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Uptake of RNA by the Root System of Tomato

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Abstract. Exogenous RNA is degraded under sterile conditions in solution to fragments greater than tetranucleotides which can be taken up by the tomato. The degradation and uptake are not equilibre at first. Equilibrium is established only after 20 hours. Fragments of high-molecular RNA are taken up more readily than those of artificially degraded RNA.

Isolated roots take up RNA fragments in the same manner as the whole tomato.

The uptake of inorganic phosphate is relatively well-understood but the uptake of organic phosphates has been investigated in some detail in recent years only. The compounds mostly studied are phytin, sodium phosphoglycerate and nucleic acids.

RATNER and SAMOILOVA (1958) studied the uptake of RNA by maize under sterile conditions. They used RNA labelled with ^{32}P which served as the sole source of phosphorus. Stimulating effects were observed in the plants. Plants grown in RNA contained more aminoacids and phosphorus and hence could form more protein and phosphate esters. The authors deduce from their experimental results that RNA is taken up by maize after exoenzymatic degradation. LEDOUX and HUART (1962) found that high-molecular RNA is taken up more readily than degraded RNA. They worked with RNA labelled with ^{14}C and ^{3}H and reached the conclusion that the whole molecule is taken up. The uptake of RNA is compared with phagocytosis by leukocytes. This view is partly supported by the work of TARASENKO and KOVALENKO (1962) who dealt with the uptake of RNA labelled with ^{32}P in the tomato. Most papers dealing with the uptake of other types of phosphorus-containing organic compounds ascribe a considerable role to phosphatase and other exoenzymes (1957, BARTH 1959, SHISHKINA 1962, STRAUS, CAMPBELL 1963). It was my task to ascertain whether RNA is taken up, in what way the uptake is achieved and whether it can replace mineral nutrition with phosphorus.

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Material and Methods

Tomato (*Lycopersicum esculentum*) cv. Immun from the breeding station at Hrdly (harvest year 1961) was cultivated in water cultures. The seeds were disinfected with chloramine (100 g per litre water) for 5 min. After several rinsings the seeds were sown on sterile agar plates (1%). For cultivation we used test tubes of 30 mm diameter and 200 mm height with a tube (55 mm) inside tied with sterile gauze and an opening into which seed was placed after three days of germination. The test tubes were placed in a wooden trough to ensure growth of roots in the dark. Knop's and White's nutrient solutions were used in the following three variants: A — complete nutrient solution with KH_2PO_4, B — nutrient solution without KH_2PO_4, C — nutrient solution without KH_2PO_4 but with RNA (55·5 mg RNA per litre solution).

We used either a commercial preparation by Merck or a ^{32}P — labelled preparation from *Saccharomyces cerevisiae*. Since sterilization caused some degradation of RNA (determined on a Zeiss spectrophotometer), we had to work aseptically and to check sterility on nutrient agar plates. The amount of RNA used (55·5 mg/litre) is equivalent to the amount of phosphate present in a complete nutrient solution ^{32}P-labelled RNA was isolated according to BELOZERSKY and PROSKURYAKOV (1951) from *Saccharomyces cerevisiae* after 48 hours of cultivation in a medium containing ^{32}P. The RNA was not purified as it would cause partial degradation. The preparation was dried freely in the laboratory. This caused the proteins to coagulate, whereby a small amount of RNA was lost but most of the RNA was separated from the protein. The preparation contained only about 2·5% DNA and less than 1% polyphosphate. This RNA preparation was dissolved in distilled water of pH 7 and added to Knop's solution. For the determination of phosphorus in the plant and solution we used MARTIN and DOTY's (1949) method modified by HAUGE, KING and CHELDELIN (1955).

The activity of root and overground part phosphatase in tomatoes was determined according to the amount of inorganic phosphate released from RNA by the action of enzymes at 20° C. The enzyme preparation was obtained according to DOBŘEMYSLOVÁ-HORNOVÁ (1960). The preparation was added to the solution with RNA and samples were removed at various time-intervals to be put into 20% trichloroacetic acid (final concentration 5%) to inactivate the enzymes. The amount of phosphorus released from RNA was then determined according to Martin and Doty.

High-voltage electrophoresis (4000 V) was used to follow the degradation of RNA (MICHALEC, KOŘÍNEK and MUSIL 1959). We used Whatman No. 3 paper washed with 0·05% EDTA and distilled water (LUŠTINEC 1961). Oligonucleotides were separated at pH 3·8 (citrate buffer) in carbon tetrachloride within 3 hours.

Explantates. Tomato seeds to be used for cultivation of roots germinated for 7 days until the root was about 2 cm long. The roots were cut off from the hypocotyl (PETRŮ and ŘETOVSKÝ 1956) and grown in 100 ml Erlenmayer flasks with 50 ml White's solution again in three variants of nutrient solution. The roots grew in the dark for 4 weeks at 20° C.

During the first 14 days their length was measured every other day and their phosphorus content determined after 4 weeks. Roots cultivated in the solution

without phosphate and RNA were transplanted after 2 weeks into a solution with RNA and the solution was examined spectrophotometrically in regular intervals and the results compared with those for the whole plant.

Results

1. Growth of sterile tomatoes in three variants of Knop's and White's nutrient solutions (to compare the results with those from explantates).

The tomatoes were grown for 4 weeks. The variants differed only in their root system. Plants in complete nutrient solution had long main roots with rich ramification. Practically no adventitious roots were formed. In the variant C the main roots were less branched than in the variant A but numerous adventitious roots were formed, their length equalling that of the principal roots. Plants in the solution without phosphate (B) had long and unbranched principal roots. A great amount of adventitious roots (very short) were formed. The difference in the phosphate content between variants A and C is not significant.

2. Uptake of high-molecular RNA by the plant.

Test tubes containing 20 ml active solution of RNA and mineral phosphate ^{32}P received one plant each under sterile conditions. Pre-cultivation carried out for 7, 14 and 30 days in nutrient media A, B and C. After 2, 6, 12 and 24 hours the plants were removed and studied by autoradiography. The intensity of phosphorus uptake from RNA decreased as follows: B, C, A (of pre-cultivation) and depended on the age of the plant. The uptake of organic phosphate lags behind that of mineral phosphate by about 2 hours. At the same time, the activity of the solution was determined and found to decrease in dependence on the duration of cultivation; the amount of P released from RNA was also estimated.

It follows from the results (Fig. 1) that RNA is degraded and that inorganic phosphate is released and is not immediately utilized.

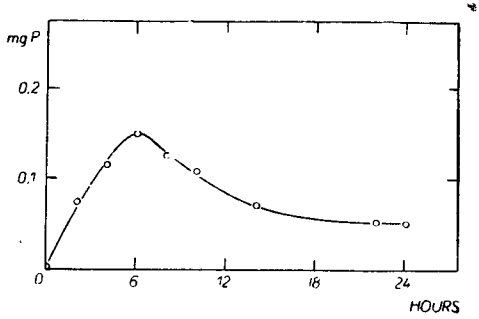

Fig. 1. Course of release of inorganic phosphate from RNA solution (55·5 ppm) as influenced by tomato roots.

It may be concluded from the results that phosphorus from RNA is taken up by the tomato probably after exoenzymatic degradation. Plants precultivated in a solution without phosphate were transferred to RNA and after 2, 6, 12, 24 and 48 hours the solution was aseptically decanted into cuvettes

of 0·5 ml volume and the absorbancy of the solution measured at 258·5Å (Fig. 2, curve 1). A rise in absorbancy indicates degradation of RNA and a decrease reveals the uptake of RNA fragments by the root system of the tomato. In addition to the foregoing, the individual samples were left to stand for the duration of the experiment (48 hours) and the solution examined after the same intervals (Fig. 2, curves 2, 3, 4, 5). The rise in absorbancy in the

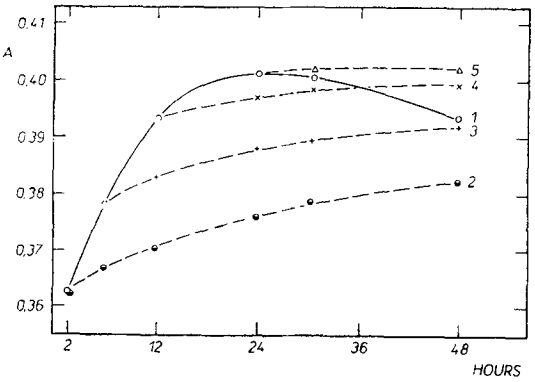

Fig. 2. Change in absorbancy of RNA solution as influenced by 14 days old tomato plants under aseptic conditions.
1. with a plant,
2., 3., 4., 5. after removal of plants.

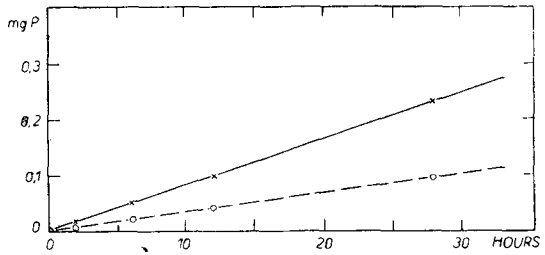

Fig. 3. Release of inorganic phosphate from RNA under the action of an enzyme extract. ———— enzyme extract from roots, — — — — — — — enzyme extract from overground parts.

individual measurements indicates exoenzymatic cleavage, since even after removing the plant degradation proceeds under sterile conditions. The amount of degraded RNA is directly proportional to the amount of released enzymes. The solution in which the plants were grown for 24 hours was subjected to electrophoresis. Only free phosphate was found, without any separation of the starting spot. Hence, it is concluded that the fragments are larger than tetranucleotides, since electrophoresis permits to determine at most tetranucleotides (MICHALEC, KOŘÍNEK 1959). After artificial degradation of RNA actually individual bases could be determined. It was, therefore, verified that the tomato enzymes can degrade RNA (Fig. 3). Knop's solution with an enzymatic

extract removed at the time of phosphate determination and Knop's solution with RNA plus trichloroacetic acid after 28 hours served as controls.

3. Uptake of degraded RNA by the tomato.

RNA was degraded by sterilization at 100° C for 30 min. at pH 8. The experiments were then conducted as in section 2. It follows from the radio-

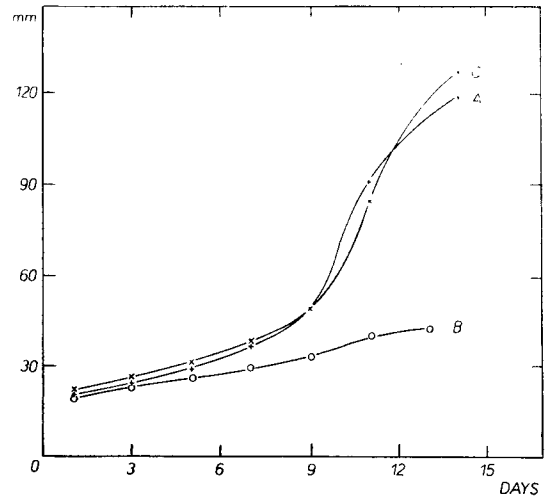

Fig. 4. Growth curves of isolated tomato root. A — White's nutrient solution with NaH_2PO_4, B — White's nutrient solution without NaH_2PO_4, C — White's nutrient solution without NaH_2PO_4 and with RNA.

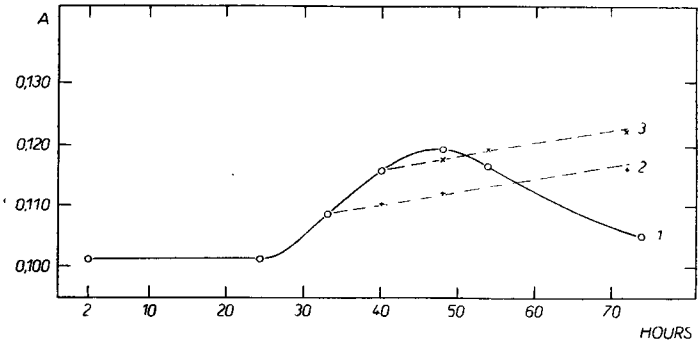

Fig. 5. Change in absorbancy of RNA solution as influenced by isolated tomato roots.
1. with an isolated tomato root,
2., 3. after removal of roots.

grams that ^{32}P from RNA is taken up only after 6 hours of cultivation in an active solution. The activity of the solution remains constant for 6 hours, whereas the amount of free phosphate rises. Between the 6th and 12th hours the amount of ^{32}P decreases and activity is also diminished. It may be said in conclusion that the uptake of phosphate from degraded RNA lags behind

the uptake of mineral phosphate by some 6 hours and behind the uptake of high-molecular RNA by some 4 hours.

4. Explantates.

Variants A and C were identical as to the character and phosphate content but differed strikingly from B (growth curves, Fig. 4). It may be seen that even roots alone will take up phosphate from RNA. The type of uptake of P from RNA was checked spectrophotometrically. Roots after 2 weeks of cultivation in a phosphate-free solution were transferred to a solution with RNA and the solution was examined at regular intervals (Fig. 5). The results obtained were compared with values from the experiment on the whole plant. RNA is taken up identically, is split exoenzymatically and the fragments taken up. Constant absorbancy from 0 to 24 hours can be explained by the assumption that the roots were impoverished by a prolonged exposure in the phosphate-free solution so that it took a certain time (24 hours) before the root began to grow and also release enzymes that would degrade RNA, as follows from the graphical representation by curves 2 and 3 of Fig. 5 which show the course of RNA degradation in the absence of roots.

Discussion

The finding that RNA is taken up by the roots is in agreement with the work of some other authors (RATNER and SAMOILOVA 1958, TARASENKO and KOVALENKO 1962, LEDOUX and HUART 1962).

The possibility of exoenzymatic cleavage of RNA in solution was mentioned by RATNER and SAMOILOVA (1958), TARASENKO and KOVALENKO (1962), STRAUS and CAMPBELL (1963), but LEDOUX and HUART (1962) are of the opinion that the entire RNA molecule is taken up.

It follows from the present results that fragments of high-molecular RNA are taken up more readily than fragments of artificially degraded RNA. This may be explained by assuming that the plant regulates its phosphatase activity depending on the amount of phosphate in the plant. If this is the case it is quite clear that the enzymes contained in the plant will degrade high-molecular compounds to such fragments as would be readily taken up by the plant. If RNA is presented in the degraded form, the preparation of such fragments for uptake takes a longer time. The observed difference in uptake is 6 hours. It is understandable that the age and stage of development of the plant affect the uptake of RNA, since as long as at least the first leaf does not emerge the plants do not require nutrients so urgently and are not even capable of degrading high-molecular organic substances. A similar effect on RNA uptake is exerted by pre-cultivation of plants.

In conclusion it may be said that RNA is taken up by the tomato in such amounts as will fully replace mineral nutrition with phosphorus even under sterile conditions.

References

BARTH, A.: Aktivita fosfatáz v průběhu submersní kultivace *Eremothetium ashbyi*. — [Phosphatase activity in the course of submerged growth of *Eremothetium ashbyi*.] — Diploma Paper, Faculty of Biology, Charles University, Praha 1959.

HAUGE, G., KING, T. E., CHELDELIN, V. H.: Oxydation of dihydroxyacetone via the pentose in *Azotobacter suboxydans*. — J. Biol. Chem. **214** : 11—26, 1951.

Dobřemyslová-Hornová, M.: Aktivita fosfatázy v pšenici za různé rychlosti růstu. [Phosphatase activity in the wheat at different growth rates.] — Diploma Paper, Faculty of Biology, Charles University, Praha 1960.

Ledoux, L., Huart, R.: Nucleic acids and protein metabolism in barley seedlings. — Biochem. biophys. Acta **2** : 185—196, 1962.

Luštinec, J.: Pentosový cyklus a glykolysa ve vztahu k regulaci růstu. [Pentose cycle and glycolysis in relation to growth regulation.] — Dissertation Czechosl. Acad. Sci., Praha 1961.

Martin, J., Doty, D. M.: Determination of inorganic phosphate. — Anal. Chem. **21** : 965, 1949.

Michalec, Č., Kořínek, J., Musil, J.: Elektroforéza na papíře a v jiných nosičích. [Electrophoresis on paper and other carriers.] — Publ. House Czechosl. Acad. Sci., Praha 1959.

Petrů, E., Řetovský, R.: Rostlinné explantáty. [Plant explantates.] — Publ. House Czechosl. Acad. Sci., Praha 1956.

Straus, J., Campbell, W. A.: Release of enzymes by plant tissue culture. – Life Science **1**: 50–62, 1963.

Белозерский А. Н., Проскуряков Н. И.: Практическое руководство по биохимии растений, 220, Москва 1951. [Belozersky, A. N., Proskuryakov, N. I.: Practical manual of plant biochemistry.

Ратнер Е. И., Самойлова С. А.: Об усвоении растениями нуклеиновых кислот и о внеклеточной фосфатазной активности корней. — Физ. растений **5**/3 : 209—220, 1958. [Ratner, E. I., Samoilova, S. A.: On the uptake of nucleic acids by plants and on extracellular phosphatase activity of roots.]

Шишкина А. П.: Активность фосфорилазы, фосфоглюкомутазы и амилазы в картофеле в зависимости от условий питания растений. — Материалы по физиологии и биохимии растений, 169—178, 1962. [Shishkina, A. P.: Activity of phosphorylase, phosphoglucomutase and amylase in the potato in dependence on the conditions of plant nutrition.]

Тарасенко Н. Д., Коваленко С. П.: О передвижении соединений типа нуклеиновых кислот по проводящей системе растений. — Физ. растений **9** : 245—247, 1962. [Tarasenko, N. D., Kovalenko, S. P.: On the transport of compounds of nucleic acid type through the conductive tissue of plants.]

Došková A., Kated ra fysiologie a půdní mikrobiologie Přírodovědecké fakulty Karlovy university a Ústav experi mentální botaniky ČSAV, Praha: **Příjem RNK kořeny rajčat.** — Biol. Plant. **8** : 110—116, **1966.**

RNK je degradována exoenzymy na štěpy větší než tetranukleotidy, které jsou rajčaty přijímány. Štěpení a příjem nejsou zpočátku v rovnováze. Rovnováha nastává až teprve po 20 hodinách. Lépe jsou přijímány štěpy z vysokomolekulární RNK než štěpy z uměle degradované RNK. Izolované kořeny přijímají štěpy RNK stejným způsobem jako celé rajče.

А. Дошкаржова, Отдел физиологии и почвенной микробиологии естественного факультета Карлова университета, Институт экспериментальной ботаники ЧСАН, Прага: **Поглощение РНК корневой системой томата.** — Biol. plant. **8** : 110—116, **1966.**

Экзоэнзимы деградируют РНК на продукты расщепления размерами больше тетрануклеотидов, которые поглощаются томатами. Расщепление и поглощение сперва неравновесны. Равновесие устанавливается лишь после 20 часов. Лучше поглощаются продукты расщепления высокомолекулярной РНК чем продукты расщепления искусственно деградированной РНК.

Эксплантаты поглощают продукты расщепления РНК также как растение томата.