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Swelling of Root Cell Walls as an Indicator of Their Functional State

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Abstract—The swelling capacity of cell walls isolated from different parts of lupine root was investigated. The water content in fragments of intact roots (Q) and swelling coefficient of standardized samples of cell walls (K^{cw}) were determined, and the dependences of Q and K^{cw} on the distance from the root tip (L) were plotted. It was shown that the change in Q value along the stretch of the lupine root reaches its maximum at distances of 1.5-6 cm or 7-12 cm from the root tip in 7-day-old and 14-day-old seedlings, respectively, whereas the K^{cw} value distribution over the root length is virtually invariable. In the radial direction, both the Q and K^{cw} values in cortex tissues are about twice higher than in the central cylinder. In our opinion, the changes of both Q and K^{cw} in the radial direction are associated with different degrees of cross-linking between polymer chains in cell wall structures of root cortex and central cylinder. The results of measurement of the K^{cw} value are consistent with the widely accepted mechanisms of water transport in roots in the radial direction. These data show that water transport through apoplast to the border between the cortex and central cylinder is accompanied by an increase in the resistance to water flow. Among other factors, this increase is due to a greater degree of cross-linking between cell wall polymers in the central cylinder. The results of measurement of the swelling coefficient of standardized cell wall samples in water and in 10 mM KCl at different pH values show that the swelling capacity of root cell walls varies according to the physicochemical properties of synthetic ion exchangers. Cell walls shrink (cell wall volume decreases) as ion concentration in solution increases and pH decreases. This causes an increase in the hydraulic resistance (or a decrease in the hydraulic conductivity) of apoplast. It was concluded that swelling is determined by the physicochemical properties of the cell wall, whereas the change in the swelling capacity induced by variation of external or internal conditions is an element of the mechanism of regulation of volume water flow in roots.

Key words: *Lupinus albus* L., roots, water content, cell wall, swelling, variation along root length, cortex, central cylinder, polymer chains, degree of cross-linking, volume water flow

It is widely recognized that the properties of cell walls are similar to the properties of weakly cross-linked cation exchangers [1-4]. On the other hand, the ion-exchange capacity of the cell wall is thought to be determined by the presence of carboxyl groups of polyuronic acids in its polymer structure [1-4]. The most important physicochemical parameters of cationites are ion-exchange capacity, ionization constant of active groups, and coefficient of polymer matrix swelling [5]. The first two parameters have been used by many researchers to describe cell wall properties and to elucidate the contribution of the cell wall to processes of primary uptake of mineral elements [1-4, 6, 7]. However, swelling capacity of cell walls has not yet been determined. Moreover, this parameter has not even been taken into account.

The swelling coefficient of synthetic ion exchangers is a function of the degree of lateral cross-linking between polymer chains, total number of ionizable groups of the ionite, degree of their dissociation, concentration of the external solution, and radius of the hydrated ion bound to the sorbent. Of these factors, the degree of lateral cross-linking between polymer chains is the major contribution to swelling capacity [5, 8]. Thus, experimental assessment of ionite swelling can be used to probe the rigidity of its three-dimensional structure or ability to change its volume upon exposure to various external factors.

It is well known that there are two pathways of water transport in plant roots: through apoplast and through symplast. The two pathways of water transport are equally important, the dominance of either of them being determined mainly by environmental conditions [9]. In the root itself, water is transported both in radial and in

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axial directions. The structure of root cells and tissues (including structure and chemical composition of cell walls) is also a subject of variation in both radial and axial directions [9, 10]. Quantitative assessment of swelling capacity of plant cell walls is thought to provide an estimate of the degree of cross-linking of its matrix. In combination with data about the anatomical structure of plant tissues and mechanisms of water and ion transport in plants, these estimates can give additional quantitative information about the contribution of the apoplast to transport of water and ions in plants.

However, there is virtually no experimental data on swelling of the plant cell wall polymer matrix or correlation between swelling and cross-linking of cell wall matrix polymer chains. In this work, we continue preceding studies of the physicochemical properties of apoplast [6, 7]. The goal of this work was to assess quantitatively the swelling capacity of cell wall polymers in cell wall preparations isolated from roots of lupine (*Lupinus*) plants of different physiological state and to estimate the possible contribution of swelling capacity of cell wall polymers to the absorption function of roots.

MATERIALS AND METHODS

Roots of lupine (variety, *Nemchinovskii belyi*) were used for this study. Lupine seeds were soaked in tap water for 3 h at room temperature and germinated in a thermostat at 27°C in the dark. Experiments were performed using 7-day-old seedlings grown in tap water (variant I) and 14-day-old seedlings grown from the age of 7 days in complete Knop solution (variant II). Ambient temperature of 20-22°C, illuminance of 110 $\mu M/m^2 \cdot sec$, and 24-h light/dark photoperiod with light phase duration of 14 h were used for both variants. Thus, plant age and ionic strength of the growth solution were varied in the experiments.

The mean root lengths in variants I and II were 8 ± 1.6 and 14 ± 2.1 cm, respectively. The whole roots were cut off and divided into fragments as follows: root tip, 0-1.5 cm; zone of lateral roots, 1.5-6 cm in variant I or 1.5-7 and 7-12 cm in variant II; basal part, 6-8 and 12-14 cm in variants I and II, respectively. Basal part fragments (7-8 and 13-14 cm in variants I and II, respectively) were divided into cortex and central cylinder tissues. Light microscopy showed that cortex tissue consisted of epidermal cells and cortex cells, whereas central cylinder tissues also contained endoderm cells.

The resulting root fragments were fixed for 5 min at 100°C, dried in the presence of a sorbent ($CaCl_2$) at 55-60°C to constant weight, and stored in closed weighing bottles until use.

Isolation of cell walls. Cell wall preparations were isolated as described earlier [6, 7]. Fixed and dried material was subjected to dynamic washing in a glass ion-exchange column (volume, V = 200 ml) with 10 mM KOH (~0.5 liter), distilled water (~2 liters), 10 mM HCl (~0.5 liter), and distilled water up to the absence of Cl^- in the effluent water. Washed material was dried in the presence of sorbent ($CaCl_2$) at 55-60°C to constant weight. Chloride ion was assayed by titration with mercury nitrate. This method of standardization (conversion of all cation-exchange groups of cell wall into H^+-form) allows the sorption capacity of ion-exchange materials with different structure of functional groups to be compared [6, 7, 11]. Light microscopy of cell wall samples isolated from different zones of the lupine roots showed that the anatomical structure of cell arrangement in the root was not disturbed, and none of the cell wall samples contained intracellular organelles.

Determination of water content in intact roots and weight coefficient of cell wall swelling in water. Fragments of intact roots or standardized cell wall preparations swollen in water were wiped with a piece of filter paper, and their fresh weight was measured (G_F and G_F^{cw}, respectively). Then, segments of intact roots prefixed for 5 min at 100°C and cell wall preparations were dried to constant weight at 50°C, and their dry weight was measured (G_D and G_D^{cw}, respectively). The coefficient of swelling of standardized cell walls (K^{cw}) and water content in fragments of intact roots (Q) were calculated as described in [5] using the following equations:

$$K^{cw} = (G_F^{cw} - G_D^{cw}) / G_D^{cw}, \quad (1)$$

$$Q = (G_F - G_D) / G_D, \quad (2)$$

where G_F and G_D are fresh and dry weights of samples (in grams), respectively, and the superscript "cw" indicates standardized cell wall.

Potentiometric titration. Potentiometric titration was performed using the method of individual weights as described in [6]. Dry samples (weight 40 ± 0.1 mg) were placed in conical flasks (volume ~50 ml), and 12.5 ml of KOH or HCl solution of different concentration but invariable ionic strength (10 mM) was added. The flasks were closed with ground stoppers. The ionic strength of the solution was maintained at a constant level by the addition of KCl solution of the required concentration. The range of variation of KOH and HCl concentration in the initial solution was 0-10 mM. Experimental samples were collected after 48 h of incubation. Standardized preparations of cell walls were wiped with filter paper, and fresh weight (G_F^{cw}) was measured. Then the cell wall preparations were dried to constant weight at 50°C, their dry weight (G_D^{cw}) was measured, and the coefficient of swelling of standardized cell walls (K^{cw}) was calculated from Eq. (1). Solution pH was measured before and after contact with the experimental samples. The pH value was

measured using a Jenway 3320 pH meter (Great Britain). Residual concentrations of H^+ and OH^- in solution were determined and used in calculations of the sorption capacity of roots or cell walls at pH_i:

$$S_i^{cat,an} = (C - C_i) \cdot V/g\,, \qquad (3)$$

where $S^{cat,an}$ is the sorption capacity for cations (S^{cat}) and anions (S^{an}), respectively (μmol per g dry weight); C and C_i are the initial and corresponding equilibrium concentrations of KOH or HCl in solution, mM; V is the solution volume, ml; g is the sample weight, g. The swelling coefficient of standardized cell wall samples was determined at each pH value, and the curve $K_i^{cw} = f(pH_i)$ was plotted.

RESULTS

It is always uncertain in studies of cell wall properties if the material isolated from plants contains true cell walls. Such uncertainty is particularly inherent for isolation methods that do not involve preliminary homogenization of plant tissues, detergents, and harsh chemical treatment. Microscopic examination is usually appropriate to address this question. Therefore, light microscopy can be regarded as a qualitative test of cell wall preparation purity if intracellular organelles are absent [2]. In this work, we assume that results of potentiometric titration of fixed tissues and standardized cell walls isolated from these tissues can be regarded as a quantitative test of the quality of the isolation method.

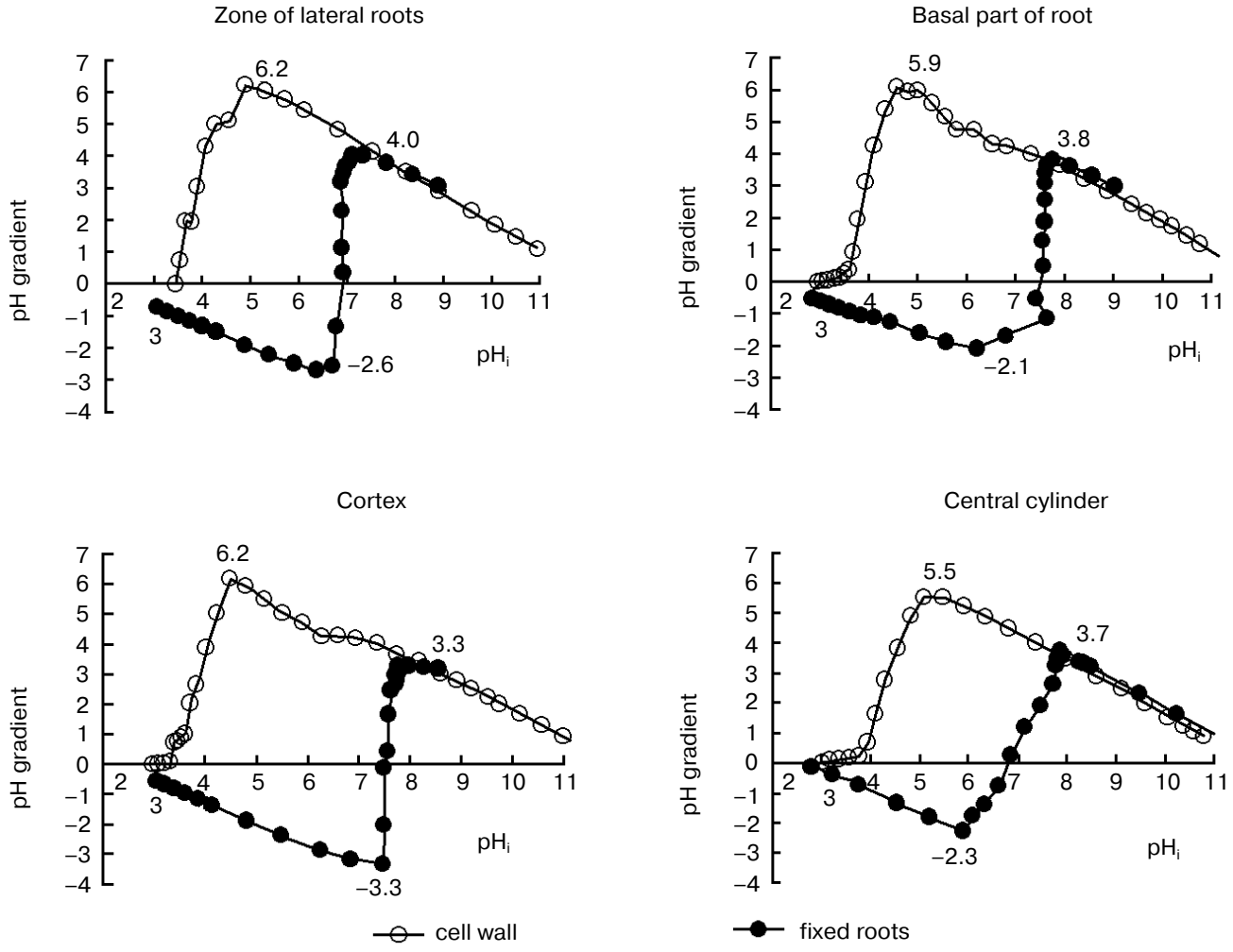

Fig. 1. Potentiometric titration of fragments of fixed roots and standardized cell walls isolated from 14-day-old seedlings grown from the age of 7 days in complete Knop solution. The zone of lateral roots and basal part were 1.5-7 and 12-14 cm from the root tip, respectively. Fragments of the basal part located 13-14 cm from the root tip were separated into cortex and central cylinder tissues. Light microscopy showed that cortex tissue consisted of epidermal cells and proper cortex cells, whereas the central cylinder tissues also contained endoderm cells. The pH gradient $\Delta pH = pH - pH_i$, where pH and pH_i are the pH values of the initial and equilibrium solutions, is shown. Positive pH gradient ($pH > pH_i$) corresponds to proton release in the exchange reaction ($R\text{-}COOH + K^+ \leftrightarrow R\text{-}COOK + H^+$), whereas negative pH gradient ($pH < pH_i$) corresponds to proton uptake by anion-exchange groups ($R\text{-}NH_2 + H^+ \leftrightarrow [R\text{-}NH_3]^+$). Experimental points are connected by empirical curves.

Experimental curves of potentiometric titration $\Delta pH = pH - pH_i = f(pH_i)$, where pH and pH_i are the pH values of the initial and equilibrium solutions, respectively, are shown in Fig. 1. It is seen that the acid–base properties of cell walls and fixed material differ from each other.

The curves measured for fixed material are distinctly sinusoidal, the ΔpH value within a narrow pH range near $pH_i \sim 7\text{-}7.5$ taking either positive or negative sign. The negative value of ΔpH is due to proton uptake by anion-exchange groups ($R\text{-}NH_2 + H^+ \rightarrow [R\text{-}NH_3]^+$). This value may exceed 3 pH units. The positive value of ΔpH is due to proton release in the exchange reaction ($R\text{-}COOH + K^+ \rightarrow R\text{-}COOK + H^+$). This value may reach 3.5-4 pH units. An experimental $pH_i = f(pH)$ curve is shown in Fig. 2. It follows from Fig. 2 that the ionogenic groups of fixed root material (and therefore, ionogenic groups of intact roots) are able to maintain the solution pH at a constant level against a background of external medium pH variation over a broad range. For example, changing the external medium pH from 3.5 to 11 caused only an insignificant (0.3 pH unit) variation in the equilibrium solution pH in all root zones located more than 1.5 cm from the root tip. The same was true in the case of cortex or central cylinder. In all types of tissues of fixed material, proton uptake by ionogenic groups of root cells or proton release were observed at equilibrium solution $pH < 7\text{-}7.5$ or $pH_i > 7\text{-}7.5$, respectively.

The experimental results show that under otherwise identical conditions, the properties of fixed material differ significantly from the properties of standardized cell walls (Fig. 1). Proton uptake by standardized cell walls is

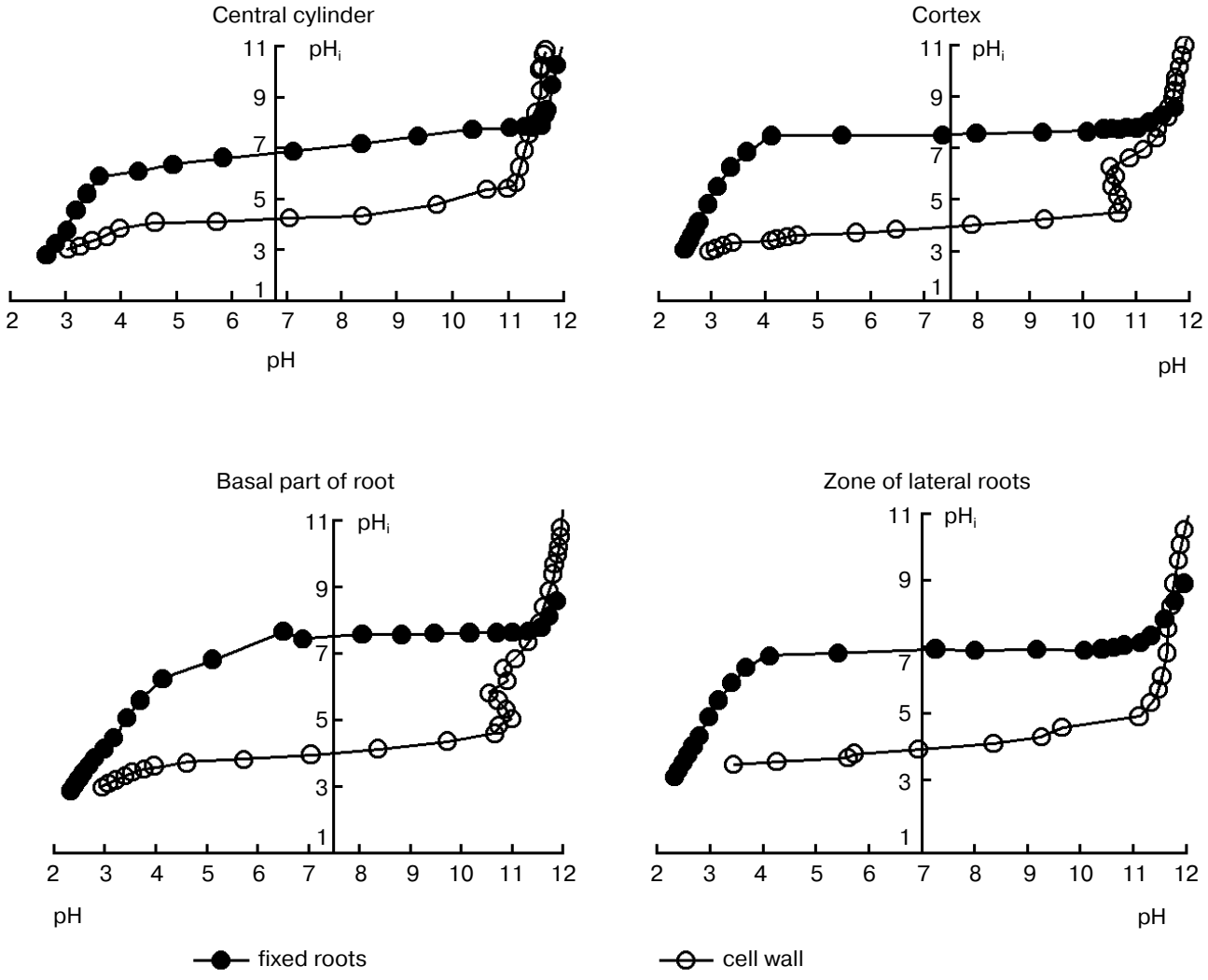

Fig. 2. Dependence of equilibrium solution pH (pH_i) on the initial solution pH as measured by potentiometric titration of fixed roots and standardized cell walls from 14-day-old lupine roots. The other symbols are as in Fig. 1. Experimental points are connected with empirical curves.

observed within the narrow range pH_i = 2.5-3.5, the absolute magnitude of the negative gradient being less than 0.03 pH unit in all cases. At pH_i > 3.5, ΔpH takes only positive values. Therefore, in contrast to fixed (intact) material, standardized cell walls contain mainly cation-exchange groups. Note, that fixed (intact) roots contain both cation-exchange and anion-exchange groups. It follows from Fig. 2 that although the ionogenic groups of cell walls are also capable of maintaining the pH value of the external solution at a constant level, this level is lower (pH_i ~ 4) than the constant pH_i level typical of fixed root material.

The estimates of the content of ionogenic groups obtained from Eq. (3) show that the maximum content of anion-exchange and cation-exchange groups in the structure of fixed (intact) roots ranges from 1.2 to 2 and from 2.3 to 3.2 mmol per g dry weight, respectively. The content of ionogenic groups varies within these ranges over the root zones. In standardized cell walls, the content of anion-exchange and cation-exchange groups in all variants was no more than 0.05 and 1.1-1.3 mmol per g dry weight, respectively.

The results discussed above show that roots treated with electrolyte solutions as described in "Materials and Methods" contain mainly cell walls, because the treated root samples are characterized by: 1) high cation-exchange capacity, and 2) low anion-uptake capacity. In other words, the ion-exchange properties of treated roots are similar to those of the cell wall preparations isolated using the method described in [3, 4].

The results of measurement of relative dry weight of cell walls are given in Fig. 3. Relative dry weight of cell walls was calculated as $G = (G_D^{cw}/G_D)\cdot 100$, where G_D^{cw} is the dry weight of standardized cell walls (g) isolated from G_D dry fixed root material (g). This parameter was found to be invariable over the whole root length except for the zone 0-1.5 cm from the root tip. These findings are consistent with the well-known fact that the cytoplasm-to-cell volume ratio in root tip cells is higher than in cells located farther from the root tip [12]. According to radial distribution, the relative weight of cell walls in central cylinder is 1.5 times higher than in cortex and independent of root age.

Dependences of water content in intact root tissues on distance from the root tip are shown in Figs. 4 and 5. In the two variants of root samples, these curves have distinctly pronounced extrema. The zone of maximum water content in variants I and II of intact fragments is located 1.5-6 and 7-12 cm from the root tip, respectively. In the two variants, the water content in root tip is 2-3 times less than in the other zones. There is also variability of the Q value in the radial direction: the Q value in the cortex is two times higher than in the central cylinder.

The swelling coefficient of standardized cell wall samples is invariable over the root length (Figs. 4 and 5), whereas its change in the radial direction is similar to the Q value variation in intact roots: K^{cw} in cortex is almost two times larger than K^{cw} in central cylinder.

The pH dependences of the swelling coefficient of isolated lupine cell walls at solution ionic strength of 10 mM

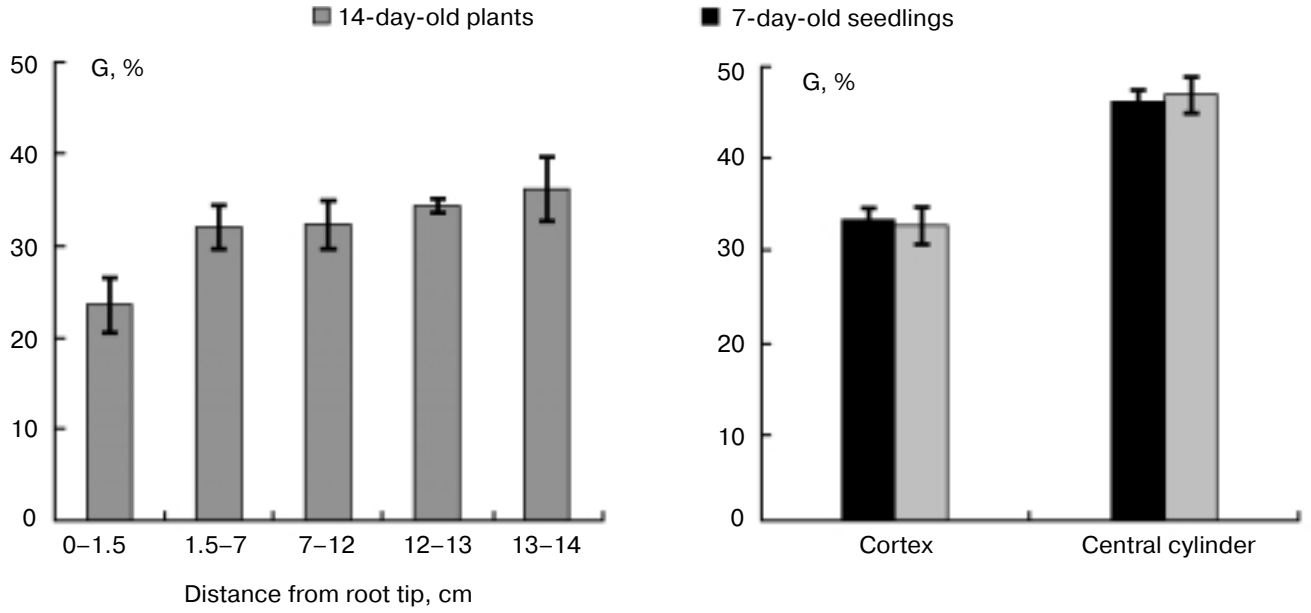

Fig. 3. Changes in relative dry weight of cell walls (G, %) in the axial and radial directions. $G = G_D^{cw}\cdot 100/G_D$, where G_D^{cw} is the dry weight of standardized cell walls (g) isolated from G_D dry fixed root material (g). Experimental results are given as arithmetic means of 5-10 measurements (bars) ± standard deviations.

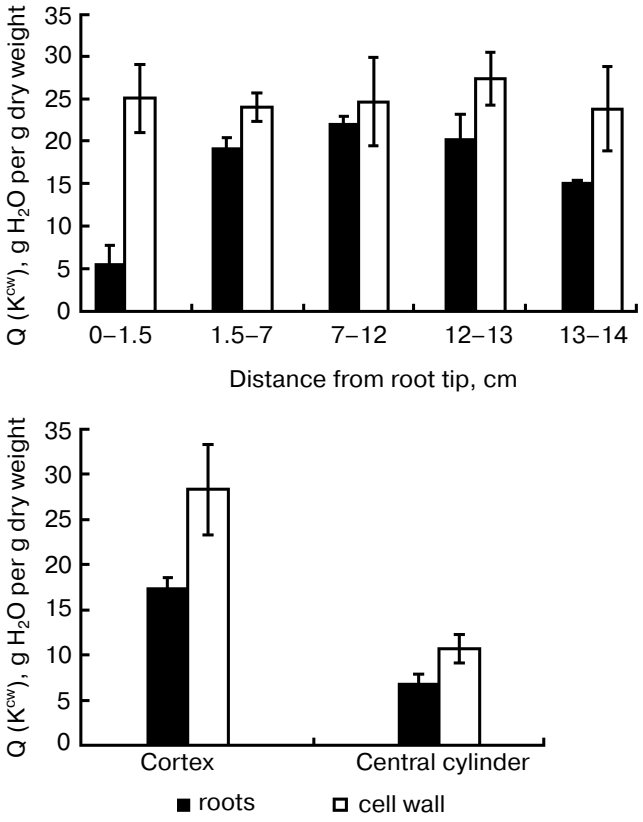

Fig. 4. Changes in water content in intact roots (Q, g H_2O per g dry weight of intact roots) and coefficient of swelling of cell walls in water (K^{cw}, g H_2O per g dry weight of cell walls) in axial and radial directions of the root of 14-day-old lupine plants. K^{cw} and Q were calculated as: $K^{cw} = (G_F^{cw} - G_D^{cw})/G_D^{cw}$; $Q = (G_F - G_D)/G_D$, where G_F and G_F^{cw} are the fresh weights of intact roots and cell walls, respectively, g; G_D and G_D^{cw} are the dry weights of intact roots and cell walls, respectively, g; superscript "cw" indicates standardized cell wall. Filled and open bars show water content in intact roots and cell walls, respectively. Experimental results are given as arithmetic means of 5-10 measurements (bars) ± standard deviations.

are shown in Figs. 6 and 7. Regardless of plant age, growth conditions, and tissue type, the swelling coefficient increases with increasing pH. The K^{cw} value in 10 mM KCl solution is significantly less than in water.

DISCUSSION

The dependences of water content in intact root tissues on the distance from the root tip have pronounced extrema (Figs. 5 and 6). The maximum and minimum water contents are observed in the zone of lateral roots and root tip, respectively. There is a sharp increase in the Q value in the zone of lateral roots and a gradual decrease in Q toward the basal part. The value of Q in root tip is 1.5-3 times less than in the other zones (the exact value of Q depends on the seedling age). Perhaps, the water content decrease in root tip is due to specific structural features of this zone (lower degree of cell vacuolization, in particular). Given the high degree of cell wall swelling and its invariability over the root region (Figs. 4 and 5), it can be suggested that a 5-7% decrease in the relative weight of cell walls in the root tip (Fig. 3) also contributes to the decrease in Q at the root tip. The dependence of the water uptake rate in 17-day-old barley roots is also characterized by the presence of pronounced extrema [13]. The

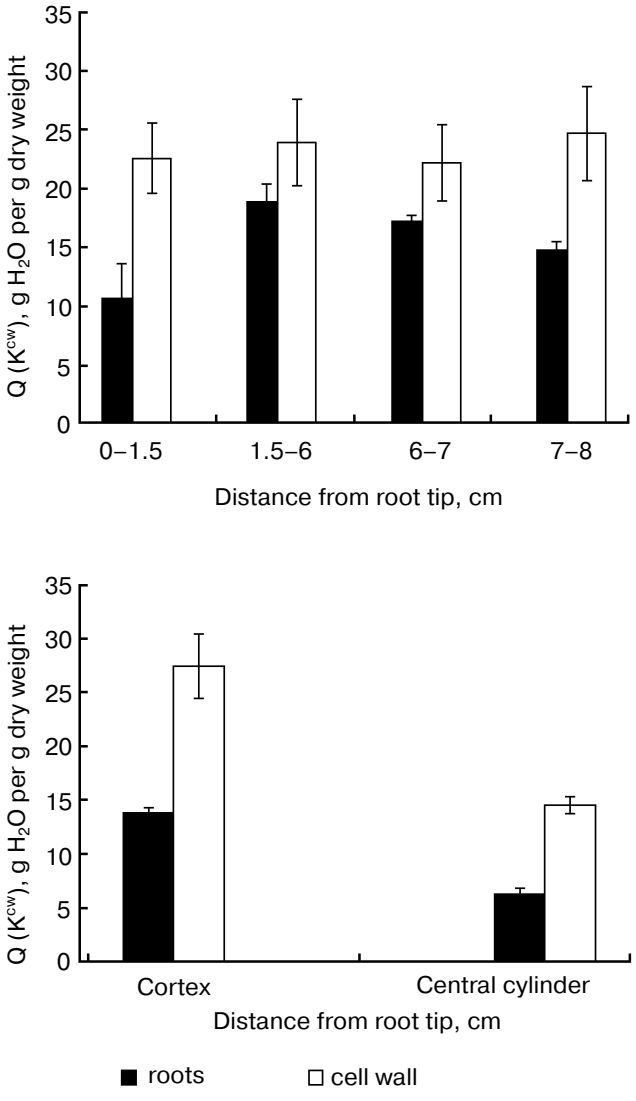

Fig. 5. Changes in water content in intact roots (Q, g H_2O per g dry weight of intact roots) and coefficient of swelling of cell walls in water (K^{cw}, g H_2O per g dry weight of cell walls) in axial and radial directions of the root of 7-day-old lupine seedlings. Filled and open rectangles show water content in intact roots and cell walls, respectively. Details are as in Fig. 4. Experimental results are given as arithmetic means of 5-10 measurements (bars) ± standard deviations.

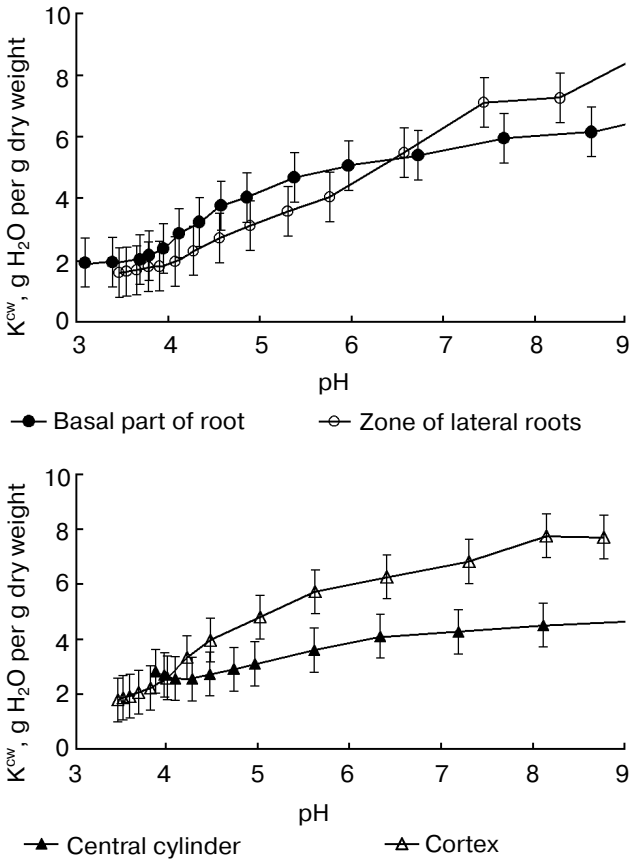

Fig. 6. The pH dependence of the swelling coefficient of standardized cell walls isolated from various fragments of 14-day-old lupine roots and measured at solution ionic strength of 10 mM. Experimental results are given as arithmetic means (bars) ± standard deviations.

maximum rate of water uptake was observed at the zone located 4-8 cm above the barley root tip [13]. Because the zone of maximum Q coincided with the zone of the minimum water flow resistance in barley roots [13], it is conceivable that the zone of maximum cell wall swelling in lupine roots also coincides with the zone of maximum rate of water uptake. The maximum water content at the zone of lateral roots can be explained by different factors, including specific structural features of the cell wall.

The pH dependences of the swelling coefficient of standardized cell walls in water (Figs. 4 and 5) and 10 mM KCl solution (Figs. 6 and 7) show that the swelling coefficient changes can be explained in terms of the physicochemical properties of ion-exchange materials [5]. In all cases, the K^{cw} value in 10 mM KCl solution was significantly less than in water. Therefore, the swelling coefficient value decreases with increasing solution ionic strength. The fact that the K^{cw} value in acidic solutions is lower than in neutral or alkaline solutions can be explained by different degrees of dissociation of ionogenic groups at different pH values [6, 7]. Indeed, the swelling coefficient at pH 2-4 does not exceed 2.0 g H_2O per g dry weight (Figs. 6 and 7), and this value virtually coincides with the swelling coefficient of loosely cross-linked carboxyl-containing ionites at low pH [8].

The dependences of swelling coefficient on concentration and pH of the external medium can be considered within the framework of macroscopic models suggested earlier for ion-exchange materials [5]. For example, according to the model suggested by Lazar and Gregor, the ionite is a laminar structure composed of flat parallel plates (linear polymer chains), the charged surfaces of the plates being linked to each other with elastic springs (sites of cross-linking between polymer chains). The interaction between ionite and solution in this model is determined by the following factors:

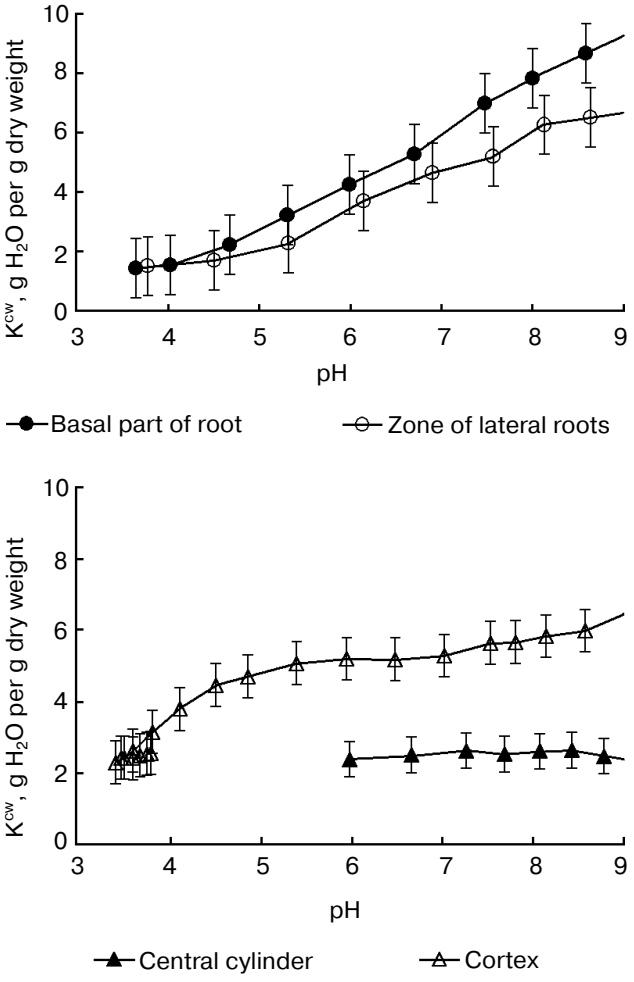

Fig. 7. The pH dependence of the swelling coefficient of standardized cell walls isolated from various fragments of 7-day-old lupine seedling and measured at the solution ionic strength of 10 mM. Experimental results are given as arithmetic mean (bars) ± standard deviations.

osmotic pressure difference between external solution and liquid in ionite pores, electrostatic repulsion, and spring pressing force. This model provides an easily understood interpretation of the swelling processes. There are two major causes of ionite volume increase. First, because ion concentration in ionite pores is rather high, these ions have high affinity for the solvent. Therefore, it can be assumed that there is high-concentration electrolyte solution in the pores, and this solution tends to be diluted. Electrostatic repulsion between like charged fixed ions is the second factor of matrix expansion. In the model of Lazar and Gregor, this factor is simulated by mutual repulsion of charged plates. As the degree of swelling increases, the effect of the two forces decreases: solution dilution in ionite pores brings the osmotic pressure in the solution–ionite system closer to the equilibrium level, whereas an increase in the distance between fixed ions causes a decrease in the electrostatic repulsion. Matrix expansion causes an increase in the force of opposite direction. In the macroscopic model, this force is simulated by spring elasticity: the higher the degree of cross-linking, the larger the spring force. An equilibrium state is attained if oppositely directed forces compensate each other. Therefore, it can be suggested that an increase in the ion concentration in the external solution causes a decrease in the osmotic pressure difference, which is determined by the concentration ratio of internal (in cell wall pores) and external solutions. The decrease in the osmotic pressure difference causes a decrease in the cell wall swelling capacity. On increasing the pH, the number of ionized groups in the lupine root cell walls increases [6, 7]. This increases the total negative charge of fixed anions, thereby increasing the force of electrostatic repulsion between the polymer chains and the swelling capacity.

The results of our experiments showed that the swelling coefficient does not change significantly along the root length (Fig. 6). The results of potentiometric titration of cell walls isolated from different zones of the root showed that along the lupine root length there is no more than 20% change in the content and structure of ionogenic groups determining the swelling coefficient value (unpublished data). As noted above, the degree of cross-linking between polymer chains is the main factor of different (or equal) efficiency of swelling of polymers in water. Therefore, it can be suggested that in spite of different chemical composition and microscopic structure of cell wall polymers, the degree of cross-linking between them is virtually the same in all zones of lupine root ($K^{cw} \approx$ const). These findings suggest that different water content in intact lupine root tissues located in different zones along the root axis (maximum water content at the zone of lateral roots) (Figs. 4 and 5) is due to specific structure of intracellular components rather than to cell wall polymer structure.

A different distribution pattern is observed in the radial direction in lupine roots. Both the Q value in intact roots and the K^{cw} value in standardized cell walls in cortex tissues are two times larger than in the central cylinder (Figs. 4 and 5), whereas the relative weight of cell walls in the central cylinder is 15% larger than in cortex (Fig. 3). It may be suggested quite confidently that radial changes in the Q value in intact roots are due to structural changes of cell wall polymers and different degree of cross-linking between polymers in central cylinder and cortex. Comparative assessment of swelling capacity clearly shows that the degree of polymer cross-linking in cortex is significantly less than in central cylinder. The results of analysis of the elemental composition of root tissues are also consistent with this conclusion (table). It was found that cortex tissues contain less carbon than central cylinder both in intact roots and in cell wall preparations. The literature contains little if any information about the composition of individual chemical elements in cell wall polymers in different root tissues. However, it can be suggested based on the chemical composition of cell walls that, in addition to carbohydrates, cell walls contain suberin, cutin, proteins, lignin, etc. [14]. Lignin is a polymer of monomers composed of 67% C, 29% O, and 5% H. It is also well known that the content of lignin in cell walls in some cases may reach 90%. The carbon content in suberin and cutin (macromolecules composed of fatty acid residues) is close to 70%. Based on the elemental composition of cellulose (40% C, 54% O, and 6% H) and lignin, it is easy to calculate that a polymer composed of 80% cellulose and 20% lignin or 60% cellulose and 40% lignin contains 46% C or 51% C, respectively. Therefore, an increase in the degree of cell wall lignification (i.e., increase in the lignin content) should be accompanied by an increase in the carbon content in cell walls. Thus, it may be suggested based on the results of this work (table) and the presently accepted concept of the apoplast structure that the higher degree of polymer cross-linking in central cylinder tissues relative to cortex tissues is due to the higher content of lignin and/or suberin in central cylinder tissues [10, 14]. It should also be noted that central cylinder differs from cortex in the content of nitrogen of hydrophilic groups. This factor may also contribute to the difference between polymer swelling capacities in these tissues (table). However, the literature on swelling of synthetic ion-exchange materials shows that an increase in the nitrogen content from 0.8 to 1.9% (typical value observed in our experiments with roots) should not cause a two- to threefold increase in the degree of polymer swelling [8]. Therefore, it is unlikely that changes in the nitrogen content alone can explain the difference in the swelling capacities between the root tissues (Fig. 4).

The absolute nitrogen content in cell walls (table) as calculated per g dry weight of intact roots is about 0.7 and

Content of N, C, and H (%) in different zones of 14-day-old lupine root and in cell walls isolated from the root. The percentage was calculated per g dry weight of roots and isolated cell walls. The samples were ground, dried at 55-60°C to constant weight, and assayed for the content of individual chemical elements. Elemental analysis was performed at the Laboratory of Elemental Analysis, School of Chemistry, Lomonosov Moscow State University, using a Carlo-Erba automatic CNH-analyzer (Italy). The assay was based on the catalytic burning of plant material at 1000°C and further separation of reaction products on a gas-chromatographic column. The results given in the table are arithmetic mean values of triplicate biological tests (plant roots from three growing sessions)

Root zone	Roots			Cell walls		
	N	C	H	N	C	H
Cortex	7.2 ± 0.3	35.1 ± 1.2	5.7 ± 0.1	1.9 ± 0.1	44.9 ± 1.0	7.2 ± 0.1
Central cylinder	4.0 ± 0.2	39.6 ± 1.0	5.7 ± 0.1	0.76 ± 0.05	50.0 ± 1.3	6.8 ± 0.1

0.4% in cortex and central cylinder, respectively. Note that relative dry weight of cell walls in cortex and central cylinder is 35 and 50%, respectively (Fig. 3). Therefore, cell walls contain about 10% of the total root nitrogen.

The estimates of the number of ionogenic groups showed that the maximum content of anion-exchange groups in both fixed and intact roots ranges from 1.2 to 2 mmol per g dry root weight. In standardized cell walls, the content of anion-exchange groups does not exceed 0.05 mmol per g cell wall dry weight. On the other hand, the results of elemental analysis show that the total nitrogen content in roots and standardized cell walls is 2.8-5 and 0.54-1.4 mmol per g dry weight, respectively (table). Therefore, the content of non-ionogenic nitrogen-containing groups (at least the nitrogen-containing groups that are not ionogenic within the pH range studied in our experiments) in fixed root tissues and standardized cell walls is up to 3 and 1.35 mmol per g dry weight, respectively. Although it is presently uncertain which compounds contain non-ionogenic nitrogen, it seems likely that the non-ionogenic nitrogen is a structural component of the polymer matrix of cell walls (e.g., amide compounds). This idea is supported by the well-known fact that synthetic ion-exchange materials containing 1,3,5-triacryloyltriazine as a cross-linking agent are highly permeable to proteins, and their structure is stable [8]. The nitrogen content in such materials may reach 2-3%, whereas the nitrogen-containing amide groups of the polymers are not ionogenic.

The experimental results on the swelling of lupine root cell walls in water and electrolyte solutions obtained in this work provide additional evidence that the cell walls can indeed be considered as a loosely cross-linked ion exchangers. In other words, the degree of cross-linking between the linear chains of the polymers is thought to be rather low. The data available from the literature on the dependence of swelling coefficient in water on the degree of cross-linking of synthetic carboxyl-containing ion-exchange materials allow estimation of the degree of cross-linking between cell-wall polymers. This value is thought to be ~0.5-1%, because it is the level typical of the synthetic carboxyl-containing ion exchangers with the same coefficient of swelling in water [5, 8].

Thus, the results of measurements of swelling of the cell wall preparations isolated from different zones of lupine root show that the degree of cross-linking between polymers is virtually invariable in the axial direction, whereas in the radial direction this parameter changes significantly (in central cylinder it is significantly higher than in the cortex tissues). The results of measurement of the K^{cw} value are fully consistent with the mechanisms of water transport in roots in the radial direction. It can be concluded that there is a stepwise increase in water flow resistance at the boundary between the primary cortex cells and the central cylinder. Among other factors, this increase is due to a higher degree of polymer cross-linking in the cell walls of the central cylinder. However, because the K^{cw} value in 10 mM KCl at pH ~7 in variant II and variant I was 4 and 2 g H_2O per g dry weight, respectively (Figs. 6 and 7), it can be concluded that the central cylinder cell walls are permeable to radial water flow. Although it is probable that a fraction of the water flow continues to move along the apoplast even when the endoderm had been passed, the volume of the fraction is significantly less than the volume of water transported through the cortex apoplast. This suggestion is consistent with the chemical structure of Caspari belts in endoderm [15]. Indeed, it was found that lignin is the main structural component of these belts. Because lignin is a hydrophilic compound, it cannot serve as an absolute barrier to water flow through the apoplast [9, 15].

According to the results obtained in this work, the degree of root cell wall swelling increases as the ionic strength of the external solution decreases. This means that the cell wall volume increases upon decreasing the ion concentration in the nutrient solution. Indeed, a decrease in the ion concentration in the nutrient solution causes an increase in the hydraulic conductance or vol-

ume flow rate of water in apoplast [16]. On the other hand, it is well known that the lower the ionic strength of the external solution, the higher the plant transpiration rate. Changes in the external solution pH are also accompanied by apoplast volume changes. The degree of cell wall swelling in acid medium is significantly less than in neutral or weakly alkaline media (cell walls shrink at low pH). It was shown in [17] that a decrease in the medium pH to about 4 causes a decrease in the hydraulic conductance (increase in the hydraulic resistance) of cell walls or a decrease in the volume flow of water through roots [16].

Thus, swelling of apoplast polymers is part of the physiological reactions of plants. Because the degree of swelling is determined by the physicochemical properties of the cell walls, it can be concluded that the swelling changes induced by variation of external or internal conditions are an element of the mechanism of regulation of volume water flow in plant roots.

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