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STUDY OF TRANSPORT PROCESSES IN PLANTS BY RADIOABSORPTION AND MICRORADIOGRAPHIC METHODS

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The passive transport processes in plants of $^{22}Na^{+}$, $^{137}Cs^{+}$, $^{45}Ca^{2+}$, $^{65}Zn^{2+}$, $^{59}Fe^{3+}$ and $^{32}PO_4^{3-}$ ions and the plant-protecting agent "Saphidon (^{14}C)" were studied by a radioabsorption method. The parameters of the passive transport processes of $^{212}Pb^{2+}$, borate and tetraborate ions in plants were measured by quantitative microradiographic methods, using photoemulsion and solid state nuclear track detectors. Ion diffusion concentration profiles within the plants were determined at various diffusion times and temperatures. The equation of linear diffusion combined with convection was used to determine the diffusion coefficients characteristic of the transport processes.

Introduction

From the aspect of the physiological functions of plants and their crop yields, the processes of material transport are of decisive importance.

Three fundamental transport processes can be distinguished in plants:[1]

(1) The flow of material in the transport fascicles of the phloem and xylem, the rate of which may attain 1 m/h.

(2) The active material trasnport, which may even be opposite in direction to that of decreasing chemical potential gradient, and where the energy necessary for the transport is covered by the biological energy.

(3) The passive material transport, which proceeds in the direction of decreasing chemical potential gradient.

The subject of our studies is the passive material transport, the first, determining step in the transport of material between the plant and its environment. In this process, the nutrients pass by diffusion from the external covering tissues through the intercellular ducts and cell walls to the cytoplasm, or to the transport system.

Experimental

In the study of passive ion transport processes in plants, we employed radioabsorption and microradiographic methods.

The essence of the radioabsorption method, first used by HEVESY and SEITH,[2] is that a radioactive isotope is applied to the surface of the sample, and the movement of the active material is followed by activity measurement as a function of time. In our radioabsorption measurements, we used the technique involving two measuring heads.[3] For examination of the transport of the ^{14}C-labelled plant-protecting agent, we developed an apparatus with a proportional counter with a measuring field of $2 \times 2\pi$.

In the radiographic investigations, samples from the plant were placed in a solution containing the ion under examination. Experimental conditions were such as to ensure only a negligible change in the ion concentration of the solution during the investigations. The transport process was frozen with the use of liquid air. After lyophilization and slicing with a microtome, radiograms of the 90 μm thick plant sections were prepared.

In the microradiographic investigations, use was made of photoemulsion, plastic solid state track detectors and an induced radiographic technique. This induced radiographic technique was employed to determine the boron concentration profile. Makrofol-E polycarbonate track detectors were placed on the boron-containing plant sections, and the samples were irradiated in a thermal neutron fluence of $2 \cdot 10^{11}$ neutron/cm^2 in the Triga Mark II Reactor in Ljubljana, using a Cd ratio of 40. The α-particles and ^{7}Li nuclei produced in the ^{10}B(n, α)^{7}Li nuclear reaction were detected in the plastic track detector. Agfa-Gevaert 34–B50 photoemulsion, Makrofol–E, Kodak LR–115 (II) and CR–39 track detectors were used for the radiographic investigations. The conditions for development of the detectors are given in Table 1.

Table 1
Developing conditions of the detectors used in our radiographic studies

Detector type	Developer	Developing time, min	Developing temperature, °C
Agfa-Gevaert 34B50	Kodak D19	3	25
CR-39	20% NaOH	120	70
Makrofol-E	15 g KOH+40 g C_2H_5OH + 45 g H_2O	10	70
Kodak-LR-115	10% NaOH	120	60

Table 2
Effective diffusion coefficients of $^{22}Na^+$, $^{137}Cs^+$ and $^{32}PO_4^{3-}$ ions in various plants at 25 °C

Diffusion medium	Effective diffusion coefficient, $\times 10^{-11}$ $m^2 \cdot s^{-1}$		
	$^{22}Na^+$	$^{137}Cs^+$	$^{32}PO_4^{3-}$
Potato	12.7	25.3	4.0
Beetroot	15.0	–	12.7
Kohlrabi	16.0	–	15.2
Celery	23.7	–	19.5
Radish (black)	7.3	–	11.8
Apple (Jonathan)	14.7	–	5.0
Onion	–	1.8	–
Parsley root			
in fibre direction	8.5	16.0	11.8
perpendicularly to fibre direction	0.7	0.8	1.3
Carrot root			
in fibre direction	9.3	8.3	14.2
perpendicularly to fibre direction	0.8	0.8	1.2

Results and discussion

Radioabsorption measurements

The passive transport processes of some nutrients and microelements, $^{22}Na^+$, $^{137}Cs^+$, $^{45}Ca^{2+}$, $^{65}Zn^{2+}$, $^{59}Fe^{3+}$ and $^{32}PO_4^{3-}$, were studied in the individual tissue parts of various plants. The effective diffusion coefficients determined from the experimental data are listed in Tables 2 and 3.

In connection with the data of Table 3 it should be noted that in the study of the maize leaf – Fe^{3+} complex system, the maize leaf was soaked for 3 h in the complex-forming solution prepared in the Department of Physical Chemistry at Kossuth Lajos University[4] and the $^{59}Fe^{3+}$ ions were transferred to the surface of the leaf following this.

Our investigations indicated that the passive ion transport processes in the plants are determined jointly by the processes of diffusion and material flow. Accordingly, to describe the passive ion transport in the plants, we used the relation[5]

$$\frac{\partial c}{\partial t} = D\frac{\partial^2 c}{\partial x^2} + v\frac{\partial c}{\partial x} \tag{1}$$

This is the equation for linear diffusion combined with convection, where
c – ion concentration in the plant;
t – diffusion time;
D – diffusion coefficient of the ion;
x – spatial coordinate;
v – velocity of convection.
By means of the transformation[6]

$$c = c^* \exp\left(\frac{v}{2D}x - \frac{v^2 t}{4D}\right) \tag{2}$$

Table 3
Effective diffusion coefficients of $^{45}Ca^{2+}$, $^{65}Zn^{2+}$ and $^{59}Fe^{3+}$ ions (with complexing agent) in plant membrane systems at 25 °C

Diffusion system	Sample thickness, $\times 10^{-5}$ m	Effective diffusion coefficient, $\times 10^{-14}$ $m^2 \cdot s^{-1}$
$^{65}Zn^{2+}$ – carrot root stelle tissue	100	1130
	200	1080
	300	1560
	400	1330
	500	1380
		$\overline{D}$ = 1300 ± 200
$^{45}Ca^{2+}$ – maize leaf	9	4.0
	18	7.2
	30	5.7
	40	6.2
	45	4.2
		$\overline{D}$ = 5.5 ± 1.4
$^{59}Fe^{3+}$ (with complexing agent) – maize leaf*	8	0.83
	18	0.95
	27	1.02
	36	1.23
	45	1.05
		$\overline{D}$ = 1.02 ± 0.15

*Without complexing agent, the diffusion coefficient of $^{59}Fe^{3+}$ in maize leaf is $(5\pm3) \cdot 10^{-17}$ $m^2 \cdot s^{-1}$.

Eq. (1) can be traced back to Fick's second law:

$$\frac{\partial c^*}{\partial t} = D \frac{\partial^2 c^*}{\partial x^2} \tag{3}$$

The solution of Fick's second law under the experimental conditions employed in the radioabsorption measurements[3] is

$$\log \frac{I_1 - I_2}{I_1 + I_2} = \log K - \frac{0.434\ \pi^2 D}{l^2}\, t \tag{4}$$

where I_1, I_2 – activities measured on the two sides of the sample;
K – constant depending on the β-ray absorption coefficient of the medium;
l – thickness of the sample.

With the above radioabsorption method it is possible to determine only an effective diffusion coefficient (D_{eff}), which is characteristic of the process of transport resulting from the joint effect ot diffusion and material flow.

By further development of the radioabsorption measurement technique, it became possible to study the passive transport of ^{14}C-labelled plant-protecting agents in plant membranes. In an apparatus with a proportional counter with a measuring field of $2 \times 2\pi$, we measured the transport of "Saphidon (^{14}C)" in various plant leaves, membranes and skins. The Saphidon emulsion employed in the measurements had the following composition: 15% Saphidon, o,o-dimethyl-mercaptomethyl-phthalimido-dithiophosphate, 25% xylene, 2% dimethyl sulfoxide, 3% acetone, 5% emulsifier, 2:1 Triton X-180 and Triton X-190, and 50% water.

Fig. 1 shows the radioabsorption measurement data relating to "Saphidon (^{14}C)", plotted in accordance with Eq. (4). The characteristic effective diffusion coefficients obtained for the passive transport processes are listed in Table 4.

The radioabsorption method permitted the fast quantitative determination of the rates of the passive transport processes in the plants. In the interval $10^{-9} - 10^{-18}\ m^2 \cdot s^{-1}$, the method can be employed for the determination of the diffusion coefficients of substances containing beta-emitting isotopes, with a standard deviation of about 30%.

Radiographic measurements

The differentiation and quantitative characterization of the diffusion and convection processes occurring in plants are possible only if the concentration profiles that develop inside the plants are established. Such concentration profiles

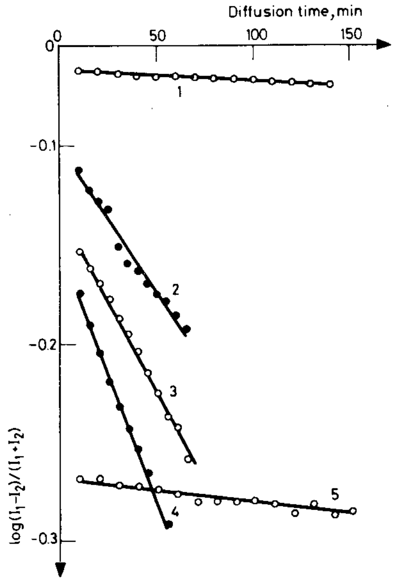

Fig. 1. Radioabsorption measurement data on "Saphidon (^{14}C)" in various plant skins. Curves: 1 – tomato skin, 2 – onion membrane, 3 – carrot root skin, 4 – parsley root skin, 5 – potato tuber skin

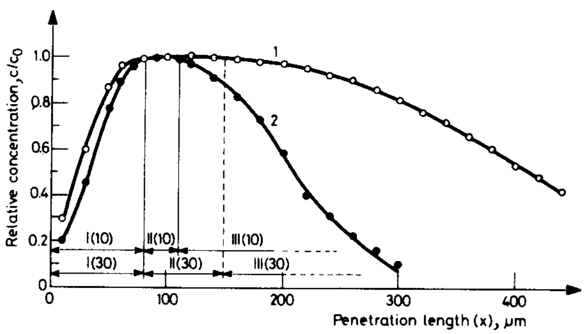

Fig. 2. Typical boron concentration distribution in skin tissues of carrot root after diffusion times of 10 (curve 1) and 30 min (curve 2) at 25 °C

and their variations in time can be followed by means of the microradiographic methods. A typical experimental result is illustrated in Fig. 2.

The profile curve corresponding to the concentration distribution developing inside the plant can be divided into three sections:[7]

(1) On proceeding from the surface of the plant towards its interior, the ion concentration progressively increases up to a maximum value. The length of this section varies in the range 20–80 μm and is independent of the diffusion time. It should be noted that our investigations revealed that when the plant and the solution containing the radioactive ions came into contact, the ions penetrated the given plants to a depth of 20–50 μm. In the calculation of the flow rate and the diffusion parameters, this must be regarded as the starting state ($t = 0$).

(2) In the second section the ion concentration is constant. The length of the section increases as the diffusion time increases. From this length (vt), the rate of convection may be calculated (generally with a large error).

(3) The third section is the diffusion zone, where the distribution of the ion concentration with regard to position and time is described to a good approximation by the relation[8]

$$c = c_0 \operatorname{erfc} \frac{x - vt}{\sqrt{2Dt}} \tag{5}$$

where c_0 is the saturation concentration measured in the second section.

For the calculation of Eq. (5), a computer program was prepared, and this was used to determine the parameters of the diffusion profiles best fitting the experimental results, together with the fitting errors. For the experimental conditions

Table 4
Effective diffusion coefficients of "Saphidon ^{14}C" in various plant membranes at 25 °C

Diffusion medium	Sample thickness, $\times 10^{-5}$ m	Effective diffusion coefficient, $\times 10^{-15}$ $m^2 \cdot s^{-1}$
Leaves		
Acacia	9.0	22.7
Pansy	19.0	348.0
Fruit peel		
Plum	10.0	1.8
Pear	16.0	175.0
Grape	16.0	206.7
Peach	25.0	340.0
Vegetable skin		
Tomato	8.5	3.3
Potato	19.0	9.2
Onion	12.0	82.8
Parsley	17.0	291.7
Carrot	22.0	350.0

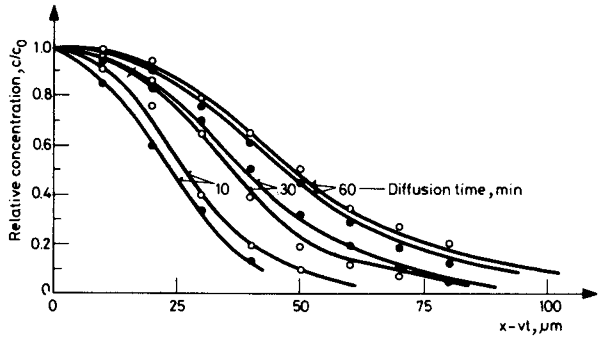

Fig. 3. Distribution of $^{212}Pb^{2+}$ ion in the diffusion zone in carrot root. The black circles were determined experimentally with Agfa-Gevaert 34B50 photoemulsion, and the empty circles with the Makrofol-E track detector, and the lines illustrate the calculated concentration distribution at 25 °C

employed in our microradiographic measurements, this fitted profile is the solution of Eq. (1).

The Pb^{2+} ion is known to be a plant poison and to pollute the environment. The transport processes of this ion were examined with a quantitative microradiographic method in carrot root. Photoemulsion and solid-state track detector techniques were used to determine the concentration profiles of the $^{212}Pb^{2+}$ ion inside the plants. The concentration distribution of the $^{212}Pb^{2+}$ ion in the diffusion zone of carrot root is presented in Fig. 3. Diffusion parameters determined with the radiographic method are listed in Tables 5 and 6.

Table 5
Diffusion parameters of $^{212}Pb^{2+}$ ion in carrot root measured with Agfa-Gevaert 34B50 photoemulsion

Diffusion time, min	Temperature, °C	Flow rate, $\times 10^{-9}$ m · s^{-1}	Diffusion coefficient, D, $\times 10^{-13}$ m^2 · s^{-1}	
			In internal tissue	In external covering tissue
10	25	6.3 ± 3.5	2.88 ± 1.23	3.58 ± 1.33
30		5.5 ± 3.1	2.70 ± 0.68	3.21 ± 0.73
60		4.4 ± 3.0	2.36 ± 0.53	2.51 ± 0.68
120		5.5 ± 3.8	2.76 ± 0.80	2.60 ± 0.85
		5.4 ± 1.7	2.68 ± 0.43	2.98 ± 0.47
60	0	3.1 ± 2.5	1.26 ± 0.35	1.56 ± 0.43
	25	4.4 ± 3.0	2.36 ± 0.53	2.51 ± 0.68
	40	6.2 ± 3.6	4.93 ± 1.68	4.58 ± 0.73

Table 6
Diffusion coefficients of $^{212}Pb^{2+}$ ion in carrot root (external covering tissue) at 25 °C, measured with CR-39, Makrofol-E and Kodak LR-115 track detectors

Diffusion time, min	Diffusion coefficient, D, $\times 10^{-13}$ $m^2 \cdot s^{-1}$		
	CR-39	Makrofol-E	Kodak LR-115
30	3.12 ± 0.71	3.63 ± 0.76	3.50 ± 1.06
60	3.09 ± 0.67	3.87 ± 0.95	3.36 ± 0.91
120	2.92 ± 0.90	3.95 ± 0.73	3.52 ± 1.24
	$\bar{D}$ = 3.04 ± 0.38	$\bar{D}$ = 3.82 ± 0.47	$\bar{D}$ = 3.46 ± 0.62

Table 7
Diffusion coefficients of borate and tetraborate ions in carrot root and potato tuber at 25 °C

Diffusion medium	Diffusion ion	Diffusion time, min	Diffusion coefficient, D, $\times 10^{-11}$ $m^2 \cdot s^{-1}$	
			In external covering tissue	In internal tissue
Carrot	Borate	5	1.98 ± 0.35	1.85 ± 0.50
		10	1.73 ± 0.66	1.60 ± 0.51
		30	1.77 ± 0.63	1.80 ± 1.05
			$\bar{D}$ = 1.83 ± 0.33	1.75 ± 0.42
	Tetraborate	5	0.97 ± 0.30	2.12 ± 0.90
		10	0.90 ± 0.18	1.97 ± 0.70
		20	0.95 ± 0.21	1.55 ± 0.36
			$\bar{D}$ = 0.94 ± 0.14	1.88 ± 0.40
Potato	Borate	10	2.30 ± 0.55	5.73 ± 2.42
		20	2.45 ± 0.53	4.18 ± 1.97
		30	2.12 ± 0.50	4.97 ± 2.35
			$\bar{D}$ = 2.29 ± 0.30	4.96 ± 1.30
	Tetraborate	5	1.15 ± 0.55	2.93 ± 1.15
		10	1.40 ± 0.56	3.33 ± 1.70
		20	1.22 ± 0.33	2.37 ± 0.83
			$\bar{D}$ = 1.26 ± 0.28	2.88 ± 0.74

Under Central European conditions, one of the most important microelements is boron; the passive transport of this element, in the form of borate and tetraborate ions, was studied in carrot and potato, an induced radiographic method being employed. The diffusion coefficients determined by this method in the systems examined are given in Table 7.

To summarize, it may be stated that the radiographic methods employed, with a spatial resolution of about 10 μm, enabled us to determine the ion concentration distributions within the plants, to differentiate between the diffusion and convection processes, and to determine the characteristic parameters of these numerically. The diffusion coefficients can be determined with a standard deviation of about 30%.

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