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Permeability of Plant Tissues to Humic Acids

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Permeabilita humusových kyselin

Do odřezaných větviček různých rostlin ponořených řeznou plochou do roztoku humusové kyseliny proniká hnědě zbarvený roztok do cév a je v nich koagulován. Během týdne se cévní svazky zbarvily do výšky 1 až 6 cm. V parenchymatických buňkách není možno přímo humusovou kyselinu zjistit; ale i buňky se žlutě nebo hnědavě zbarvenou blanou jsou živé (dají se plasmolysovat).

Kyselina humusová značená aktivním uhlíkem C^{14} se v rostlinách šíří celkem stejně v nesterilních i sterilních kulturách, a to rovněž především cévními svazky.

Summary

When small branches of different plants were dipped after cutting into a solution of humic acid, a brown substance penetrated into vessels and coagulated there. The vascular bundles were stained to the height of 1—6 cm after a week. The presence of the humic acid in the parenchymal cells could not be demonstrated directly, but cells with a yellow or brown cell wall remained alive (they could be plasmolysed).

Humic acid, labelled with C^{14} was distributed in the same way in sterile and in non-sterile plant cultures, mainly through vascular bundles.

Introduction

It is not easy to investigate the penetration of different compounds into the cell; the investigation of the permeability of such chemically undefined compounds as humic acids or of other humic fractions is still more difficult. We do not possess a specific reagent for demonstrating their penetration. We can, nevertheless, use different methods to observe this at least partially.

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Material, Methods and Results

It was not possible to demonstrate the penetration of humic acids through the tissue membrane in the liquid above the membrane by the modified method of BROOKS (PRÁT 1960). Since we find it difficult to use such a simple method for tracing the permeability of humic acid through plant tissues in water, there is even less chance of observing them in the plant cell itself.

Humic acids are coagulated in acid medium; it was, therefore, promising to use plants with a very acid reaction for such experiments (though humic acids are soluble in some organic acids). But this was of no use. The acid reaction of the tissue and perhaps also the relatively high amount of calcium are perhaps the reason why so little of the humic acid penetrates into the cell, so that there is no perceptible coagulation (*Begonia*, *Bryophyllum*, *Pelargonium*).

Even in the big internodial cells of *Nitella*, cultivated for a shorter or longer time in different concentrations of humic acid, there was no perceptible humic acid in the cell sap.

Experiments with radioactive humic and fulvic acids have shown that active compounds are taken up from the nutrient solution by maize roots. They diffuse in their roots very slowly. They appear in the leaves after several days in very low concentration, both in the experiments with humic acids and with fulvic acids. The resorption by the leaves was very slow or imperceptible (PRÁT and POSPÍŠIL 1959, PRÁT 1960).

These experiments were repeated with plants in sterile cultures with the same result as under non-sterile conditions. Radioactive compounds thus also show the permeability of humic acids in cultures without bacteria (*Polytrichum commune*, *Dicksonia antarctica*, *Asplenium septentrionale*, *Dryopteris filix-mas*, *Lycopersicum esculentum*, *Cucurbita pepo*, *Cattleya*). We may thus suppose that humic and fulvic acids are not disintegrated by bacteria before penetrating into the cell.

We were hitherto not able to demonstrate the activity in tissues of the callus (explantates) of the carrot, but we have found it in tomato roots. Root segments from explantates of *Lycopersicum esculentum* cult. immun., prepared in sterile cultures by Dr. E. PETRŮ (Inst. of Experiment. Botany ČSAV), were cultivated for 10—20 days in a solution with added radioactive humic acid. Mr. L. BENEŠ (Biophysical inst. ČSAV), has prepared microautoradiograms of the slides. They showed active granules inside the root cells.

All these experiments only show how radioactive carbon spreads. They cannot give any information about the form (molecule) where it is bound during transport. It is thus necessary to follow the molecules of humic acid itself.

In all these experiments the commercial preparation "Humussäure Riedel de Haen Selze Hannover" was used. Its solution flocculated totally when HCl or H_2SO_4 were added; it follows that it did not contain any fulvic acid. It contained, however, a relatively high amount of hymatomelanic acid.

Humic acid has an advantage: its colour is a dark brown and its solutions are likewise dark brownish. It is thus possible to follow this colouring directly without any reaction at all, the only condition being the use of high concentrations. If other conditions are favourable, there is no danger of any damage to the cells. There are several reports in the literature about damage to plants caused by higher concentrations of humous compounds; several instances could be cited. But it seems that sometimes other conditions, such as too high an acidity or alkalinity or the composition of the nutrient solution, are responsible.

The solution of potassium humate in distilled water is not poisonous even in high concentrations. Under favourable conditions (pH between 5—7) a good preparation of humic acid may be used in concentrations as high as 1000 mg./l. The normal formation of adventitious roots, but slightly retarded if compared with the control in pure water or almost the same, shows that the plants are by no means damaged by this high concentration (*Coleus hybridus*, *Tradescantia zebrina*, *T. fulminensis*, *Impatiens sultani*, *Pelargonium zonale*, *Cucurbita pepo*, *Bryophyllum crenatum*). Rooting cuttings showed no difference from the control.

The permeability is observed for a far shorter time than is necessary for the formation of the roots. We may thus take the cells as quite normal and use the dark colour of the humic acids as a control of their penetration into the plant. If we dip cut branches of different plants into the solution, the solution penetrates into the conductive tissues where dark brown coagulation occurs. If we dip the cut part of branches of different plants in the solution, in a few hours or days they show traces of humic acid in the vascular bundles, especially in vessels. In vessels dark brown coagulation is formed, filling nearly all their lumen. Reactions in different plant species or individuals were different; in *Pelargonium* the vascular bundles were stained in two

days to a height of 1—3 cm, in *Impatiens* about twice as much, in *Coleus* 2—6 cm. But the colouring did not reach further or only very little even after a week or more. In all cases it is necessary to compare the tested plant with the control in water; there are cases where the vessels get a brownish colouring even in water (usually in *Pelargonium*), but such a colouring is not intense and does not exceed a very narrow strip at the base. In humates very intense staining may be observed up to 1—2 cm. in height during 24 hours. The cell wall of parenchymatous cells gets a brown colour only 3—5 layers from the cut surface. If the staining continues further, cell walls round intercellular spaces are coloured.

The coagulation of humic acid is striking in vascular bundles, especially in vessels of different plants: *Pelargonium zonale, Coleus hybridus, Tradescantia fluminensis, T. purpusi, Impatiens sultani.* In *Cucurbita, Brassica napus* v. *chinensis* there is a less distinct reaction. A very good and distinct reaction was observed in the big vessels of *Bryophyllum crenatum.*

In cut roots we can trace the spreading of the coagulation in vascular bundles from both sides, if we either dip only the root slice in the glass with the humate, or place the cut root so that the cut part is situated 1 mm. below the level of the solution. In the controls with water the brownish colouring of the cut vessels is first perceptible only after 12 hours and only on the surface. During this time the colouring of the vascular bundles in humic acid reached 1—3 mm. and was very intense; during the next few days it continued to the distance of 1—2 cm.

Very good results may be obtained with cut roots of sugar beet, less good is the upper part of the root at the junction with the stem. Cuttings of sugar beet roots, dipped in a 1000 mg./l. solution of potasium humate, show dark brown staining in the cut vascular bundles already after one hour.

It is necessary to work as far as possible under sterile conditions, as an infection may cause an intense colouring of the vessels and cells of the surrounding tissues even in the controls.

Less good were the red beet *Beta vulgaris rubra* and the carrot *Daucus carota.* In the roots of *Scorzonera hispanica* the vessels of the cut roots were not stained further than 1 cm. even after five months.

It is significant that the humic acid spreads in cut, open vessels only; it does not penetrate into the vessels through the bark tissues, not even when the epidermis was removed and the bark cells were in direct contact with the solution (*Pelargonium, Coleus, Impatiens*). Even when the solution of the humic acid was injected into the hollow stem or petiole (*Cucurbita maxima*), it did not penetrate into the vessels, but a small wound is sufficient and the brown coagulations appear in vessels. If leaves are broken or cut, the vascular bundles are stained in a basipetal direction on the same or even longer distance through the leaf scar than through the cut basic part of the stem.

The solution of humic acid can be poured in the hollow of a cut stem-internodium. The best material for this experiment is *Solanum tuberosum.* But even in this case no penetration of the humic acid through the undamaged parenchyma of the vascular bundle could be observed with certainty. If scarified, the vessels showed distinct intense coagulations of humic acid.

For the time being we are not able to say anything definite about the nature of the coagulated material in the vessels. It is probable that it is not simple coagulation, since the material dissolves only slowly and with difficulty in hydroxide and in chloralhydrate. It might be some result of oxidation and polymerisation, but we have hitherto no experimental evidence for this. It is worth mentioning that coagulations usually originate in young, narrower vessels, not in the widest ones.

The results in a series of experiments where the solution of humic acid was poured into a pit in a pith were similar. In apple (*Pirus malus*) a yellow brown coloration of cell walls of several surface layers of cells was observed. Vascular bundles were indistinct, but cell walls of the protracted cells surrounding the bundles were stained yellow-brown.

In bulbs of *Brassica oleracea gongylodes* brown colouring was found on cell walls of one or two cell layers in the cut place, but irregular yellow-brown coloration was observed up to the fifth or tenth layer around the intercellular spaces. Cells, surrounding vessels, but not the actual vessels were intensely brown.

In some cases meristemisation was observed under cells with brownish coloured cell wals. In *Brassica* and red beet (*Beta vulgaris rubra*) below one or two layers of brown cells several layers of thin flat cells with entirely unstained walls were observed. It is difficult to tell whether it is a layer separating the dying surface cell layers or the result of the stimulating effect of humic acids. The first possibility is more probable judging from the experiments on the function of humic acids in regeneration (PRÁT 1962).

In some plants distinctly yellow or brown staining of cell walls was observed even in cells still living, where plasmolysis could take place, as in *Beta vulgaris rubra.*

The anthocyans do not perceptibly react with humic acid; we could thus trace no changes in cells whose walls were impregnated with humic acid in *Beta vulgaris rubra, Begonia rex* or *Begonia crassicaulis.* But the presence of anthocyans helps the observation of plasmolysis (by 0·5 N saccharose). This shows that even cells with very intensely coloured walls were living.

Fluorescence was of not much use in these experiments. But it is worth mentioning that after the zone of yellowish coloured, non-fluorescent cells, three or four cell layers were often observed showing a distinct yellow-greenish fluorescence. It seems that some fraction, split as in a kind of chromatography, penetrates into the tissue. No better results were obtained even in combination with acridine orange.

Discussion

Experiments with radioactive humic acid have shown that it penetrates into plants very slowly. This experience as well as the fact that the results in sterile cultures are similar show that whole molecules are spreading. Even if we suppose that the big molecule is split, these experiments provide information about the maximum speed possible for the spreading of active carbon compounds. It is important that even in roots the speed of the active compounds is low. The velocity in stems and leaves is a few centimeters distance in several days. This is surprisingly low compared with other organic compounds; the rate of movement of C^{14}- labelled photosynthetic products varies from 40 to 120 cm/hr (KURSANOV 1961). Saccharose produced by photosynthesis spreads to a distance of 96 cm. in an hour (see NELSON 1962). Minimum velocity exceeding 2 cm. per sec. fully deserves the term "rapid translocation" (NELSON, PERKINS, GORHAM (1958); SHIROYA, NELSON, KROTKOV 1962). The velocity of spread of the radioactivity or brown colouring of the humic acid is far lower than that.

The penetration of the commercial preparation of humic acid in vascular bundles of cuttings was very slow too, different in different plants, about 1—2 cm. a day, 1—6 cm. a week. It may be that the velocity of penetration was lowered by the coagulation in vessels.

All the experiments clearly show the significance of the conductive tissues, especially of vessels for the penetration of humic acids. The same was found for the penetration of virus bodies, but the velocity of virus spread is much higher (see MITCHEL, SCHNEIDER, GAUCH 1960). It is hardly possible to compare these experiments because the concentration of the humic acid must be very high before we can trace it in vessels. But even the experiments with radioactive humic acid of very low concetration show that it spreads in plants very slowly.

In this connection the experiments of SLADKÝ (1959), showing the influence of the sprinkling of leaves on the development of root system are of some interest. One would suppose that humic and fulvic acids can diffuse a long distance. But it is possible that humic acids penetrate into the leaf tissues only and that the roots only get the products of the changed metabolism of the leaves, which influence their growth. Such problems call for methods permitting the investigation of even low concentrations of humic acids in different tissues.

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Пермеабилитет гумусовых кислот

В сосуды отрезанных веток разных растений, опущенных поверхностью среза в раствор гумусовой кислоты проникает коричнего окрашенный раствор и коагулируется в них. В течение недели сосудистые пучки окрасились на высоту 1—6 см. В паренхиматических клетках не возможно прямо установить гумусовую кислоту; однако, и клетки с желто или коричневато окрашенной оболочкой являются живыми (обладают свойством плазмолиза).

Гумусовая кислота меченная активным углеродом С 14 распространяется в растениях в общем одинаковым образом в стерильных и нестерильных культурах, опять прежде всего сосудистыми пучками.