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Diffusion of an Organic Cation into Root Cell Walls

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Abstract—Uptake of a cationic dye (methylene blue) by isolated root cell walls, roots of whole transpiring seedlings, and excised roots was investigated using 7-day-old seedlings of cucumber, maize, and wheat. The number of ionogenic groups per 1 g dry and wet weight of the root cell walls, their swelling capacity (K_{cw}), time-dependence of methylene blue (M_{cw}) ion exchange capacity, and diffusion coefficients of the cation diffusion in the polymer matrix of the cell walls (D_{cw}) were determined. The M_{cw} value depended on pH (or carboxyl group dissociation); it changed in accordance with the number of carboxyl groups per 1 g cell wall dry weight. This parameter decreased in the order: cucumber > wheat > maize. For description of experimental kinetic curves and calculation of cation diffusion coefficients, the equation for ion diffusion into a cylinder of infinite length was used. The chosen model adequately described cation diffusion in cell walls and roots. Diffusion coefficient values for cucumber, wheat, and maize were $3.1 \cdot 10^{-8}$, $1.3 \cdot 10^{-8}$, and $8.4 \cdot 10^{-8}$ cm^2/sec, respectively. There was a statistically significant linear dependence between K_{cw} and D_{cw} values, which characterize the same property of the polymer matrix, rigidity of its polymer structure or the degree of cross-linkage or permeability. This also confirms the right choice of the model selected for calculation of methylene blue diffusion coefficients, because K_{cw} and D_{cw} values were obtained in independent experiments. The coefficients determined for methylene blue diffusion in transpiring seedling roots (D_{ts}) and excised roots (D_{er}) depended on the plant species. The rate of methylene blue diffusion into the excised roots was either 1.5-fold lower (cucumber) or 3-4-times lower (maize, wheat) than in cell walls. The values of diffusion coefficients in roots of whole seedlings were comparable which those for the cell walls. On the basis of the experimental data and results of calculations, it is concluded that the mechanism of methylene blue uptake by plant roots involves ion exchange reactions between the organic cation and cell wall carboxyl groups, and the uptake rate is determined by the cation diffusion in the polymer matrix of the cell walls.

Key words: diffusion coefficients, cell wall, root, dye

In plant roots, water and ions are transported via the apoplast and the symplast. These two pathways for mineral nutrient ions are equivalent, and predominance of one of them is mainly determined by environmental conditions and the type of transported compound [1]. Under certain conditions, the apoplast pathway of water and ion movement predominates; it is mainly determined by properties of cell walls. Properties of cell walls as cation exchangers can be characterized by such physicochemical parameters as ion-exchange capacity, ionization constants for active groups, swelling coefficient of polymer matrix, and coefficient of ion diffusion in the matrix [2, 3]. Two first parameters for characterization of properties of the cell wall and their effects on mineral ion uptake have been studied in several laboratories [4-11]. Little is known about ion diffusion in cell walls [2, 9, 12]. Many authors believe [13] that diffusion is the determining stage during ion movement in the root; however, no experimental data on the rate of ion diffusion in the matrix of plant cell wall and the effect of this property on ion transport are available.

Several stages of ion-exchange processes involving cross-linked three-dimensional ionites (including plant cell wall) are generally recognized [14]. They include: 1) delivery of the adsorbed ion from solution to ionite surface, which involves a combination of diffusion and convection (mass transduction, film kinetics); 2) delivery of the adsorbed ion (usually via diffusion) from the ionite surface to some point in its volume, where exchange (mass transfer, gel kinetics) occurs; 3) the ion-exchange reaction itself; 4) derivation of desorbed ion (as a rule due to diffusion) from the desorption place to the ionite surface (gel kinetics); 5) derivation of the desorbed ion from the ionite surface into solution (diffusion, convection, film kinetics). Generalized consideration of all these stages of the ion exchange is very difficult, so the known

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kinetic principle of a limiting stage is often used for simplification [14]. Since stage 3 represents a chemical reaction, it usually proceeds quite quickly. Diffusion of a substance in the liquid is relatively slow process; diffusion in the porous solid gel occurs much slower. This is the main reason by which the overall rate of ion-exchange process is determined by stages of mass transfer and mass flow. Thus, it is relevant to suggest that principles of kinetics of ion-exchange processes can be used to evaluate ion diffusion in the polymer matrix of cell walls. This process, often mentioned in papers on mineral nutrition, has not yet been quantitatively investigated.

In the present study, which continues our previous studies of physicochemical properties of apoplast [7-11], we have quantitatively evaluated ion diffusion in the cell wall and its contribution to the absorbing function of the root. The dye methylene blue has been used as a chemical reagent for monitoring of the diffusion process. Earlier this dye was used for evaluation of total and working absorbing surface of plant roots [15] and the study of diffusion processes in synthetic ion-exchange materials [16].

MATERIALS AND METHODS

Roots of wheat *Triticum aestivum* L., maize *Zea mays* L., and cucumber *Cucumis sativus* L. were used in the study. Grain and seeds were soaked in tape water for 3 h at room temperature and germinated at 27.5°C in darkness. Experiments were carried out at 20-22°C using 7-day-old seedlings grown in tape water at concentration of K^+, NO_3^-, Cl^-, Na^+, PO_4^{3-} of ~0.2 mM. Illumination was 110 μM/sec per m^2 for 14 h per day and solutions were aerated during 8 h per day.

Root cell walls, roots of whole seedlings, and excised roots were used in experiments. For correct comparison of methylene blue uptake by these preparations and analysis of kinetic curves, the experimental values were referred to a unit of root wet weight. In experiments with excised roots, 1 g of wet roots was used. In experiments with roots of whole transpiring seedlings we used the amount of plants with root mass of 1 g, and in experiments with cell walls the amount of wet material of cell wall isolated from 1 g of root wet weight was used.

Cell walls were isolated and standardized as described earlier [7, 8] with some modifications. The excised roots were dried with filter paper. After determination of their wet weight, the roots were placed into an Erlenmeyer flask (~0.5 liter) and treated with ~200 ml of 1% KOH for 24 h, then the roots were washed 3-4 times with distilled water (~200 ml), treated with 200 ml 1% HCl for 24 h and washed again with distilled water until complete removal of chloride [7]. The latter was determined by titration with $Hg(NO_3)_2$. This approach, which allows converting cell wall cation exchangeable groups into H^+-form, is widely used in experiments with synthetic cation exchangers; it is especially useful in comparative studies of adsorption features of ion-exchange materials with various structures of functional groups [7-11].

Microscopic study of cell walls isolated from the roots revealed lack of intracellular structures and intactness of anatomical positioning of cells in the roots subjected to the above-mentioned treatments.

Methylene blue uptake by excised roots, transpiring seedling roots, and cell walls isolated from roots. Cell walls swollen in water or plant roots were dried with filter paper. After determination of wet weight, they were placed into 0.1 mM solution of methylene blue (0.032 mg/ml). (Mass of transpiring seedling roots was determined after experiments.) The experiments were carried out under constant shaking (60 oscillations/min). Aliquots of the mixture (1 ml) were taken after 2, 4, 6, 10, 15, 20, 30, 40, 50, 60, 120, and 180 min incubation and diluted for spectrophotometric determination of methylene blue concentration at 650 nm using a Uniplan analyzer (Russia). The amount of methylene blue accumulated by plant preparation was calculated using the following formula:

$$M_i^t = \frac{C_o V_o - C_i^t V_i^t - \sum_{i=3}^{n} \left(C_{i-1}^t V_{al} \right)}{319 \cdot g},$$

where M_i^t is the amount of methylene blue accumulated during time interval t (μmol per 1 g root wet weight); C_0 and C_i^t are dye concentration (μg/ml) at zero time ($t = 0$) and after incubation time (t_i); V_0 and V_i^t represent initial volume (ml) and the volume at the moment of the aliquot collection, respectively; n is the number of aliquots; i is the aliquot number; V_{al} is the aliquot volume (ml); C_{i-1} is methylene blue concentration (μg/ml); g is root weight (in grams); 319 is the relative molecular mass of methylene blue.

Determination of maximal uptake of methylene blue by isolated cell walls. The isolated cell walls dried with filter paper were placed into Erlenmeyer flasks (~250 ml), containing 150 ml 0.1 mM methylene blue solution. After incubation for two days, the plant material was removed and dye concentration was determined as described above. Maximal uptake was calculated using the following formula:

$$M_{\max} = \frac{\left(C_{\text{in}} - C_{\text{f}} \right) \cdot V}{319 \cdot g},$$

where M_{max} is maximal ion-exchange capacity (in μmol per 1 g wet weight) of cell walls with respect to methylene blue under certain conditions (pH, dye concentration, root/solution ratio, temperature), C_{in} and C_f represent initial (C_{in}) and final (C_f) concentrations of methylene blue (μg/ml), V is the volume of the incubation mixture

(150 ml), *g* is weight of roots used for isolation of cell walls (in grams), 319 is relative mass of methylene blue.

This method was used for experimentation with porous anionite containing primary, secondary, and tertiary amino groups. Results demonstrated that given experimental conditions and sorbent/solution ratio 1 : 150 did not influence methylene blue concentration over 24 h exposure. This suggests that the amount of dye bound to cell walls due to nonionic interactions (flow into pores, adsorption due to van der Waals forces) is negligibly low.

Potentiometric titration was carried out using the method of separate weights as described [7-9]. Samples of cell wall weights dried to constant mass 40 ± 0.1 mg were placed into Erlenmeyer flasks (~50 ml) equipped with ground-glass stoppers and filled with 12.5 ml of either KOH or HCl at constant ionic strength (10 mM) which was prepared from corresponding KCl solutions. Concentrations of acid or alkaline varied from 0 to 10 mM. After 24 h, samples were separated from the solution in which pH value was determined using a Jenway pH meter, model 3320 (England). The concentration of alkaline or acid was determined using titration with bromothymol blue. The adsorption capacity of cell wall at fixed pH values was calculated by changes of H^+ or OH^- concentrations using the following formula:

$$S_{cat(an)} = \frac{(C_{in} - C_{eq}) \cdot V}{g},$$

where $S_{cat(an)}$ is the cation (anion) adsorption capacity (in μmol per 1 g dry mass of cell walls), C_{in} and C_{eq} are initial and equilibrium concentrations of KOH or HCl in solution (mM), V is the volume (ml), and g is sample weight (in grams).

Ionization constants of cation exchange groups and their amounts in cell wall were determined using potentiometric titration curves as described [7-10].

Determination of water content in roots and weight coefficient of cell wall swelling in water. Excised roots or cell walls swelled in water were dried with filter paper and weighed. (Their weights are designated as G^F and G^F_{cw}, respectively.) The roots were then fixed at 100°C for 5 min, and cell walls were dried at 50°C to constant mass. (Their dry masses are designated G^D and G^D_{cw}, respectively.) Weight coefficient of cell wall swelling (K_{cw}) and water content in roots (Q) were determined using the following formulas [11]:

$$K_{cw} = \frac{G^F_{cw} - G^D_{cw}}{G^D_{cw}},$$

$$Q = \frac{G^F - G^D}{G^D},$$

where G^F and G^D are wet and dry mass of samples (in grams), index cw means cell wall. Data on ratio of wet and dry masses of roots and isolated cell walls allows referring experimental data to wet and dry masses of roots or isolated cell walls.

RESULTS AND DISCUSSION

Diffusion in cell walls. Figure 1 shows kinetic curves of methylene blue uptake by cell walls of cucumber, wheat, and maize roots. In all variants of the measurements, there was exponential time-dependent dye uptake by cell walls (M_{cw}). The M_{cw} values were in the following order: cucumber > wheat > maize. Since ionization of methylene blue results in formation of a colored cation, it is reasonable to suggest that its uptake occurs via exchange reaction between this cation and protonated carboxyl groups of the cell walls. Data on pH dependence of methylene blue uptake seem to support this suggestion (Fig. 2). In all cases, change in pH value was accompanied by corresponding change of the amount of methylene blue in cell walls. Such pH-dependent behavior of adsorption capacity suggests that methylene blue binding to cell walls is an ion-exchange process and, consequently, the adsorption value should be linked to the number of ionogenic groups in cell walls.

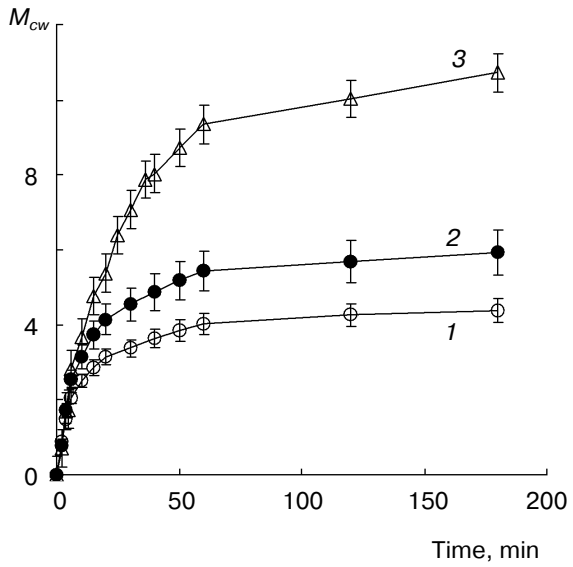

Fig. 1. Time course of methylene blue uptake (M_{cw}) by cell walls isolated from maize (*1*), wheat (*2*), and cucumber (*3*) roots. In all experiments the volume of solution and dye concentration were 150 ml and 0.1 mM, respectively. Results (μmol per 1 g root wet weight) represent mean ± SD of 3-5 experiments.

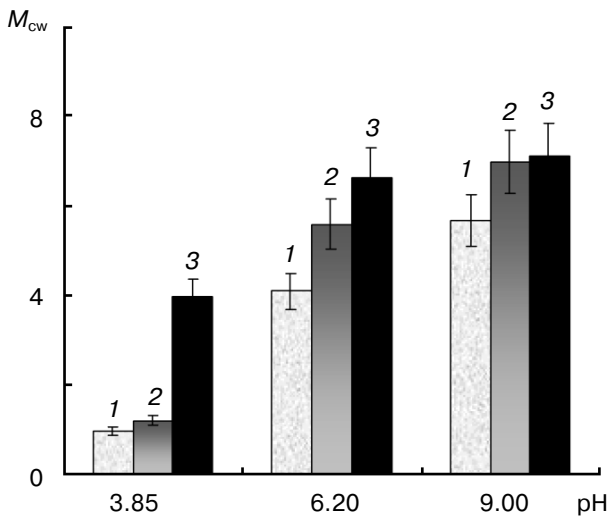

Fig. 2. Effect of pH on methylene blue uptake (M_{cw}) by cell walls isolated from maize (*1*), wheat (*2*), and cucumber (*3*) roots during 3 h exposure to 150 ml of standard 0.1 mM dye solution. Results (μmol per 1 g root wet weight) represent mean ± SD.

To confirm the suggestion of ionogenic nature of dye binding we determined the number of carboxyl groups that can be potentially involved in cation-exchange reactions with external medium cations under given conditions. In accordance with our previous reports, the results of the present study suggest that the number of anion-exchangeable groups (S_1) in the cell wall structure is one order of magnitude less than that of cation-exchangeable groups (Table 1). The polymer structure of root cell walls of all plants studied contains four types of ionogenic groups: amino groups with pK_a ~ 3, carboxyl groups of α-D-polygalacturonic acid with pK_a ~ 5, the second type of carboxyl groups with pK_a ~ 7.3, and phenolic groups with pK_a ~ 10 (Table 1). Calculations made using titration data (Table 1) and ratios of wet and dry masses of roots and isolated cell walls (Table 2) demonstrate that in all cases the amount cell wall bound dye was 1.5-3-fold higher than the number of carboxyl groups of α-D-polygalacturonic acid. This suggests involvement of both types of carboxylic groups into methylene blue cation binding. It should be noted that within the range of pH and the dye concentration employed in this study, cation-exchange hydroxyl (phenolic) groups do not participate in the ion-exchange reactions because their pK_a values are 10-10.3 [7-11].

The number of various carboxyl groups ($S_2 + S_3$) of cucumber that may be involved into exchange reactions with external cations (including methylene blue under physiological conditions) is 1.3-1.5-fold higher than in wheat and maize (Table 1). The order of changes of total number of carboxyl groups (Table 1) corresponds to the order of methylene blue binding capacity by cucumber, wheat, and maize cell walls (Fig. 1). Thus, the titration results confirm that the pH dependence of methylene blue adsorption is related to dissociation of cell wall carboxyl groups: the higher the pH value the higher the number of ionized groups (which may be involved in exchange reactions) and amount of methylene blue adsorbed by the root cell walls is.

The ion-exchange mechanism of methylene blue binding is also confirmed by the dependence of maximal ion-exchange (methylene blue) capacity of cell walls (M_{max}) on total number of carboxyl groups ($S = S_2 + S_3$) which may be potentially involved in the exchange reactions (Fig. 3). Results of statistical treatment indicate that under our experimental conditions maximal capacity of

Table 1. Number of various ionogenic groups (S_j) in cell walls isolated from maize, cucumber, and wheat

Species	S_j				
	I				II
	S_1	S_2	S_3	S_4	$S_2 + S_3$
Cucumber	70 ± 10	250 ± 30	800 ± 60	200 ± 25	24 ± 1
Wheat	140 ± 8	100 ± 15	350 ± 50	550 ± 60	18 ± 1
Maize	90 ± 10	30 ± 12	450 ± 20	800 ± 80	16 ± 1

Note: *j* is group type: *j*1) amino groups (pK_{a1} ~ 3); *j*2) carboxyl groups of α-D-polygalacturonic acid (pK_{a2} ~ 5); *j*3) second type carboxyl groups (pK_{a3} ~ 7); *j*4) phenolic groups (pK_{a4} ~ 10). The values of S_j expressed as μmol per 1 g dry weight cell walls were determined by titration data (I); the sum ($S_2 + S_3$) expressed as μmol per 1 g wet weight cell walls (II) was calculated using data of (I) with consideration of ratio between dry and wet weight of cell walls (Table 2).

Table 2. Water content in root tissue (Q), coefficient of cell wall swelling in water (K_{cw}), and relative dry mass of cell walls (G)

Species	Q	K_{cw}	G, %
Maize	11 ± 2	14 ± 1	60 ± 1
Cucumber	25 ± 2	7.3 ± 0.7	63 ± 2
Wheat	16 ± 3	6.4 ± 0.2	66 ± 3

Note: Q and K_{cw} are expressed as grams H_2O per 1 g dry weight of roots and their cell walls, respectively. $K_{cw} = (G^F_{cw} - G^D_{cw})G^D_{cw}$; $Q = (G^F - G^D)/G^D$; $G = (G^D_{cw}/G^D)\cdot 100$, where G^F and G^D are wet and dry weight of samples (in grams), and index cw means cell wall.

cell walls by methylene blue and number of cell wall carboxyl groups are related parameters: the correlation coefficient for $M_{max} = f(S)$ is 0.989.

Thus, all these considerations provide convincing evidence that the mechanism underlying methylene blue binding involves ion-exchange reactions in the cell wall and the experimental kinetic curves can be analyzed by equations describing ion-exchange kinetics [14].

Using known approaches for analysis of kinetic curves, it is possible to evaluate the rate-limiting stage of the whole process. For this purpose we have transformed the kinetic curves into the dependences $F = f(t)$ (Fig. 4), where F is the degree of completeness of the process or conversion degree. It is defined as the amount of methylene blue adsorbed during period t and referred to maximal amount of the dye which can be absorbed by the ion-exchange material under given conditions (methylene blue concentration, pH value, ionic strength, root/solution ratio). In these plots the experimental curves are also characterized by exponential behavior, but methylene blue adsorption by cell walls of the plants studied reduced in a different order: wheat ≈ maize > cucumber (Fig. 4).

Semi-logarithmic presentation of kinetic curves $F = f(t)$, $\ln(1 - F) = f(t)$, allows the determination of the rate-limiting stage for methylene blue accumulation by root cell walls. If in these plots the experimental dependences represent a straight line that issues from the zero point,

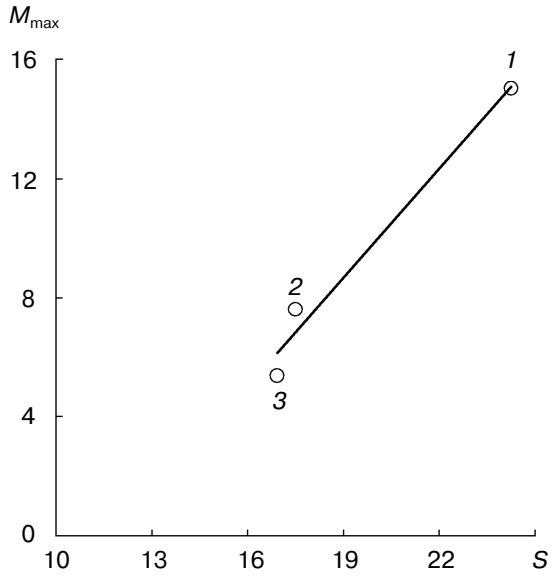

Fig. 3. The dependence of maximal uptake of methylene blue cation (M_{max}) by cell walls under given experimental conditions (dye concentration, pH, ionic strength, roots/solution ratio) on total number of carboxylic groups ($S = S_1 + S_2$, Table 1) in cucumber (*1*), wheat (*2*), and maize (*3*) cell walls. In all cases the volume of 0.1 mM methylene blue solution was 150 ml. The cell walls used in experiments were isolated from 1 g wet weight of excised roots. The results are expressed as μmol per 1 g wet weight. Points represent experimental results and solid line shows trend ($M_{max} = 1.22S + 14.6$; $r_{corr} = 0.989$).

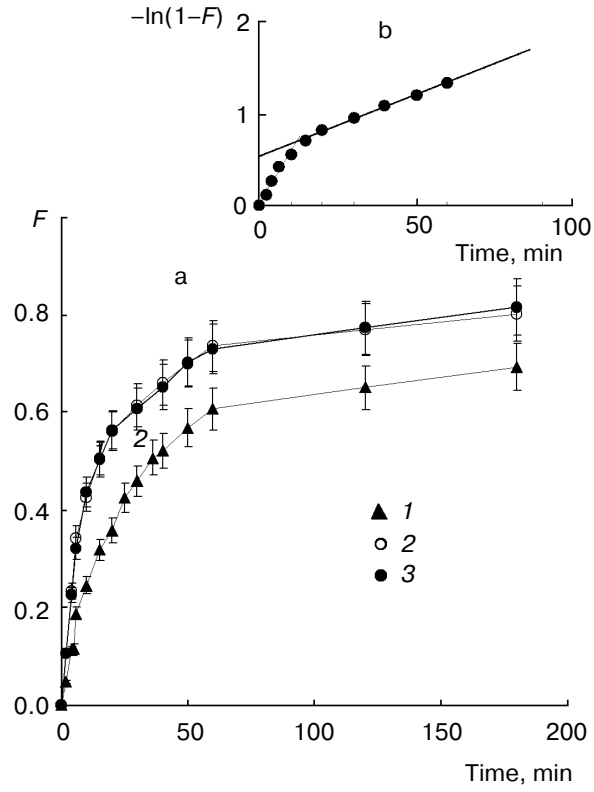

Fig. 4. a) Kinetic curves of methylene blue uptake by cell walls isolated from roots of cucumber (*1*), wheat (*2*), and maize (*3*) expressed as $F = f(t)$, where F is conversion degree, which is determined as the ratio of maximal uptake of methylene blue at time t referred to maximal capacity of cell walls by methylene blue. Results are expressed as mean ± SD. b) Anamorphoses of the kinetic curves in semi-logarithmic plots obtained for maize cell walls.

the whole process is limited by the rate of chemical reaction or diffusion in the film, otherwise the process is determined by pure diffusion events (internal and external diffusion) [14]. The dependences $\ln(1 - F) = f(t)$ obtained in the present study are represented by the broken line (Fig. 4b). This suggests that methylene blue accumulation represents mixed diffusion. In this case, the diffusion coefficient can be calculated from the final part of the kinetic curve. The following equation for ion diffusion in or out of a cylinder of infinite length of R radius can be used for calculation of diffusion [14]:

$$F = 1 - \sum_{n=1}^{\infty} \frac{4}{\mu_n^2} \exp(-\mu_n^2 F_o) , \qquad (1)$$

where $F_0 = Dt/R^2$; D is the diffusion coefficient for ion in ionite (cm^2/sec); t is time (sec); μ_n are roots of a characteristic equation ($\mu_1 = 2.4048$, $\mu_2 = 5.5201$, $\mu_3 = 8.6537$).

Let us represent the shape of a root as an infinitely long cylinder. This assumption is valid because the ratio of length to diameter of the root is much more than 100 (the average length of wheat, cucumber, and maize roots is 71 ± 31, 157 ± 22, and 145 ± 48 mm, and the average diameter of these plant roots is 0.37 ± 0.06, 0.69 ± 0.07, 0.94 ± 0.08 mm, respectively). Under these conditions the diffusion coefficient can be calculated using experimental points of the kinetic curve obtained for very long term diffusion, which is described by the first member of Eq. (1) [14]:

$$F = 1 - \mu_1^2 \exp(-\frac{\mu_1^2 Dt}{R^2}) . \qquad (2)$$

Logarithmic presentation of Eq. (2) is:

$$\ln(1 - F) = 2 \ln \mu_1 - \frac{\mu_1^2 D}{R^2} t . \qquad (3)$$

If experimental curves plotted as $\ln(1 - F) = f(t)$ represent straight lines, their slope (tangent) may be used for calculation of the diffusion coefficient. According to our calculations the dependences $\ln(1 - F) = f(t)$ actually represent straight lines (see correlation coefficients of Table 3). The values of constant A determined by the segment cut by the straight line $\ln(1 - F) = f(t)$ on the ordinate axis is an additional argument for applicability of the intradiffusion model for description of methylene blue accumulation by cell walls (Table 3). In all cases A values are within 0.6-0.74 (for infinite length cylinder $A = 0.69$); they also confirm correctness of the model applied, assumptions made, and applicability of diffusion principles to the process of methylene blue uptake [14].

The coefficient of methylene blue diffusion in root cell walls depends on plant species (Table 4). In maize this parameter is 2.5- and 6-fold higher than in cucumber and wheat, respectively. Since parameter D has not been quantitatively evaluated in plant roots, the parameters obtained in the present study have been compared with those for synthetic ion-exchange materials. Using sulfur-containing ionites as an example, it was found that methylene blue diffusion coefficient depends on ionite cross-linkage; the higher the rigidity of polymer ionite matrix structure the lower the rate of ion movement in the polymer is [16]. The diffusion coefficient for methylene blue cation in sulfur-containing cationites varied from $3 \cdot 10^{-10}$ to $4 \cdot 10^{-12}$ cm^2/sec in dependence on the amount of cross-linking reagent (2-16%). According to our data (Table 4), plant cell wall is more permeable for methylene blue than the synthetic ionites because the diffusion coefficients in cell walls are 2-4 orders of magnitude higher. This is obviously determined by characteristic structure of cell wall matrix, which is characterized by lower cross-linkage compared with the synthetic ion-exchange materials.

Values of diffusion coefficients obtained in the present study (Table 4) together with results on root cell wall swelling in water (Table 2) provide additional arguments

Table 3. Regression coefficients for the dependence $\ln(1 - F) = \ln A + Bt$ for root cell walls (r_{corr} is correlation coefficient)

Species	$-B$	A	r_{corr}
Cucumber	0.0108	0.74	0.991
Wheat	0.0138	0.63	0.989
Maize	0.0132	0.66	0.994

Table 4. Coefficients of methylene blue diffusion (D) in cell walls (I), transpiring seedling roots (II), and excised roots (III)

Species	$D \times 10^8$, cm^2/sec		
	I	II	III
Cucumber	3.1	2.3	2.0
Wheat	1.3	2.5	0.41
Maize	8.4	9.4	2.4

Note: The diffusion coefficient D was calculated using the dependence $\ln(1 - F) = \ln A + Bt$, where $D = B \cdot R^2/\mu_1^2$ (R is root radii in cm; $\mu_1 = 2.4048$ [14]).

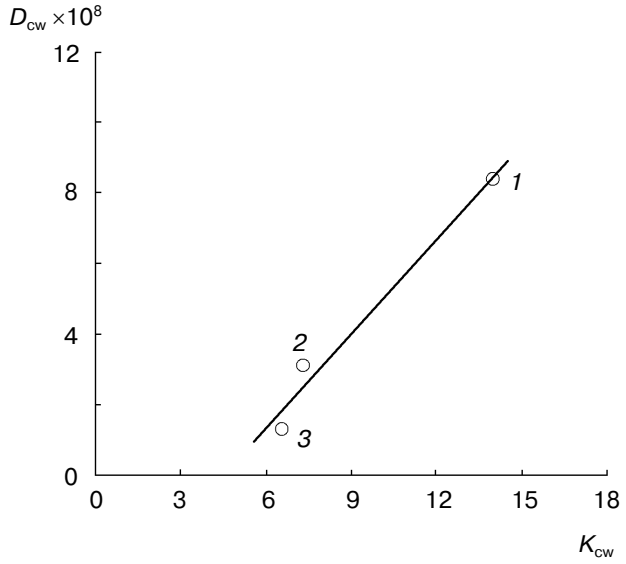

Fig. 5. Dependence of coefficients of methylene blue diffusion (D_{cw}) in root cell walls of maize (*1*), cucumber (*2*), and wheat (*3*) on coefficient of cell wall swelling in water (K_{cw}, grams H_2O per 1 g dry weight of cell walls). The coefficients D_{cw} and K_{cw} characterized the same property of the polymer matrix, the rigidity of its polymeric structure (or degree of its cross-linkage, or permeability). Calculations revealed statistically significant relationship, which confirms the correctness of the model for the calculation of diffusion coefficients because K_{cw} and D_{cw} were obtained in independent experiments. Points indicate experimental results and solid line shows trend ($D_{cw} = 0.89K_{cw} - 3.94$, $r_{corr} = 0.99$).

supporting our conclusion that cell walls represent less cross-linked ion exchanger or they are characterized by low degree of cross-links between linear chains of polymers (~1%). The coefficient of cell wall swelling in water and the coefficient of methylene blue diffusion in cell walls are parameters characterizing the same property of a matrix, rigidity of its polymeric structure (or cross-linkage or permeability). This suggests the existence of correlation between K_{cw} (Table 2) and D_{cw} (Table 4). Our calculation actually revealed the existence of statistically significant relationship between these parameters (Fig. 5). This also supports the correct choice of the model for calculation of diffusion coefficients, because K_{cw} and D_{cw} values were obtained in independent experiments. These data also provide convincing evidence that the swelling coefficients in water can serve as quantitative characteristics for permeability of both cell wall and synthetic ion-exchange materials.

Table 5. Correctness of calculated and experimental kinetic curves of methylene blue uptake by root cell walls

Species	*b*	*a*	r_{corr}
Cucumber	0.97	0.010	0.950
Wheat	1.09	0.019	0.966
Maize	1.12	0.014	0.964

Note: *a* and *b* are regression coefficients of the equation $F_{calc} = b \cdot F_{exp} + a$, where F_{calc} and F_{exp} are values of conversion degree calculated using Eq. (1) with means of diffusion coefficients taken from Table 4 and experimentally obtained (Fig. 1), respectively; r_{corr} is the correlation coefficient.

Using diffusion coefficient values, data on root radii, and Eq. (1), calculated curves for methylene blue uptake by cell wall have been obtained (Table 5). In all cases they satisfactorily correspond to the experimental data (relative error did not exceed 20%). This suggests that internal diffusion of methylene blue cation in the matrix of the root cell walls actually represents the rate limiting stage of dye uptake.

One of physiological functions of cell walls as the ion-exchange material consists of accumulation of ions from the environment. Using data of the present study, it is possible to evaluate quantitatively methylene blue cation concentrating ability of cell wall phase at low ionic strength of the external medium. Using data on relative mass of cell walls, their swelling coefficients in water (Table 2), and assuming that density of dry substance of cell walls and intact roots is the same and is roughly equal to unity, it is possible to evaluate cell wall volume per 1 g wet weight of the intact root. In wheat, cucumber, and maize this parameter is 0.44, 0.26, and 0.55 ml, respectively. This volume of root cell walls of these plants maximally accumulates 7.6, 15.0, and, 5.4 μmol of methylene blue, respectively (Table 6). In this case methylene blue concentration in cell walls of wheat, cucumber, and maize roots is 17, 60, and 10 mM. Under these conditions and assumptions the concentrating coefficient $k_{conc} = C_{cw}/C_0$ (where C_{cw} and C_0 represent voluminous concentrations of methylene blue in cell wall and in initial solution (0.1 mM)) is 170, 600, and 100 in wheat, cucumber, and maize, respectively. This means that k_{conc} for maize and wheat is, respectively, 6- and 3.5-fold lower than that for cucumber. Although methylene blue cation is significantly smaller than mineral nutrition cations, these results clearly demonstrate that cell wall accumulates cations during the first stage of cation consumption by cells. Previously, we demonstrated that cation concentration in cell wall depends on the concentration of the cation in the external medium (the higher the concentration the lower the ion concentrating in cell walls is) and the accumulation degree is determined by pH and ion composition of external medium and extracellular space [10]. At potassium concentration (C_K) in the medium of 1 mM and pH 7.0 its concentration in cell walls is 20-60-fold higher (depending on plant species) than in the initial medium. Increase of C_K to 10 mM was accompanied by corresponding decrease of k_{conc} (by one order of mag-

Table 6. Maximal ion-exchange capacity of cell walls for methylene blue (M_{max}) under given experimental conditions (dye concentration, pH, ionic strength of solution and root/solution ratio)

Species	M_{max}	M_{cw}	M_{ts}	M_{er}
Cucumber	15.0 ± 1.0	10.4 ± 0.8	11.4 ± 1.4	9.1 ± 0.4
Wheat	7.6 ± 0.4	5.9 ± 0.5	7.5 ± 1.2	4.1 ± 0.4
Maize	5.4 ± 0.5	4.4 ± 0.3	4.6 ± 0.6	2.1 ± 0.5

Note: M_{cw}, M_{ts}, and M_{er} are values of methylene blue uptake by cell walls, roots of transpiring seedlings, and excised roots, respectively, during 3-h contact of samples with 150 ml of 0.1 mM methylene blue. Results expressed as μmol per 1 g wet weight represent mean ± of 3-9 experiments.

nitude) [10]. Since we employed methylene blue concentration of 0.1 mM, the values of k_{conc} (100-600) obtained in the present study are consistent with previous results.

Thus, the cell wall is the compartment accumulating mineral nutrition cations and their concentrating degree depends on plant species and growing conditions.

Diffusion in the root. Kinetic curves of methylene blue uptake by the whole seedlings ($M_{ts} = f(t)$) and excised roots ($M_{er} = f(t)$) are characterized by pronounced exponential behavior (Fig. 6). In all variants, changes in M_{ts} and M_{er} were of the same order as in the case of isolated cell walls: cucumber > wheat > maize. The excised roots were characterized by the lowest accumulation capacity with respect to methylene blue accumulation (Figs. 1 and 6). Nevertheless, during 3 h incubation the amount of methylene blue in the cell walls, whole seedling roots, and in excised roots did not exceed maximally possible amount of methylene blue (M_{max}), which may be adsorbed on cell walls under given experimental conditions (Table 6). There were insignificant differences between M_{cw} and M_{er} values of cucumber and maize, whereas in wheat they reached 20%. These differences may be attributed to penetration of methylene blue inside cells or to the effect of transpiring flow. Methylene blue is traditionally used in physiological experiments. Several viewpoints exist on the problem of penetration of the dye inside cells. Some authors believe that methylene blue can penetrate inside cells [15], other support the opposite

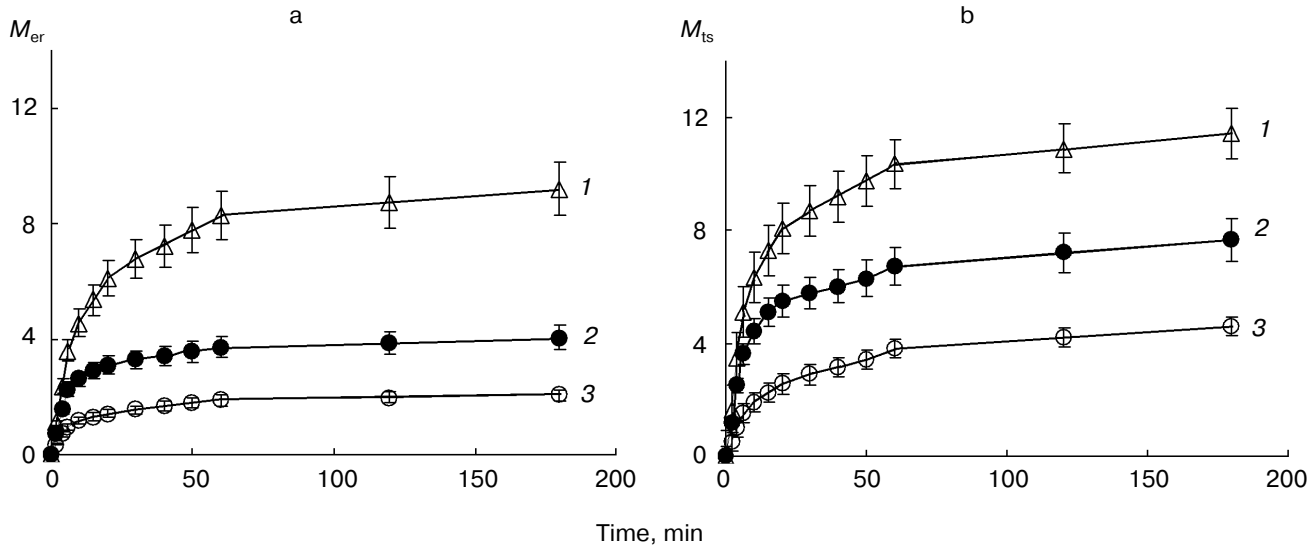

Fig. 6. Time course of methylene blue uptake by excised roots (a) and transpiring seedling roots (b). In all experiments the volume of 0.1 mM methylene blue solution and mass of roots were 150 ml and 1 g wet weight, respectively. M_{ts} and M_{er} are values of methylene blue uptake by transpiring seedling and excised roots of cucumber (*1*), wheat (*2*), and maize (*3*). Results expressed as μmol methylene blue per 1 g wet weight represent mean ± SD of 3-9 experiments.

viewpoint [17]. However, it should be noted that both viewpoints are based on microscopic studies (employing light microscope), but not on quantitative evaluations. The other important point is that various authors used different dye concentrations ranged from 0.1 to 5 mM, different root/solution ratio, and various time intervals selected for root contact with solution. These variations in experimental conditions may contribute to conclusions made by different groups of authors. We did not find in the available literature any indication of quantitative characterization of proportion of dye penetrated inside cells. In our microscopic studies of root cross-sections, we did not observe any (even weak) staining of internal content of the root cells contacting 0.1 mM methylene blue solution. However, this observation does not mean that the dye cannot penetrate inside cells. Due to intensive deep blue staining of walls the conclusion that methylene blue does not penetrate inside cells is also very subjective. Results of our experiments suggest that intracellular methylene blue concentration is about 0.5 μg/ml. This concentration is visually recognized as "colorless". If this value is used for basic calculations of methylene blue distribution in cell and cell walls, we may conclude that the amount of dye inside cells is less than the error of spectrophotometric measurements (2-3%). On the basis of these considerations, we came to the conclusion that differences in M_{cw} and M_{er} values obtained for wheat do not originate from methylene blue inside cells.

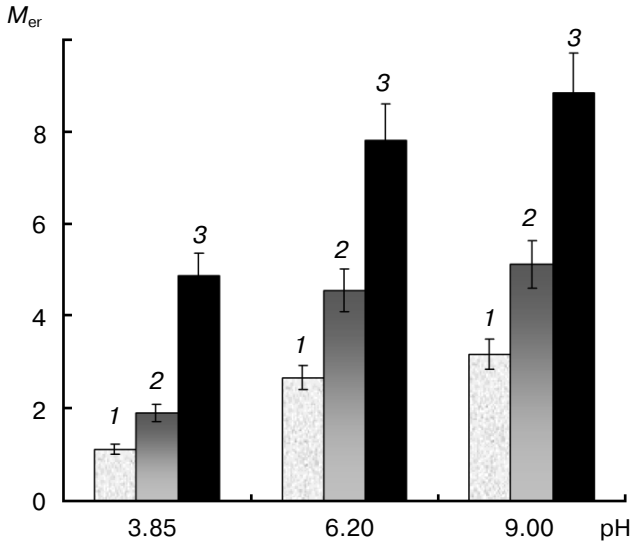

Fig. 7. Effect of pH on methylene blue uptake by excised roots (M_{er}) of maize (*1*), wheat (*2*), and cucumber (*3*) during incubation with 150 ml of 0.1 mM methylene blue for 3 h. In all experiments 1 g wet weight of roots was used. Results expressed as μmol methylene blue per 1 g wet weight represent mean ± SD of three experiments.

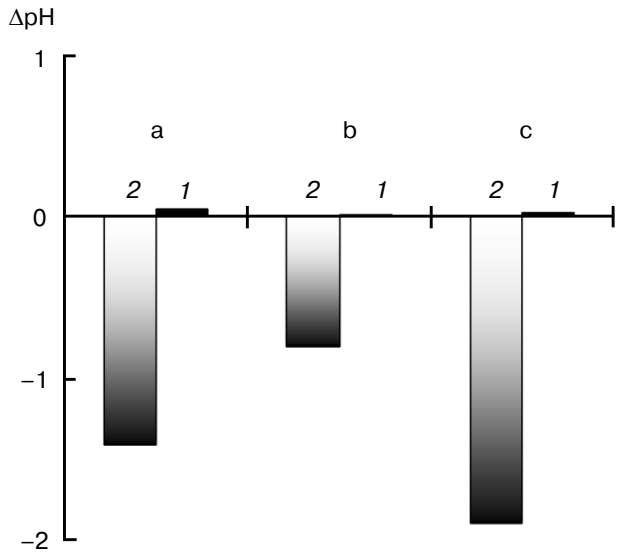

Fig. 8. Change in pH during exposure of cell walls (*1*) and roots (*2*) of wheat (a), maize (b), and cucumber (c) to methylene blue solution for 3 h. The ordinate axis represents $\Delta pH = pH_{in} - pH_f$, where pH_{in} and pH_f are initial (3.85) and final (after termination of contact of samples with solution) pH values.

The value of methylene blue uptake by excised roots (M_{er}) strongly depends on pH: increase or decrease in pH_{in} values are accompanied by corresponding increase or decrease in M_{er}, and the pH dependence of methylene blue accumulation is similar to that found for isolated cell walls (Fig. 7). It is possible that in excised roots (as well as in cell walls) increase or decrease in the ability to accumulate methylene blue is related to change in dissociation degree of carboxyl groups, which occurs during change in external pH (of the contacting solution). However, in weakly acidic solutions (pH 3.85) methylene blue accumulating ability of excised roots is higher than in cell walls. This reflects more complex structure of the ion exchanger; this is due to pH values that are formed during contacts between plant preparation and solution (Fig. 8). In the experiments with the excised roots pH value increased by 1-1.4, whereas in the case of cell walls this parameter decreased by 0.05 (Fig. 8). It is possible that in the excised roots anion-exchange groups bind protons ($-RNH_2 + H^+ \rightarrow -RNH_3^+$), involved in the exchange reaction of methylene blue cation for proton of ionogenic groups, and therefore they maintain apoplast pH and ionization of carboxyl groups at a certain level. So we did not observe sharp decrease in dye uptake (Fig. 7). We suggested that in neutral solutions methylene blue accumulation by excised roots should be higher than in the case of isolated cell walls (due to pH decrease which stems exchange reaction of methylene blue cation for proton). However, in all variants at pH 6 during exposure for 3 h, M_{cw} value

constantly exceeded M_{er}. This suggests that the rate of methylene blue transport to reaction centers of excised root cell walls decreased. It should be noted that geometrical shapes of the excised root and its isolated cell wall coincide, because cell wall isolation did not include such treatments like thermal treatment, grinding, homogenization, or mechanical damage. Cell walls were isolated from the same mass of excised roots which were used in the experiments. This means that number of reactive groups in cell walls (i.e., number of functional groups, which may bind methylene blue cation under given conditions) should be the same in both variants. Thus, for each plant species differences in kinetic behavior of $M = f(t)$ plots between excised roots and cell walls reflect different rates of dye accumulation by these systems.

We suggested that the mechanism of methylene blue uptake by roots involves ion-exchange reactions between ionized groups of cell walls and the organic cation. So, using the above-described approaches we calculated coefficients of methylene blue diffusion in the whole seedling roots and in the excised roots (Table 4). As in the case of cell walls, the diffusion coefficient varied in these species in the same order: maize > cucumber > wheat. However, the rate of methylene blue diffusion in the excised roots was from 1.5 (cucumber) to 3-4 (maize, wheat) times lower, whereas diffusion coefficients for the whole seedling roots were comparable to those obtained for the isolated cell walls. These results suggest that as in cell walls the movement of methylene blue cation in roots of transpiring seedlings occurs via apoplast. Direct proportional dependence between diffusion coefficients in whole seedling roots and cell wall swelling in water (Fig. 9a) is additional evidence for this conclusion. Analysis of the dependence $D = f(K_{cw})$ suggests the existence of statistically significant relationship between these parameters. Lack of such relationship in the case of the excised roots (Fig. 9b) may indicate that the rate of methylene blue movement to the reaction centers of cell wall is determined not only by resistance of cell wall matrix.

Using the determined coefficients for methylene blue diffusion in roots of transpiring seedlings and Eq. (1), we calculated theoretical curves of methylene blue uptake. According to our calculations, the deviation of experimental and calculated values did not exceed 3% (Fig. 10). This also supports our hypothesis on the diffusion process and ion-exchange mechanism of the interaction between the organic cation (methylene blue) and cation exchangeable groups of cell walls.

Microscopic study of transpiring seedling roots and excised roots exposed to methylene blue solution for different time intervals revealed that in all cases epidermal cells, two-four layers of cortex tissue and cell walls of all tissues of the central cylinder, including endoderm, were intensively stained in blue color. Cortex layer cells adjacent to endoderm remained unstained over the whole period of this experiment. These results confirm our conclusion that organic cation movement occurs via apoplast. There are at least three sites which may be responsible for ion passage into the stele via apoplast (avoiding endoderm cells): exoderm and endoderm carrying cells, immature endoderm cells in the root apex, and side root apoplast of the basal part of the root [17].

It is possible that in excised roots methylene blue cation movement occurs mainly via diffusion, whereas in roots of transpiring seedlings this process occurs via diffusion and mass flow. The contribution of mass flow to

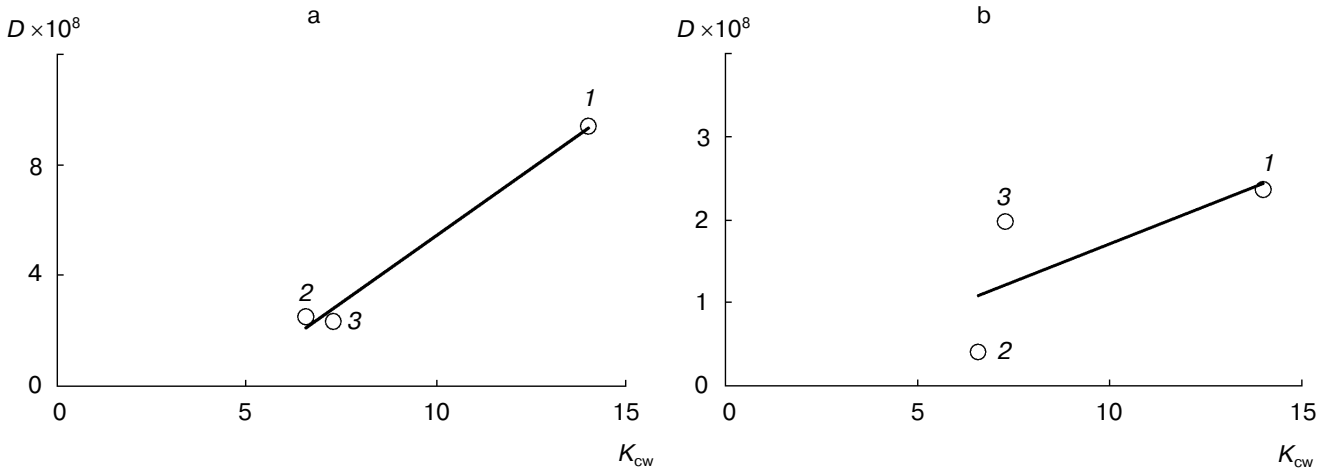

Fig. 9. Relationship between cell wall swelling (K_{cw}, in grams H_2O per 1 g dry weight of cell walls) and coefficient of methylene blue diffusion in roots of transpiring seedlings (a) and excised roots (b) of maize (*1*), wheat (*2*), and cucumber (*3*). Points indicate experimental results, whereas the solid line shows the trend; r_{corr} is correlation coefficient ($y = 0.97x - 4.2$, $r_{corr} = 0.995$ (a); $y = 0.18x - 0.06$, $r_{corr} = 0.71$ (b)).

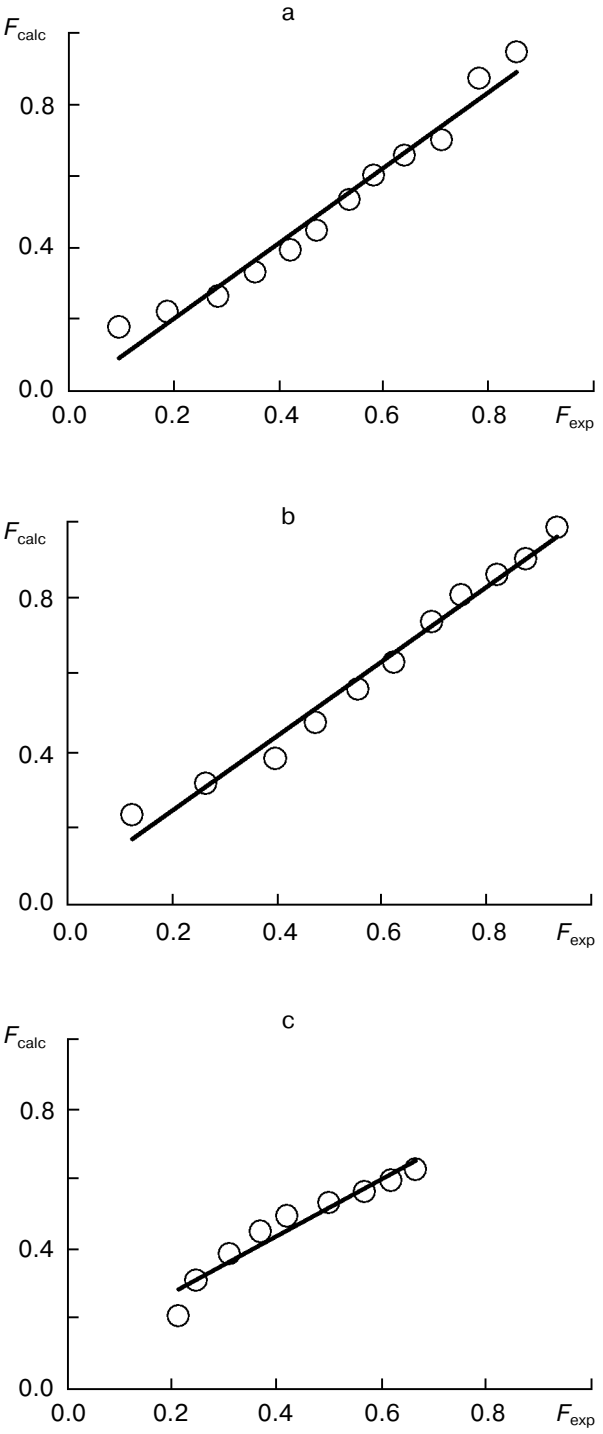

Fig. 10. Correspondence between calculated and experimental results; F_{calc} and F_{exp} are values of conversion degree, either calculated by Eq. (1) using diffusion coefficient values from Table 4 or obtained experimentally during methylene blue cation uptake by roots of transpiring seedlings of maize (a), wheat (b), and cucumber (c). Points indicate experimental results, whereas solid line shows trend; r_{corr} is correlation coefficient ($y = 1.05x - 0.006$, $r_{corr} = 0.984$ (a); $y = 0.97x + 0.049$, $r_{corr} = 0.990$ (b); $y = 0.81x + 0.11$, $r_{corr} = 0.965$ (c)).

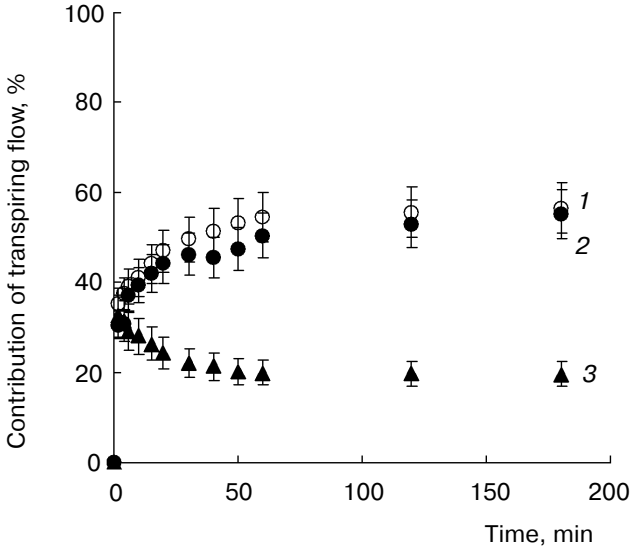

Fig. 11. Contribution of mass flow (T) to methylene blue movement in roots of transpiring seedlings. $T^t = [(M^t_{ts} - M^t_{er})/M^t_{ts}]\cdot 100$, where M^t_{ts} and M^t_{er} are values of methylene blue uptake by roots of transpiring seedlings and excised roots of wheat (*1*), maize (*2*), and cucumber (*3*), expressed as μmol per 1 g wet weight of roots.

methylene blue movement is characterized by pronounced species-specificity (Fig. 11). In cucumber the contribution of this component to methylene blue uptake is somewhat lower (~20-25%) than in wheat and maize (~35-45%). It should be noted that different behavior of roots of the whole seedlings and the excised roots is determined not only by contribution of mass flow into this process but also by general impairment in the functioning of the plant as integral biological system (particularly by reduction of upper terminal motor, lack of assimilate inflow to roots, etc.). So, using the data obtained we suggest that mass flow contributes to ion movement in plant roots. At the same time, excised roots cannot be considered as a model adequately reflecting processes of accumulation and transport of nutrition elements.

During contact of cell walls with methylene blue solution, the ion-exchange interaction between dye cation and cell wall carboxyl groups occurs. This notion is supported by: 1) the effect of pH on the amount of accumulated dye (Fig. 2); 2) existence of statistically significant relationship between number of carboxyl groups in cell walls and maximal ion-exchange capacity of cell walls by methylene blue under given experimental conditions (Fig. 3). Cation diffusion in cell walls is the rate-limiting step of methylene blue uptake. The latter conclusion is supported by kinetic analysis of the dependence $\ln(1 - F) = f(t)$, direct proportional dependence between cation diffusion coefficient and coefficient of cell wall swelling

in water (Fig. 5), and adequate description of methylene blue uptake by cell walls using the equation of internal diffusion (Table 5).

The mechanism of methylene blue cation binding by roots of transpiring seedlings is mainly based on ion-exchange interaction of this cation with carboxyl groups of cell walls. This was confirmed by the pH effect on methylene blue uptake (Fig. 7), the correspondence between the amount of accumulated dye and number of carboxyl groups in cell walls (Table 6). Methylene blue cation moves mainly via root apoplast and the rate of cation accumulation is determined by its diffusion in cell wall matrix. This is supported by lack of big differences between kinetic curves obtained for isolated cell walls and transpiring seedling roots (Figs. 1 and 6), direct proportional dependence between cation diffusion coefficient for transpiring seedling roots and coefficient of cell wall swelling in water (Fig. 9), adequate description of methylene blue uptake by roots using the equation of internal diffusion (Fig. 10), and results of the microscopic study.

In the physiology of mineral nutrition, it is generally accepted that by studying kinetics of ion accumulation it is possible to discriminate its transport in cell walls and transport through the cell membrane [18-20]. Two phases are recognized in the typical exponential curve of time-dependent cation accumulation. The first (fast) phase is characterized by half-saturation period ($t_{1/2}$) of several minutes, whereas the second (slow) phase has $t_{1/2}$ of several hours. Each phase reflects processes in certain compartments: phase I reflects transport via apoplast (in apparent free space), and phase II reflects transport via symplast (in cytoplasm). Results of our study suggest that diffusion, the slowest stage of ion exchange, is very important for ion accumulation by plant roots, and exponential behavior of the kinetic curve is not the criterion for discrimination of ways of ions transportation (via symplast or apoplast) [19, 20]. Cation transport in cell walls is not a rapid process as suggested earlier [19]. According to results of the present report, during 3 h exposure cell walls are saturated with methylene blue by 60-90% (depending on plant species). In spite of significant differences between the main nutrient mineral cations and methylene blue, it is possible that under certain conditions some of their proportion is also transported via apoplast both in radial and axial directions and in this case diffusion processes will have decisive importance. For example, under conditions of altered mineral nutrition (e.g., sudden increase of cation concentration in the soil solution) cell walls may serve as a temporary reserved pool for cations; the size of this pool may be experimentally determined. In this case diffusion will determine the rate of its filling.

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