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Ion exchange properties of *Cicer arietinum* L. root cell walls under different environmental salt conditions

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Ion exchange properties of the polymeric matrix of cell walls isolated from roots of 20-day-old *Cicer arietinum* L. plants grown in nutrient solution in the presence of 0.5 and 80 mM NaCl were studied by the potentiometric method. The ion exchange capacity (S_i) and the swelling coefficient (K^{cw}) of root cell walls were estimated at various pH values (from 2 to 12) and at different means of solution ion strength (between 10 and 250 mM). To analyse polysigmoid titration curves $pH_i = f(S_i)$, Gregor's equation was used. It was shown that Gregor's model fits quite well the experimental data. The total quantities of cation exchange (S_t^{cat}) and anion exchange (S_t^{an}) groups were determined in root cell walls. It was found that in plant root cell walls there are 3 cation-exchange groups (two types of carboxyl groups and a phenolic group) and one anion-exchange group (amine group). The quantity of functional groups of each type (ΔS^j) was estimated, and the corresponding values of pK_a^j were calculated. It has been shown that for carboxyl groups of polygalacturinic acid arranged in the cell wall structure, the acid properties are enhanced by increasing the electrolyte concentration. Swelling of root cell walls changes with pH and NaCl concentration in the solution. The findings are discussed from the standpoint of involvement of root cell walls in plant response to salinity.

Key words: *Cicer arietinum,* cell wall polymeric matrix, root, ionogenic groups, p*K*, salinity

INTRODUCTION

The cell wall is now considered to be an elaborated multifunctional system that can be a source of signals for triggering the defense reactions of the plant [1, 2]. The cell wall is a compartment that is the first to contact an external solution and modify its composition by exchange reactions between the ion-exchange groups of the wall polymeric matrix and the medium ions and, thus, regulate the entrance of substances into the cell.

Recent investigations on the composition and properties of polysaccharides, structural proteins and enzymes constituting the cell wall have resulted in considerable progress in knowledge of the cell wall on the molecular level. Nevertheless, information about effects of various stressors on processes in this compartment is very scarce.

Problems associated with the interaction of the cell wall with pathogens [3] are most exhaustively studied. But little is known about changes in the cell walls generated under the influence of abiotic stressors, salt stress in particular.

Publications about specific features of the plant cell wall functioning as natural ion exchangers under salinity conditions are few in number [4–6]. The ion-exchange properties of root cell walls of the halophyte *Suaeda altissima* (L.) Pall and tolerance glycophyte *Spinacia oleracea* L. were shown to depend on the presence of four types of functional groups. The ion-exchange capacity of the root walls of these plants was found to change due to an increase in the content of polygalacturonic acid groups in response to increase in salt concentration in the medium [5, 6]. To determine whether this response of these plants to salinity was specific, in the present work the ion-exchange properties of the root cell walls of a typical glycophyte (*Cicer arietinum* L.) were studied at varied levels of environmental salinity.

MATERIALS AND METHODS

Roots were obtained from 20-day-old chickpea plants (*Cicer arietinum* L.) grown in Pryanishnikov medium. When 3-day-old, the plants were subjected to salinity by addition of NaCl into the nutrient solution every 2–3 days, providing the increase in NaCl concentration in the pot to be no more than 20 mM. The final NaCl concentrations in the nutrient medium were 0.5 and 80 mM.

Cell walls were isolated from roots as described in [7]. Excised roots were placed into a glass ion-exchange column (V = 250 ml), successively washed in 1% alkali and acid solutions and in distilled water until the disappearance of Cl^- in the washing water, and then dried to constant weight in the presence of $CaCl_2$ at 55–60 °C.

To assess the quality of cell wall isolation, the preparations were stained with the fluorescent dye DAPI (4′, 6-diamidino-2-phenylindole) (Sigma, USA) and examined microscopically: no intracellular structures were found in any of the preparations (data not shown).

The qualitative and quantitative composition of ion-exchange groups of the cell walls was determined by potentiometric titration using separate weights [7]. Dry weights of the

cell wall preparations (40 ± 0.1 mg) were placed in 50-ml glass flasks with glass stoppers and filled with 12.5 ml of KOH or HCl solutions of varied concentration but with the same ionic strength provided by appropriate NaCl solutions. The concentration of the alkali and acid varied from 0 to 10 mM. After 48 h, the plant preparations were separated from the solution which was used to determine pH with a Model 3320 pH meter (Jenway, England) and the concentration of the remaining acid or alkali by titration with methyl red as the indicator. By changes in the H^+ or OH^- concentrations the sorption capacity of the cell wall was calculated at a fixed pH_i value using the formula:

$$S_i = (C^{in} - C^{eq})V / g, \quad (1)$$

where S_i is the cation-exchange capacity of the samples at the corresponding value of pH_i, μmol/g dry weight of cell walls; C^{in} and C^{eq} are the initial and equilibrium concentrations of KOH or HCl in the solution, mM; V is the solution volume, ml; g is the sample weight, g. For the root cell walls of the plants grown under different salinity conditions (0.5 and 80 mM NaCl) the potentiometric curves were obtained at the solution ionic strength of 10, 100 and 250 mM.

Titration curves were calculated as described elsewhere [5, 7]. The number of each type of functional group (ΔS^j) was determined from experimental curves of pH dependence on the cell wall sorption capacity. Contents of free amino acids were determined by non-aqueous titration in acetic acid [8].

Calculation of pK_a^j by potentiometric curves. The experimental potentiometric curve was subdivided into j monosigmoidal fragments according to differential curve $\frac{dS_i}{d\mathrm{pH}_i} = f(\mathrm{pH}_i)$. Each differential curve had several maxima and several minima (inflexion points). Each minimum was approximated to either the initial or the final ionization point of the specific group in cell walls [5, 7]. For example (Fig. 1), the point pH_1 (pH ≈ 3.3) was the final ionization point of the first ionogenic group and at the same time the initial ionization point for the second ionogenic group. The point pH_2 (pH ≈ 5.6) was the final ionization point of the second ionogenic group and at the same the initial ionization point for the third ionogenic group, and so on. The difference in the ion exchange capacity between the initial and the final points showed the amount of ionogenic groups of the j th type (ΔS^j). Thus, the character of the differential curves showed how many ion exchange groups existed in the cell walls, so ΔS^j values could be found and the ionization degree (α) could be calculated as

$$\alpha = \frac{S_i^j}{\Delta S^j}, \quad (2)$$

where S_i^j is the amount of the ionized ionogenic group of j-type at pH_i.

To calculate the ionization constant for each ionogenic group (pK_a^j), Gregor's equation was used [9]:

$$\mathrm{pH} = \mathrm{p}Ka + n\log_{10}\left(\frac{\alpha}{1-\alpha}\right), \quad (3)$$

where pK_a is an apparent ionization constant of the polymer ionogenic group, α is the dissociation degree, n is a constant depending on the polymeric matrix structure and the counter-ion nature. On calculating the corresponding value of $\log10[\alpha_i / (1 - \alpha_i)]$ for every pH_i value and using regression analysis, the pK_a^j and n^j values were obtained for each step of ionization. Using the obtained values of the parameters (ΔS^j, pK_a^j, n^j), the calculated curves of the $S_i = f(pH_i)$ dependence were determined for all points of the experimental pH_i values using the summarizing equation [10]:

$$S_i^{cal} = S_t^{cat} - \sum_{j,i=1}^{k,m} \Delta S^j \left[1 + 10^{\frac{(pK_a^j - pH_i)}{n^j}}\right], \quad (4)$$

where S_t^{cat} is the maximal cation-exchange capacity of the cell walls; ΔS^j is the amount of j-type ionogenic groups; S_i^{cal} is the calculated ion-exchange capacity of the cell wall at the corresponding pH_i value. S_t^{cat}, ΔS^j and S_i^{cal} are expressed in μmol per 1 g dry weight of cell walls; pK_a^j is the apparent ionization constant of the j-type ionogenic groups; n^j is the constant of Eq. (3) for the j-type ionogenic groups; k is the number of points on the potentiometric curve; m is the number of ionogenic group types.

The adequacy of the approach used for description of the acid–base equilibrium was assessed by regression analysis determining the parameters of the equation:

$$S_i^{cal} = B\ S_i^{exp} + A, \quad (5)$$

where S_i^{exp} and S_i^{cal} are the ion-exchange capacity, μmol/g dry weight of cell walls, experimental and calculated from Eq. (4) at the corresponding value of pH_i; A and B are regression parameters.

Water content in plant tissues (Q) and weight coefficients of cell wall swelling in water (K_w^{cw}) and solutions (K_s^{cw}) were determined as described in [7]. The values of the parameters K_w^{cw}, K_s^{cw} and Q were determined by the formulas:

$$K_{w(s)}^{cw} = \frac{G_F^{cw} - G_D^{cw}}{G_D^{cw}}, \quad (6)$$

$$Q = \frac{G_F - G_D}{G_D}, \quad (7)$$

$$G = G_D^{cw} / G_D 100, \quad (8)$$

where G_F and G_D are fresh and dry weights of the samples, g; the index *cw* indicates the cell wall.

RESULTS

In the experimental titration curves of root cell walls (Fig. 2) of chickpea plants grown at different salt concentrations in the nutrition medium, the range of positive S_i values corresponds to protons release from the cell walls according to the reaction: ~COOH + Na+ → ~COONa + H+, where ~ denotes the polymeric chain. At pH > 10.5 the cation-exchange capacity reaches the maximum (S_t^{cat}), and S_t^{cat} characterizes the total amount of acidic groups in the polymeric structure, which are capable of being involved in the exchange reactions at appropriate pH values of the environment (Table 1).

In accordance with the differential curves obtained from the experimental ones, the amount of cation-exchange groups of

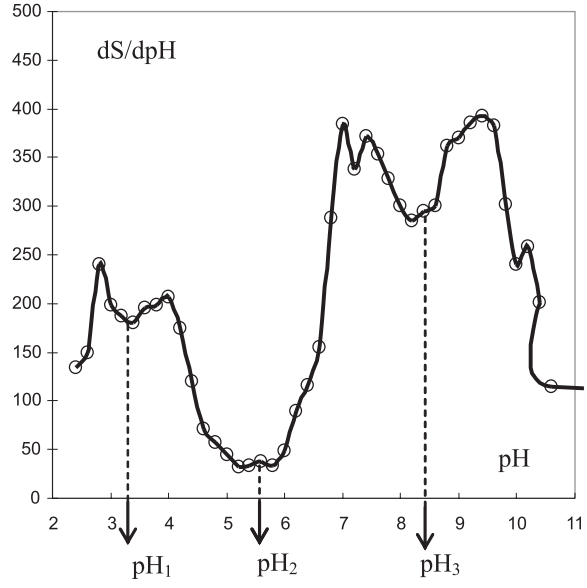

Fig. 1. Typical differential curve computed from experimental potentiometric titration curve. Explanation in the text (Materials and Methods, calculation pK^j_a by potentiometric curves)

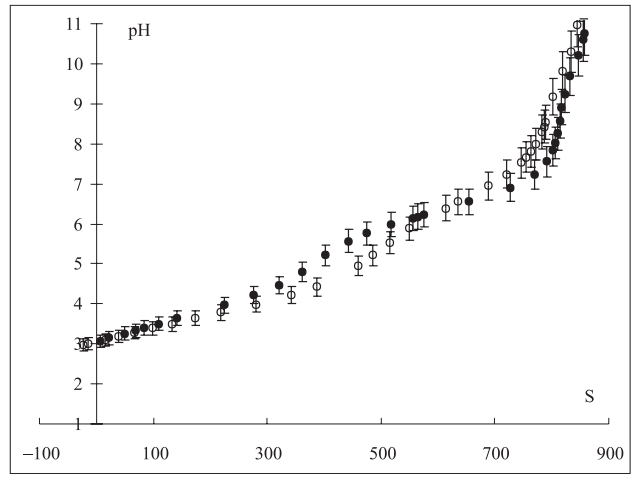

Fig. 2. Potentiometric titration curves of the polymeric matrix of root cell walls of chickpea plants grown at 0.5 (closed circles) and 80 (open circles) mM NaCl in the medium. The ionic strength of the solution on titration was 100 mM. *S* is the ion exchange capacity of the polymeric matrix of the cell walls, μmol/g dry weight of cell walls

Table 1. **Effect of NaCl concentration in the growth medium (C^{NaCl}, mM) on the content of amino groups (ΔS^1), carboxyl groups of polygalacturonic acid (ΔS^2), carboxyl groups of oxycinnamic acid (ΔS^3), and phenol groups (ΔS^4) in the polymeric matrix of the cell walls isolated from chickpea roots. S_t^{cat} and S_t^{an} are the total cation exchange and anion exchange capacity, respectively, of the cell wall matrix. S_t^{cat}, S_t^{an}, ΔS^2, ΔS^3, ΔS^4 are expressed in μmol/g dry weight of the cell walls; ± is the standard deviation**

C^{NaCl}	$S_t^{an} = \Delta S^1$	ΔS^2	ΔS^3	ΔS^4	$S_t^{cat} = \Delta S^2 + \Delta S^3 + \Delta S^4$
0.5	708 ± 13	433 ± 42	373 ± 51	55 ± 23	870 ± 80
80	883 ± 21	497 ± 25	223 ± 105	50 ± 20	830 ± 65

Table 2. **Effect of ionic strength of the solution (*I*, mM) on the mean dissociation constant (pK_a^j) of cation exchange groups of the cell wall polymeric matrix of chickpea. *J* – the type of ionogenic group: 2 – carboxyl groups of polygalacturonic acid; 3 – carboxyl groups of oxycinnamic acids; 4 – phenol groups**

I	pK_a^j		
	j		
	2	3	4
10	4.20 ± 0.12	7.05 ± 0.23	10.03 ± 0.23
100	3.76 ± 0.17	6.71 ± 0.36	9.86 ± 0.14
250	3.34 ± 0.06	6.93 ± 0.16	10.02 ± 0.13

Table 3. **Water content in root tissues (*Q*), the swelling coefficient of root cell walls in water (K_w^{cw}) and their relative dry weight (*G*) in chickpea plants depending on NaCl concentration in the nutrient solution (C^{NaCl}, mM). *Q* and K_w^{cw} are expressed in g H_2O per 1 g dry weight of the root tissue and cell walls, respectively. Values of the parameters K_w^{cw}, K_s^{cw} and *Q* were determined by formulas 5–7 (see Materials and Methods)**

C^{NaCl}	Q	K_w^{cw}	G, %
0.5	17.1 ± 1.0	17.7 ± 2.2	39 ± 4
80	16.8 ± 3.9	17.0 ± 2.4	30 ± 4

each type (ΔS^j) was determined in the cell wall polymeric matrix (Table 1; the method was described in [6, 7, 11]).

The calculations have shown that the model chosen completely conforms to the experimental data, and this is confirmed by values of the coefficient correlation (r^{corr}) of the dependences $S_i^{cal} = f(S_i^{exp})$ and also values of the coefficients *A* and *B* in Eq. (5) (data not shown). In all variants $r^{corr} \geq 0.99$, the value of *A* is not more than the experiment error, and $B \approx 1$.

In the chickpea, the ion-exchange capacity of the root cell walls increased with increasing the salt concentration in the solution. Depending on the salt concentration, this parameter changed from 70 to 165–190 μmol/g dry weight of cell walls in the interval of ionic strength changes from 10 to 1000 mM (Fig. 3).

The increase in NaCl concentration in the medium was associated with the reduced relative dry weight of root cell walls (*G*), whereas the swelling of the cell wall polymeric matrix in water (K_s^{cw}) only weakly depended on the salt concentration of the nutrient solution (Table 3). The capacity of the chickpea cell wall polymeric matrix for swelling in solutions sharply increased with increase in pH and decreased with increase in the ionic strength of the solution; however, this parameter did't depend on NaCl concentration in the nutrient medium (an example for a plant grown at 0.5 mM NaCI in the medium see in Fig. 4).

DISCUSSION

The polymeric matrix structure of root cell walls of the glycophyte tested includes four types of ionogenic groups capable of being involved in exchange reactions with environmental ions under certain conditions: three types of these groups exchange cations and the fourth type exchanges anions (Table 1). The total

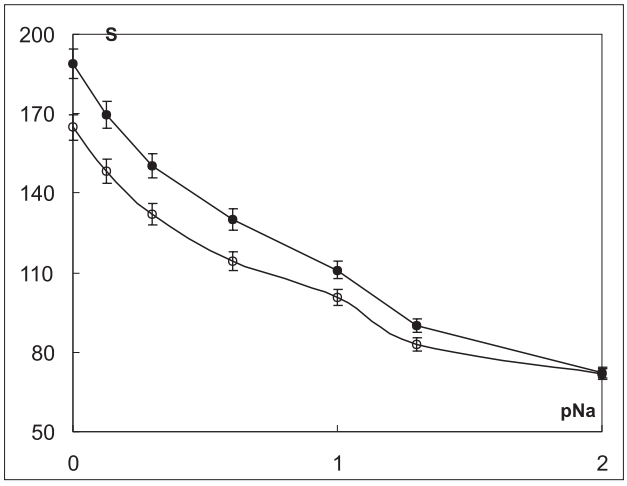

Fig. 3. Effect of salt in titration solution (pNa) on ion exchange capacity (*S*) of chickpea root cell walls. Walls were isolated from chickpea plants grown at different salt concentrations in the growth medium (NaCl, mM): 0.5 – open circles; 80 – closed circles. pNa = $-\log_{10}(C^{NaCl})$, C^{NaCl} – NaCl concentration in external solution, M. Points show experimental data. Solid lines are trend lines

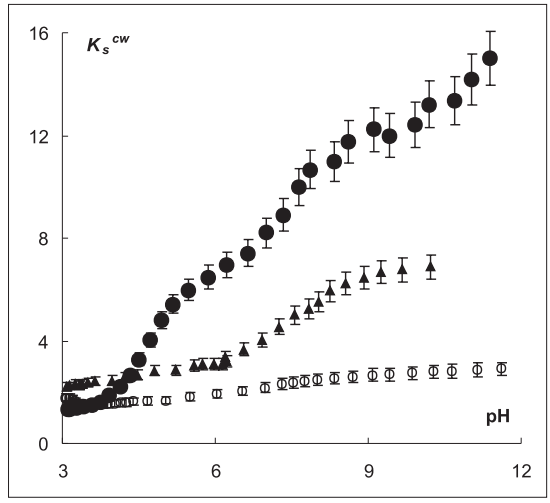

Fig. 4. Effects of pH and ionic strength of solution (*I*) on the swelling coefficient of the polymeric matrix of cell walls (K_s^{cw}, g H_2O per 1 g dry weight of cell walls) isolated from roots of chickpea plants grown at 0.5 mM NaCl. *I* = 10 (closed circles), 100 (closed triangle) and 250 mM (open circles). Bars – standard deviations

amount of anion exchange groups (S_t^{an}) varies from 710 to 880 μmol per 1 g dry weight of cell walls depending on salt concentration in the medium (0.5 or 80 mM NaCl). The total amount of cation exchange groups does not depend on salinity level in the medium (Table 1).

Based on the calculated values of pK_a^j (Table 2), data on the chemical composition of cell walls [12] and on the properties of cell walls of other plant roots [5–7], it is suggested that groups with pK_a^2 are carboxyl groups of polygalacturonic acid, groups with pK_a^3 are carboxyl groups of oxycinnamic acids, and groups with pK_a^4 are phenol groups. The environmental salt concentration has no effect on the qualitative composition of the ion exchange groups of the extracellular matrix of these plants: amino groups, two types of carboxyl groups, and phenol groups are present in all cases.

The content of polygalacturonic acid carboxyl groups in the cell walls of the glycophyte increased with salt concentration in the medium (Table 1), whereas the content of cinnamic acid carboxyl groups decreased. Such changes in the content of carboxyl groups seemed to be a response reaction of the plants under study to salinity. With increase in salt concentration, the content of phenol groups did not change in the cell walls.

Changes in the solution ionic strength from 10 to 250 mM were associated with changes in the apparent ionization constant of polygalacturonic acid carboxyl groups from 4.2 to 3.3, whereas the pK_a of two other cation exchange groups did not depend on the osmotic pressure of the external solution (Table 2). In previous works [13–15], the dissociation constant of polygalacturonic acid carboxyl groups has been calculated by Gelferich's equation [16]: $pK_a = pK_a' + \log(C^{Na+}) - \log(X / 2)$, where pK_a is the ionization constant of the ionogenic group in a weak acid ion exchanger approximately equal to the dissociation constant of a similar group in a soluble polymeric acid; pK_a' is the pH value corresponding to dissociation of 50% of ionogenic groups (in our case this value is pK_a^2, Table 2); C^{Na+} is the sodium concentration in the solution, M; *X* is the concentration of active groups in the ionite. Thus, the plot of the $pK_a' = f[\log(C^{Na+})]$ dependence has to intercept the ordinate axis at pK_a. Analysis of the dependence $pK_a^2 = f[\log(C^{Na+})]$ for polygalacturonic acid carboxyl groups of the glycophyte cell walls gives pK_a = 3.1. This value is close to values obtained by other researchers [13–15, 17]. According to the conclusion from Gelferich's equation, this means that in *Cicer arietinum,* groups with pK_a^2 really are carboxyl groups of polygalacturonic acid in a three-dimensional polymeric structure.

In the cell walls for polygalacturonic acid carboxyl groups pK_a decreased or their acidic properties strengthened with an increase in NaCl concentration in the nutrient solution. Hence, in response to salinity, the ion exchange capacity of chickpea cell walls had to increase. This statement was confirmed by the observed dependences of ion exchange capacity of the isolated glycophyte cell walls on NaCl concentration in the external solution (Fig. 3).

Our data are of great use in estimating the concentration of protons in apoplast, which (i. e. H^+ concentration) often rises in response to fluctuations of environmental conditions due to ion exchange reaction. The obtained results show that an increase of salt concentration in external solution, for example from 10 to 100 mM, results in an increase of proton concentration within extracellular water space up to 7 mM (data not shown). It is broadly accepted that any decrease in apoplast pH results in stimulation of various transport processes [21]. For example, it was shown that that the activity of plasma membrane K-selective inward-rectifying channels (KIRCs) in barley roots depends on apoplastic pH: potassium current increases upon acidification of the external solution [22]. Thus, apoplastic pH change the membrane potential, i. e. the electrical forces driving the transport processes through the membrane. Our data show that an increase in salinity brings about a rise of apoplastic proton concentration due to exchange reactions between sodium ions of external solution and protons of carboxyl groups of the cell walls. Such sudden changes in apoplast H^+ concentration are very likely to affect the ion transport into cells.

The plant cell wall is a natural weakly cross-linked ion exchanger [5–7]. Swelling is an important physicochemical feature of a polymer as an ion exchanger. The swelling of ion exchangers in aqueous solution is caused by the presence of hydrophilic groups, whereas the insolubility is caused by the presence of cross-links. The swelling degree of a synthetical ion exchanger depends on the ionite properties and the composition of the external solution. The capacity for swelling increases with a decrease in the cross-linking degree, an increase in the total amount of ionogenic groups and degree of their dissociation, and a decrease in the solution concentration. The capacity for swelling also depends on the radius of the hydrated ion used for filling the sorbent [16].

The present work shows that the swelling coefficient of chickpea cell walls strongly depends on the ionic strength of the external solution. Independently of salt concentration in the medium, the K_s^{cw} value increases with a decrease in the ionic strength of the solution and increase in pH (or α) (Fig. 4). In all cases, the cell wall swelling was minimal in the acidic region. This means that the cell walls shrink or diminish in volume with a decrease in pH in the apoplast or external medium. Thus, according to our data, the volume of the root cell walls is not constant but depends on the ionic conditions and the pH of the external solution and apoplast.

Thus, a decrease in the pH of the solution and / or an increase in its ionic strength led to a decreased swelling of the cell walls (Fig. 4). On the other hand, acidification of the medium decreased the hydraulic conductance of cell walls [19]. It is also shown that at a low ionic strength of the external solution (high rate of transpiration) the apoplast pathway of water movement is prevalent, because under these conditions the low hydraulic resistance of the root provides for a rapid water absorption by the plant roots [20]. Based on the literature data and on our findings, we conclude that in chickpea the swelling of the polymeric matrix of the root cell walls is directly correlated with the water flow, and the important physiological function of the root cell walls in this plant may be associated with the regulation of water movement through the root apoplast. This property of the cell wall polymeric matrix is especially important for roots, which are responsible for absorption of water and dissolved substances. Changes in the hydraulic conductance with an increase in the environmental salt concentration seem to be an essential factor in the adaptation of the glycophytes to salt stress.

Finally, a dramatic increase of NaCl content in the environment will decrease the pH of the extracellular water space because of exchange reactions between sodium ions entering from the external solution and protons of the cell wall carboxyl groups and decrease the swelling of the cell walls. According to our results, we conclude that ion exchange processes in cell walls are an important part of the mechanism of *C. arietinum* adaptation to salinity.

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