution (0.01 M phosphate buffer, pH 7) was changed four times, then the extract was centrifuged once more for 20 min at 12 000 g. The sediment was discarded and the supernatant was used as crude enzyme extract. All the above described procedures were performed at a temperature of 0° to +3°C. Proteolytic activity was determined by the method described by Rosen (1957). One milliliter of the enzyme extract was incubated with 1 ml of 1 per cent bovine hemoglobin and 1 ml 0.2 M phosphate buffer, pH 7.0 on a water bath at 40°C for 90 min. The reaction was stopped by adding 1 ml of 20 per cent trichloroacetic acid, and after centrifugation at 3 000 g for 15 min enzymatic activity was determined in the supernatant. To 1 ml of supernatant 0.5 ml of cyan acetate and 0.5 ml of ninhydrin solution prepared after Mejbaum-Katzenellenbogen and Mochnacka (1969) were added. The mixture was boiled for 15 min on a water bath at 100°C. Immediately after removal from the water bath 6 ml of isopropanol diluted 1 : 1 with distilled water were added and the whole was thoroughly shaken. After cooling to room temperature extinction was measured on a Spekol spectrophotometer at wavelength 570 nm, and the amount of free NH_2 groups was read from the standard curve for L-leucin. Enzyme activity was expressed as total activity (amount of product per gram of plant material) or as specific activity (amount of product released in the incubation reaction during 1 h per 1 mg protein).

RNA depolymerase activity. The plant material was extracted with acetate buffer, pH 5.0 with 0.25 M sucrose in a proportion of 5 ml extracting solution per 1 g of tissue. The extract was filtered through fourfold cheesecloth and centrifuged at 13 000 g. The whole procedure was performed at 0°C. The supernatant was used for determination of enzyme activity according to Anfinsen et al. (1954).

The clear supernatant in the amount of 0.1 ml was made up with water to 1 ml centrifuge tubes. To the each sample 1 ml of freshly prepared orcin reagent (100 ml of conc. HCl + 57 mg $FeCl_3$ + 10 mg orcin per 1 ml of acid) was added. The samples were incubated for 20 min on a boiling water bath. After cooling incubation mixture was diluted with distilled water to 4 ml. Extinction was measured in a Spekol spectrophotometer at 670 nm and the amount of free ribose was read from the standard curve (Mejbaum-Katzenellenbogen, Mochnacka, 1969). The enzyme activity was expressed as specific activity (amount of product released in incubation reaction per 1 mg of protein). The amount of protein in the extract was determined after Lowry et al. (1951). As standard served bovine albumin, fraction V.

Actinomycin D and chloramfenicol were used in concentration of 10 μg per 1 ml.

Reagents. All the reagents used were Sigma Chemical Company products.

RESULTS

The cut leaves were immersed by their petioles for 24 h in an auxin solution or in water and then transferred for 9 days to tap water.

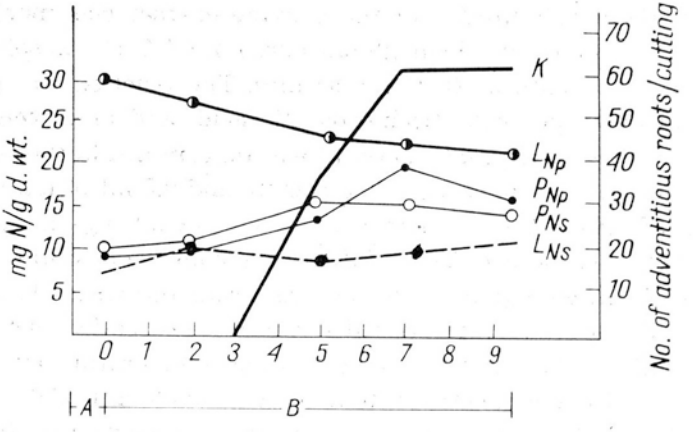

Fig. 1. Influence of 10^{-4} M IAA stimulating adventitious roots formation and changes in content of nitrogen fractions A — "induction time", B — incubation time, L_{Ns} — soluble nitrogen in leaf blades, L_{Np} — insoluble nitrogen in leaf blades, P_{Ns} — soluble nitrogen in petioles, P_{Np} — insoluble nitrogen in petioles, K — number of adventitious roots

Figures 1 and 2 show the changes in the content of the soluble (N_s) and insoluble (N_p) nitrogen fractions, induced by indolylacetic acid (Fig. 1) and the same values for the controls (Fig. 2) in the course of development of adventitious roots as well as the number of roots formed in both combinations.

As seen from the data in figure 1, the decrease in the insoluble nitrogen fraction content in the leaf blades stimulated with auxin starts almost at once after transfer of the leaves to water after 24-h induction and is rather large up to the initiation of adventitious roots (5th day). In the blades of leaves not stimulated with auxin (Fig. 2) the decrease of insoluble nitrogen content starts as late as 6 days after incubation of the petioles in water. The rate of insoluble nitrogen disappearance from the blades of leaves treated with auxin is markedly diminished after development of adventitious roots and ceases almost completely as the number of these roots increases and their growth proceeds. A similar relation is not observed in leaves not subjected to induction (Fig. 2). Simultaneously with the decrease in insoluble nitrogen content in the leaf blades treated with IAA, the amount of this fraction in the basal part of the petiole increases up to the 7th day after induction, that is to the moment of formation of the maximal number of adventitious roots. A similar relation is not noted in leaves not subjected to induction.

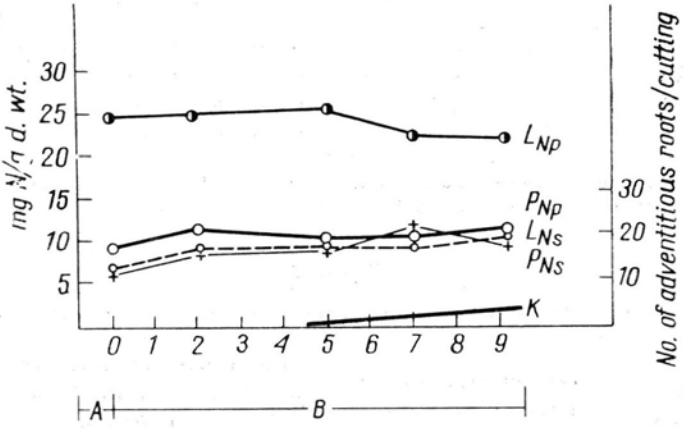

Fig. 2. Root formation and changes in nitrogen fraction, in control combination (leaves not stimulated with auxin) A — "induction time" (H_2O), B — incubation time, L_{Ns} — soluble nitrogen in leaf blades, L_{Np} — insoluble nitrogen in leaf blades, P_{Ns} — soluble nitrogen in petioles, P_{Np} — insoluble nitrogen in petioles, K — number of adventitious roots

The amount of soluble nitrogen (N_s) in the leaf blades treated with IAA is about the same as that in leaves not subjected to induction, this indicating that the products of insoluble nitrogen breakdown in the blades of leaves stimulated with IAA are transported to the petioles. The amount of soluble nitrogen in the basal part of the petiole of IAA-treated leaves steadily rises to about the 5th day and then stabilizes at a constant level. It is worth noting that on the 5th day the decrease of insoluble nitrogen in leaves treated with IAA is stopped. No similar relations were observed in the blades of leaves not treated with auxin.

Investigations of enzyme activity were preceded by establishment of the period indispensable for initiation of adventitious roots primordia by auxin ("induction time"). For this purpose actinomycin D was used as a known inhibitor of DNA-dependent RNA synthesis and chloramfenicol as inhibitor of protein synthesis.

Tomato leaves after 24-h induction with auxin were directly transferred to water or to inhibitor solution for the incubation period, or transferred to water for a period of 1, 2 or 3 days and then to inhibitor solution (Tables 1 and 2). In all combinations the appearing adventitious roots were counted every day.

Table 1

Influence of actinomycin D on adventitious roots formation after induction with auxin

Induction with	Day on which act. D was added to the incubation solution	Mean number of adventitious roots per cutting on paritcular days of incubation									
		1	2	3	4	5	6	7	8	9	10
IAA	—	0	0	0	3	12	19	30	30	43	48
IAA	1	0	0	0	0	0	0	0	0	0	0
IAA	2	0	0	0	0	0	0	0	0	0	0
IAA	3	0	0	0	1	10	10	18	18	24	27

The petioles of the cuttings were placed after exposure for 24 h to 10^{-4} M auxin, in water or in actinomycin D (10 μg/ml) and in water from which they were transferred after 1 or 2 days to actinomycin D solution

Table 2

Effect of chloramphenicol on adventitious roots formation after induction with auxin

Induction with	Day on which chloramphenicol was added to incubation solution	Mean number of adventitious roots per cutting on particular days after incubation									
		1	2	3	4	5	6	7	8	9	10
IAA	—	0	0	0	7	13	30	68	70	70	70
IAA	1	0	0	0	0	0	0	0	0	0	0
IAA	2	0	0	0	0	0	0	0	0	0	0
IAA	3	0	0	0	0	0	0	0	0	0	0
IAA	4	0	0	0	6	9	18	42	57	61	61

The petioles of the cuttings were placed after 24 h of exposure to auxin (10^{-4} M) in water or in chloramphenicol solution (10 μg/ml) and in water to which after 1, 2 and 4 days chloramphenicol was added

Actinomycin D applied immediately or on the 2nd day after soaking the petioles in IAA solution completely suppres the effect of auxin as factor inducing root formation. The addition of actinomycin D into the incubation solution on the 3rd day after induction with auxin did not prevent root formation. A similar relation occurred in respect to chloram-

fenicol, with the difference that the latter substance inhibited the inductory effect of auxin for 3 days after soaking (Table 2).

Since the above described experiments indicate that synthesis of protein specific for root formation occurs in the first 2 days after induction with auxin, further experiments on enzyme activity were restricted to activity measurements within 3—4 days after incubation with auxin.

The total proteolytic activity of the leaf petiols, notwithstanding whether they were stimulated with auxin or not stimulated, drastically increased 24 h after detachment of the leaves from the plant (Fig. 3). In fur-

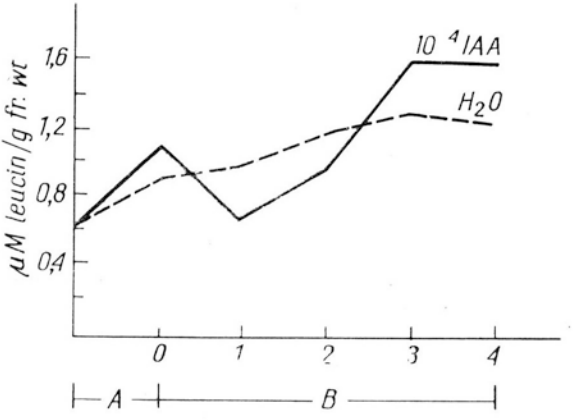

Fig. 3. Changes in proteolytic activity in basal part of petiole during 4-day incubation of tomato leaves stimulated with auxin and unstimulated ones. A — "induction time", B — incubation time

ther days of measurement there occur, however, significant changes in enzyme activity in both combinations. In the petioles of control leaves (not stimulated) total proteolytic activity steadily rises to the 3rd day of incubation and then remains at a constant level. On the other hand, in petioles of auxin-stimulated leaves, after an initial intensive increase of proteolytic activity during induction with auxin, a rapid decrease of enzyme activity by about 40 per cent as compared with the control is observed. Two days after induction with auxin, in spite of a certain rise of activity, its level is lower than in the control. It is only on the third day that the activity rises considerably and like in the control stabilizes at this higher level.

The course of proteolytic activity in the leaf blades is shown in Table 3. Twenty four hours after detachment of the leaf from the plant proteolytic activity in the blades increased both when the leaves were soaked in auxin and in water. In the combination without stimulation a higher rise of enzyme activity was observed, but in the successive days after transfer of the leaves to neutral conditions (tap water) the activity in the leaves stimulated with IAA remained at a much higher level than in the controls and was constant for about 3 days after incubation.

Table 3

Effect of 10^{-4} M IAA on proteolytic activity in leaf blades during the period of 4-day incubation after exposure to auxin

Combination	Detachment of leaves 0	Exposure to auxin h 24	Incubation time, days			
			1	2	3	4
Control	75.8	116.0	28.1	76.0	37.2	63.4
IAA 10^{-4} M	75.8	83.4	102.0	96.4	113.0	72.2

Enzyme activity expressed as specific activity in amount of μmoles leucin per mg of protein per hour

Thus, auxin reduced the proteolytic activity in the petiole with its simultaneous increase in the leaf blade.

In order to gain a better knowledge of the variations in the proteolytic activity and to demonstrate whether newly formed enzymic protein is involved, an experiment was performed in which, after induction with auxin for 24 h, the leaves were transferred to water or to an actinomycin D solution of 10 μg per 1 ml concentration. In the combination with actinomycin D the adventitious roots, as was to be expected, did not develop. In the course of the first 3 days after treatment with IAA proteolytic activity was measured in the basal part of the petiole and in the leaf blade. As seen from the data in Table 4, the presence of actinomycin D in the incubation medium after induction with auxin enhanced proteolytic activity both in the petioles and the blades. A similar increase of proteolytic activity caused by actinomycin D was observed in non induced blades and petioles (Table 5).

The experiment with 3 replications concerning root formation with simultaneous measurement of changes in the activity of the RNA-decomposing enzymes in the basal part of the petiole showed that in the petioles of leaves treated with auxin a marked fall of the RNA depolymerases activity occurs (Diagram 4). This fall reaches about 32 per cent of the initial value, and the enzyme activity remains at this low level for 2 days, thus for the time necessary for synthesis of nucleic acids active probably in

Table 4

Proteolytic activity in petioles and leaf blades after exposure to auxin in the presence of actinomycin D in the incubation solution

Part of cutting	Induction with	Incubation with	Days after exposure to auxin		
			1	2	3
Petioles	IAA	H_2O	57.1	33.4	36.2
Petioles	IAA	Act. D	75.7	80.1	73.2
Leaf blades	IAA	H_2O	102.5	96.0	113.9
Leaf blades	IAA	Act. D	115.8	152.8	208.3

Explanations as in Table 3

Table 5

Proteolytic activity in petioles and leaf blades not exposed to auxin, in presence of actinomycin *D* in the incubation medium

Part of cutting	Induction with	Incubation with	Days after exposure to water		
			1	2	3
Petioles	H_2O	H_2O	66.6	33.2	40.1
Petioles	H_2O	Act. D	90.7	73,2	68.4
Leaf blades	H_2O	H_2O	28.8	76.4	37.2
Leaf blades	H_2O	Act. D	30.6	107.0	160.5

Explanations as in Table 3 and 4

root formation. At the same time in the control (unstimulated leaves) the enzyme activity drastically increases up to as much as 212 per cent as

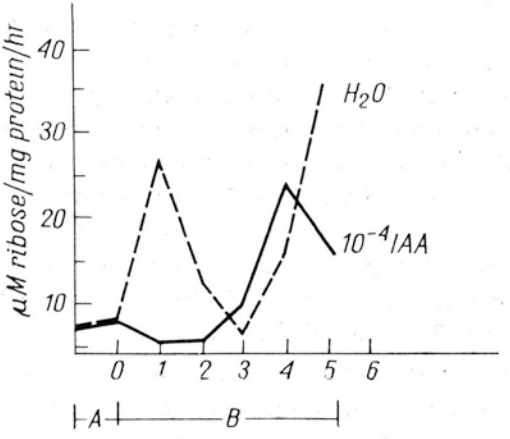

Fig. 4. Changes in RNA depolymerase activity in petioles in the course of adventitious root emergence in cuttings treated with auxin and untreated. A — "induction time".

compared with the initial value. From the second day (in the combination with auxin induction) and on the 3rd day (in the control combination) an increased enzyme activity was observed.

DISCUSSION

The here described experiments show that in the course of root formation on tomato cuttings there occur in them intensive transformations of nitrogen compounds. Significant differences are noted in the content of nitrogen fractions in cuttings stimulated with auxin and in untreated ones. The fact that in leaf blades of cuttings previously stimulated with auxin an intensive breakdown of the insoluble nitrogen fraction and an increase

of this fraction content in the petioles occur indicates that auxin causes a mobilization of nitrogen reserves in the blade and probably their transport to the petiole. The experiments thus indicate that the vigorous formation of adventitious roots on leaf petioles exposed to the action of auxin is due not only to the hydrolysis of protein substances in the leaf blade and transfer of the products of hydrolysis to the petioles, but also to simultaneous protein synthesis at the site of development of root primordia (Figure 1). No such relation was observed in the case of leaves not treated with auxin (Figure 2). These observations are in agreement with the results of Overbeek (1946) concerning root formation by Hibiscus rosa sinensis branches, those of Frankel (1974) with bean hypocotyls and the experiments of Chibnal (1954) with the use of labelled nitrogen.

The results of experiments in which chloramfenicol and actinomycin D solution were used as inhibitors abolishing many effects of auxin (Tanimoto, 1968; Coartney, 1967; Nooden, 1965; Patterson 1967; Buczek, 1969, 1971) suggest that for root formation the period following immediately induction with auxin is extremely important. This period for tomato leaf cuttings lasts 2—3 days. During this time auxin elicited an enhanced protein breakdown (decrease in the insoluble nitrogen fraction) in leaf blade and an increase of the content of this fraction in the basal part of the petiole. These results find confirmation in the experiments which showed that in leaves immersed for 24 h in IAA there occurs an important increase of proteolytic activity in the blades as compared with controls, whereas this activity declines in the petioles as compared with that in controls (Table 3, Diagram 3).

It is a known fact (Chibnal, 1954) that detachment of leaves from the mother plant enhances in them proteolytic activity. As demonstrated by the present experiments, an increase of proteolytic activity is observed both in the blade and the petiole of leaves directly after their cutting (Figure 3, Table 3). Soaking of the petioles in IAA, however, almost immediately inhibited the enzyme activity in the petioles and increased that in the leaf blades. On the other hand, experiments with actinomycin D suggest that it is not induction or inhibition of enzymatic protein synthesis that comes into play here, but auxin seems rather to exert an inhibitory influence indirectly on the activity of proteolytic enzymes at the site of root emergence, and simultaneously to enhance their activity in the leaf blades. Green (1973), Gurusiddaiah (1972) and Ryan (1968, 1970, 1973) established that under the influence of injury an enhanced production of inhibitors occurs in plants, counteracting proteolytic enzymes. The enhanced proteolytic activity in blades exposed to IAA may result from the fact that auxin can reduce proteolytic inhibitor accumulation (Ryan, 1973) and in this way the proteolytic activity in the leaf blade may be enhanced.

Since experiments with actinomycin D (Tables 4 and 5) seem rather to indicate a constant presence of proteolytic enzymes, it may be assumed that the antibiotic applied has an inhibitory effect on the synthesis of protease inhibitor proteins, and in this connection an increased activity of proteolytic enzymes is observed.

It would seem that enhanced accumulation of protein degradation products in the petioles of leaves stimulated with auxin at the cost of breakdown of the insoluble nitrogen fraction in the leaf blade may be the cause of the decline in proteolytic activity in the petioles of cuttings exposed to auxin.

It is believed, therefore, that for the process of rooting a depression of proteolytic activity in tomato leaf petioles is very important, that is at the site of formation of adventitious roots primordia.

The present investigations seem to indicate that auxin in some way so far unclear depresses the proteolytic activity in the petioles during the "induction time". It is not excluded that this may be a general property of auxin, since, as shown by our studies, the activity of RNA depolymerases also decreased in the petioles treated with IAA exactly in the period important for root formation, referred to here as the "induction time". The depressing influence of auxin on RNA depolymerase activity is particularly pronounced when compared with the activity of these enzymes in control petioles (not treated with auxin).

Depression of RN-ase activity by phytohormones has been observed in many cases (Fletcher, 1965; Srivastava, 1965, 1968; Osborne, 1962; Pilet, 1970, 1971; Gordon, 1973).

The depression of RNA depolymerase and proteolytic enzymes activity at the site of adventitious roots formation is extremely important in the processes of rooting when numerous cell divisions, protein and nucleic acids syntheses occur in the petioles.

Tomato leaves detached from the mother plant and incubated in water without previous treatment with IAA showed in the present experiments a similar metabolism as that exhibited by dying tissues or fragments which, beside increasing protein degradation (Sacher, 1965, 1969, 1973; Hochkeppel, 1973, Pitt, 1974) show a raised ribonuclease level (Udwardy, 1969, 1972; Phillips, 1969).

It is not excluded that some of the observed changes in enzyme activity are not the immediate cause of adventitious roots development, but may rather be their consequence and the result of metabolic changes evoked by this process. Nevertheless all the facts form together a certain logical picture. Treatment of tomato leaf cuttings with auxin induces root formation after about 4—6 days. The appearance of adventitious roots is preceded by metabolic changes which involve among other processes, the breakdown of the insoluble nitrogen fraction in the leaf blade, rapid transfer of these decomposition products to the petiole, intensive synthesis of

protein compounds in the basal part of the petiole and depression of proteolitic enzymes and RNA depolymerase activity at the site of emergence of root primordia. Since no such relations were observed in leaf cuttings not subjected to induction with auxin, it may be suggested that IAA induces directly or indirectly a number of metabolic changes leading as consequence to the formation of adventitious roots.

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REFERENCES

Anfinsen C. B., Redfield R. R., Choate W. L., Dage J., Carroll W. R. 1954. Studies on the gross structure, cross-linkages and terminal sequences in ribonuclease. J. Biol. Chem. 207: 201—210.

Basu R. N., Pal S. R. 1966. An analysis of the effect of sucrose feeding on root formation in soft (green wood) and semihard wood (ripe wood) cuttings of Ipomoea carnea Jacq. Proc. All India Symp. Hort. Calcutta, 45—53.

Basu R. N., Roychoudhury N., Sen P. K.. 1968. Analysis of the effect of ringing and auxin treatment on rooting of mango (*Mangifera indica* L.) seedling cuttings. Proc. int. Symp. Plant Growth Substances. 1967, Calcutta. 271—280.

Beevers L. 1968. Protein degradation and proteolytic actiivty ingerminating pea cotyledons. Phytochem. 7: 1837—1844.

Buczek J. 1969. Effect of inhibitor of RNA and protein synthesis of IAA and EDTA — induced elongation growth. J. Exp. Bot. Oxford 20: 52—55.

Buczek J. 1971. Action of Actinomycin D on auxin-induced growth of *Avena coleoptile* section. Acta. Soc. Bot. Pol. 40: 615—622.

Chibnall A. C. 1954. Protein metabolism in rooted rummer-bean leaves. New Phytol. 53: 31.

Coartney J. S., Morre D. J., Key J. L. 1967. Inhibition of RNA synthesis and auxin-induced cell wall extensibility and growth by actinomycin D. Plant Physiol. 42: 434—439.

Fletcher R. A. 1965. Regulation of protein and nucleic acid synthesis by gibberellin during leaf senescence. Nature 207: 1176—1177.

Frankel C. 1974. Isozymic changes in relation to root initiation in mung bean. Can. J. Bot. 52: 2—6.

Gordon S. A., Buess E. M. 1973. Correlative inhibition of root emergence in the gamma-irradiated coleus leaf III. effects of auxin on the radiation-induced changes in RNA, RNA metabolism and rooting. Radiation Botany 13: 283—286.

Green T. R., Ryan C. A. 1973. Wound-induced proteinase inhibitor in tomato leaves. Plant Physiol. 51: 19—21.

Gregory F. G., Samantari B. 1950. Factors concerned in the rooting response of isolated leaves. J. Exp. Bot. 1: 159—193.

Gurusiddaiah S., Kuo T., Ryan C. A. 1972. Immunological comparisons of chymotrypsin inhibitor I among several genera of the *Solanacea*. Plant Physiol. 50: 627—631.

Hochkeppel H. K. 1973. Isolierung einer Endopeptidase aus alternden Tabaklattern und ihre Beziehung zum Vergilben. Z. Pflanzenphysiol. 69: 329—343.

Lowry O. H., Rosenbrough N. J., Farr A. L., Randall R. J. 1951. Protein measurement with Folin phenol reagent. J. Biol. Chem. 193: 265—275.

Mejbaum-Katzenellenbogen W., Mochnacka I. 1969. Kurs praktyczny z biochemii. PWN Warszawa.

Nanda K. K., Purohit A. N., Tandon R., Bala A. 1968. Mechanism of auxin action in rooting of cuttings. Proc. int. Symp. Plant Growth Substances 1967, Calcutta 201—209.

Nooden L. D., Thimann K. V. 1965. Inhibition of protein synthesis and of auxin-induced growth by chloramfenicol. Plant Physiol. 40: 193—201.

Overbeek J. van., Gordon S. A., Gregory L. E. 1946. An analysis of the function of the leaf in the process of root formation in cuttings. Amer. J. Bot. 33: 100—107.

Patterson B. D., Trawavas A. J. 1967. Changes in pattern of protein synthesis induced by 3-indolylacetic acid. Plant Physiol. 42: 1081—1086.

Phillips D. R., Horton R. F., Fletcher R. A. 1969. Ribonuclease and chlorophyllase activities in senescing leaves. Physiol. Plant. 22: 1050—1054.

Pilet P. E., Braun R. 1970. Ribonuclease activity and auxin effect in the lens roots. Physiol. Plant. 23: 245—250.

Pilet P. E. 1971. The effect of auxin and abscisic acid on the catabolism of RNA. J. Exp. Bot. 21: 446—451.

Pitt D. 1974. Activation and de novo synthesis of ribonuclease following mechanical damage to leaves of *Solanum tuberosum* L. Planta 117: 43—55.

Puritch G. S., Barker A. V. 1967. Structure and function of tomato leaf chloroplasts during ammonium toxicity. Plant Physiol. 42: 1229—1238.

Rosen H. 1957. A modyfied ninhydrin colorymetric analysis for amino acids. Arch. Biochem. Biophys. 67: 10—15.

Ryan C. A. 1968. Synthesis of chymotrypsin inhibitor I in potato leaflets induced by detachment. Plant Physiol. 43: 1899—1865.

Ryan C. A., Huisman W. 1970. The regulation of synthesis and storage of chymotrypsin inhibitor I in leaves of potato and tomato plants. Plant Physiol. 54: 484—489.

Ryan C. A. 1973. Proteolytic enzymes and their inhibitors in plants Ann. Rev. Plant Physiol. 24: 173—196.

Sacher J. A. 1965. Senescence: Hormonal control of RNA and protein synthesis in excised bean pod tissue. Amer. J. Bot. 52: 841—848.

Sacher J. A. 1969. Hormonal control of senescence of bean endocarp Auxin supression of RNase. Plant Physiol. 44: 313—314.

Sacher J. A. 1973. Senescence and postharvest physiology. Ann. Rev. Plant Physiol. 24: 197—224.

Srivastava B. I. S. 1965. The effect of kinetin on nucleic acids and nucleases of excised barley leaves. Plant Physiol. 540: 62—64.

Srivastava B. I. S. 1968. Acceleration of senescence and of the increase of chromatin-asociated nucleases in excised barley leaves by abscisin II and its reversal by kinetin. Biochem. Biophys. Acta 169: 534—536.

Tanimoto E. 1968. Effect of auxin on cell wall degrading enzymes. Physiol. Plant. 21: 820—826.

Udvardy J., Farkas G. L., Marre E. 1969. On RNase and other hydrolytic enzymes in excised *Avena* leaf tissue. Plant Cell Physiol. 10: 375—385.

Udvardy J., Farkas G. L. 1972. Abscisic acid stimulation of the formation of an ageing-specyfic nuclease in *Avena* leaves. J. Exp. Bot. 23: 914—920.

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Zmiany niektórych frakcji azotu, aktywności proteaz oraz depolimeraz RNA, w sadzonkach indukowanych auksyną podczas regeneracji korzeni przybyszowych

Streszczenie

24 godzinna indukcja auksyną 10^{-4} M sadzonek liściowych z pomidorów wpływa wyraźnie na zwiększenie ilości korzeni przybyszowych. Zaindukowane auksyną sadzonki wykazują szybki ubytek azotu nierozpuszczalnego w blaszce liściowej. Równocześnie obserwuje się wzrost zawartości frakcji azotu nierozpuszczalnego i wzrost frakcji azotu rozpuszczalnego w bazalnej części ogonka. Podobnej zależności nie obserwuje się w sadzonkach nie poddanych działaniu auksyny. Doświadczenia z aktynomycyną D i chloramfenikolem wskazują, że okres 2—3 dni po indukcji auksyną jest istotny dla syntezy specyficznego białka, które w efekcie wpływa na zainicjowanie wykształcania korzeni przybyszowych. W okresie tym aktywność proteolityczna w blaszkach liści zaindukowanych auksyną wykazuje wyższy poziom niż w blaszkach liści nieindukowanych, co zgodne jest z obserwowanym ubytkiem frakcji azotu nierozpuszczalnego w tych blaszkach. W ogonkach liści indukowanych procesy proteolityczne są mniej aktywne niż w ogonkach liści nieindukowanych IAA, co również odzwierciedla się w zwiększonej syntezie azotu nierozpuszczalnego w ogonkach liści moczonych w IAA w porównaniu do ogonków sadzonek moczonych w tym czasie w wodzie. W czasie inicjowania zawiązków korzeniowych w kombinacjach z indukcją auksyną następuje również obniżenie aktywności depolimeraz RNA w bazalnej części ogonków.

Należy zatem stwierdzić, że moczenie ogonków sadzonek w roztworze IAA wyzwala w sadzonkach szereg przemian natury genetycznej, które w konsekwencji prowadzą do powstania charakterystycznego typu przemiany materii umożliwiającego inicjację korzeni przybyszowych.