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Ion-Exchange Properties of Cell Walls of *Spinacia oleracea* L. Roots under Different Environmental Salt Conditions

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Abstract—Ion-exchange properties of the polymeric matrix of cell walls isolated from roots of 55-day-old *Spinacia oleracea* L. (Matador cv.) plants grown in nutrient solution in the presence of 0.5, 150, and 250 mM NaCl and from roots of *Suaeda altissima* L. Pall plants of the same age grown in the presence of 0.5 and 250 mM NaCl were studied. The ion-exchange capacity of the spinach cell walls was determined at pH values from 2 to 12 and different ionic strength of the solution (10 and 250 mM NaCl). In the structure of the root cell walls, four types of ionogenic groups were found: amine, two types of carboxyl (the first being galacturonic acid residue), and phenolic groups. The content of each type of group and their ionization constants were evaluated. The ion-exchange properties of spinach and the halophyte *Suaeda altissima* L. Pall were compared, and the qualitative composition of the ion-exchange groups in the cell walls of roots of these plants appeared to be the same and not depend on conditions of the root nutrition. The content of carboxyl groups of polygalacturonic acid changed in the cell walls of the glycophyte and halophyte depending on the salt concentration in the medium. These changes in the composition of functional groups of the cell wall polymers seemed to be a response of these plants to salt and were more pronounced in the halophyte. A sharp increase in the NaCl concentration in the medium caused a decrease in pH in the extracellular water space as a result of exchange reactions between sodium ions entering from the external solution and protons of carboxyl groups of the cell walls. The findings are discussed from the standpoint of involvement of root cell walls of different plant species in response to salinity.

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Key words: *Spinacia oleracea*, *Suaeda altissima*, cell wall polymeric matrix, ionogenic groups, ionization constant, salinity

Studies on the mechanisms of plant adaptation to unfavorable environmental factors including such abiotic stressors as high salt concentrations are now an intensively developed trend in plant physiology. High concentrations of salt in the environment are known to induce osmotic stress and ionic disbalance, which limit plant growth and productivity. Water deficiency produced under conditions of salt stress first of all affects the ability of cells to grow by extension. Growth under such conditions is associated with both regulation of water and osmotic homeostasis and changes in the cell wall properties [1].

The cell wall is now considered an elaborated multifunctional system, which can be a source of signals for triggering the defense reactions of the plant [2, 3]. The cell wall is a compartment that is the first to contact the 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 [4-7] are the best studied. But little is known about changes in the cell walls generated under the influence of abiotic stressors, in particular, salt stress.

Publications about specific features of the plant cell wall functioning as natural ion exchangers under salinity conditions are few in number [8, 9]. The ion-exchange properties of cell walls of *Suaeda altissima* (L.) Pall roots were shown to depend on the presence of four types of

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functional groups, and the ion-exchange capacity of the root walls of this halophyte was found to change due to increase in the amount of polygalacturonic acid groups in response to increase in salt concentration in the medium. To determine whether this response to salinity was specific for the halophyte *S. altissima*, in the present work the ion-exchange properties of the root cell walls of the glycophyte *Spinacia oleracea* L. of the same family were studied at varied levels of environmental salinity.

MATERIALS AND METHODS

The study was performed on 55-day-old plants of the glycophyte spinach *Spinacia oleracea* L., Matador cultivar (Chenopodiaceae). Seeds were sprouted in humid vermiculite for two weeks, and then the seedlings were planted onto a nutrient solution [10]. The plants were grown in a hothouse at the day temperature of 25°C and the night temperature of 18°C under conditions of natural illumination and additional illumination with DRL-1000 lamps, at the illuminance of 25-30 klx. When 4-week-old, the plants were subjected to salinity by addition of NaCl into the nutrient solution every 2-3 days providing the increase in the NaCl concentration in the pot to be not more than 50 mM. The NaCl concentrations in the nutrient medium were 0.5, 150, and 250 mM. The ion-exchange properties of the halophyte seablite *S. altissima* plants grown under similar conditions in the presence of 0.5 and 250 mM NaCl were determined earlier [8].

Cell walls were isolated from roots as described in [11, 12]. The 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 the 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.

The qualitative and quantitative composition of ion-exchange groups of the cell walls was determined by potentiometric titration using separate weights [12]. 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 the appropriate solutions of NaCl. The concentration of the alkali and acid was varied from 0 to 10 mM. After 48 h, the roots 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 bromothymol blue as the indicator. By changes in the H^+ or OH^- concentrations the sorption capacity of the cell wall was calculated at the 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, 150, and 250 mM NaCl), the potentiometric curves were obtained at the solution ionic strength of 10 and 250 mM.

Titration curves were calculated as described in [8, 12]. The amount 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 groups were determined by non-aqueous titration in acetic acid [13].

Ionization constants of ionogenic groups were calculated using the Henderson–Hasselbach equation as modified by Gregor [14]:

$$pH = pK_a + n\log_{10}[\alpha/(1-\alpha)], \quad (2)$$

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 $\log_{10}[\alpha_i/(1-\alpha_i)]$ for every pH_i value and using regression analysis, the pK_a^j and n^j values were obtained for each j-step of the 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 [15]:

$$S_i^{calc} = S_t^{cat} - \sum_{j,i=1}^{k,m} \Delta S^j [1 + 10^{(pK_a^j - pH_i)/n^j}]^{-1}, \quad (3)$$

where S_t^{cat} is the maximal cation-exchange capacity of the cell walls; ΔS^j is the amount of the j-type ionogenic groups; S_i^{calc} is the calculated ion-exchange capacity of the cell wall at the corresponding pH_i value; S_t^{cat}, ΔS^j, and S_i^{calc} are expressed in μmol/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. (2) 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 parameters of the equation:

$$S_i^{calc} = B \cdot S_i^{exp} + A, \quad (4)$$

where S_i^{exp} and S_i^{calc} are the ion-exchange capacity (in μmol/g dry weight of cell walls) experimental and calculated from Eq. (3) at the corresponding value of pH_i; A and B are regression parameters.

Determination of cell wall ion-exchange capacity at different concentrations of NaCl in solution. Dry weights of cell wall preparations (40 ± 0.1 mg) were placed in 50-ml glass flasks with glass stoppers and filled with 12.5 ml of solutions with different NaCl concentration. After 48 h, the samples were separated from the solution. In the solutions, pH was determined with the Jenway pH meter before and after contact with the samples. The sorption capacity of the cell walls was calculated from the change in the H^+ concentration in the solution by the formula:

$$S = |[10^{(-pH^{in})} - 10^{(-pH^{eq})}] \times 1000\ V|\ g^{-1}, \qquad (5)$$

where S is the cation-exchange capacity of the cell walls, μmol/g dry weight of cell walls; pH^{in} and pH^{eq} are the initial and corresponding equilibrium pH value of the solutions; V is the solution volume, ml; g is the sample weight, g.

Water content in plant tissues (Q), weight coefficients of cell wall swelling in water (K_w^{cw}) and solutions (K_s^{cw}), and relative dry weight (G) of cell walls (fraction of dry weight of cell wall from dry weight of tissue used for isolation of cell walls) were determined as described in [12]. Values of the parameters K_w^{cw}, K_s^{cw}, Q, and G were determined by the formulas [16]:

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

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

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

where G_F and G_D are fresh and dry weight of the samples, g; the index "cw" indicates the cell wall. The K_s^{cw} values were obtained at different values of pH and ionic strength of the solutions (10 and 250 mM).

The results were processed statistically using the SPSS program, version 13.

RESULTS

In the experimental titration curves of root cell walls (Fig. 1) of the spinach plants grown at different salt concentrations, the range of positive S_i values corresponds to releasing of protons from the cell walls according to the reaction:

$$\sim COOH + Na^+ \rightarrow \sim COONa + H^+,$$

where ~ denotes the polymeric chain. At pH > 10.8 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 the appropriate pH values of the environment.

In accordance with the differential curves obtained from the experimental ones, the amount of cation-exchange groups of each type (ΔS^j) was determined in the cell wall polymeric matrix (Tables 1 and 2; the method was described in [8, 12, 13]). 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 $S^{calc} = f(S^{exp})$ and also values of coefficients A and B in Eq. (4) (Table 3). In all variants $r^{corr} \rightarrow 1$, the value of A is not more than the experiment error, and $B \rightarrow 1$.

In the glycophyte, the ion-exchange capacity of the root cell walls sharply increased with the increase in the salt concentration in the nutrient solution. Depending on the salt concentration, this parameter changed from 30 to 170 μmol/g dry weight of cell walls in the interval of ionic strength changes from 5 to 1000 mM (Fig. 2).

The increase in the NaCl concentration in the medium was associated with a slightly reduced swelling of the cell wall polymeric matrix in water (K_w^{cw}), whereas the relative dry weight of the root cell walls (G) only weakly depended on the salt concentration of the nutrient solution (Table 4).

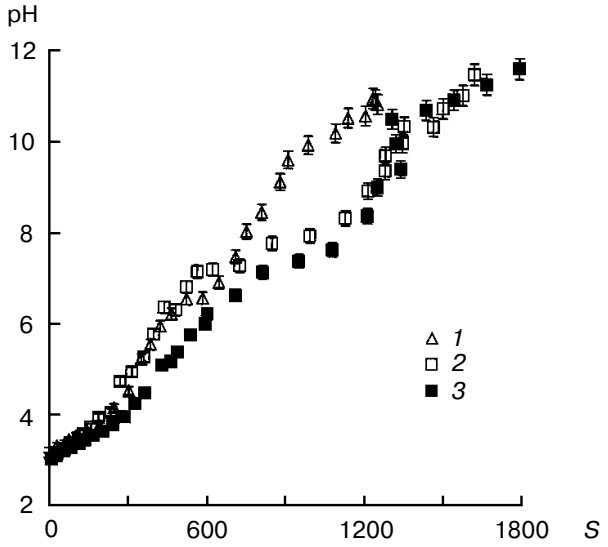

Fig. 1. Potentiometric titration curves of the polymeric matrix of root cell walls of spinach plants grown at 0.5 (*1*), 150 (*2*), and 250 mM NaCl (*3*) in the medium. The ionic strength of the solution on titration was 250 mM. S is the ion-exchange capacity of the polymeric matrix of cell walls, μmol/g dry weight of cell walls.

Table 1. Effect of NaCl concentration in the growth medium (C^{NaCl}, mM) on the contents of amino groups (ΔS^1), carboxyl groups of polygalacturonic acid (ΔS^2), carboxyl groups of oxycinnamic acid (ΔS^3), and phenolic groups (ΔS^4) in the polymeric matrix of the cell walls isolated from spinach roots

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	832 ± 66	300 ± 14	400 ± 14	540 ± 127	1240 ± 58
150	833 ± 32	345 ± 28	475 ± 99	445 ± 162	1265 ± 110
250	621 ± 90	420 ± 80	670 ± 80	670 ± 77	1760 ± 168

Note: 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, and ΔS^4 are expressed in μmol/g dry weight of the cell walls; ± is the standard deviation.

Table 2. Parameters of acid–base equilibrium for root cell walls of spinach plants grown at different salt concentrations in the nutrient solution (C^{NaCl})

C^{NaCl}	j	pK_a^j	n^j	r_{corr}^j	k	ΔS^j
0.5	2	3.80	0.87	0.981	7	310
	3	6.97	0.81	0.989	7	400
	4	10.10	1.50	0.980	11	630
150	2	3.90	1.2	0.962	7	325
	3	7.24	0.97	0.965	7	545
	4	9.23	1.27	0.872	12	560
250	2	3.76	0.75	0.974	11	420
	3	6.78	1.4	0.991	8	670
	4	10.30	1.98	0.865	8	670

Note: The ionic strength of solutions during potentiometric titration was 250 mM. *j*) The type of ionogenic group in the polymeric matrix of the cell walls: 2, 3) carboxyl groups of α-D-polygalacturonic acid and oxycinnamic acids, respectively; 4) phenolic groups; pK_a^j) the ionization constant of the *j*-type group; n^j) the constant of Eq. (2) for the *j*-type group; r_{corr}^j) the correlation coefficient; *k*) the number of points on the curve; ΔS^j) the amount of the *j*-type groups, μmol/g dry weight of cell walls.

Table 3. Adequacy of experimental and calculated curves of potentiometric titration of cell walls isolated from roots of spinach plants grown at different salt concentrations (C^{NaCl}, mM) of the nutrient solution

C^{NaCl}	I	A	B	r_{corr}^j	k
0.5	10	−18.07	1.057	0.993	29
150	10	−13.77	1.016	0.996	28
0.5	250	−6.818	0.993	0.997	29
150	250	16.75	0.954	0.982	31
250	250	−21.55	1.095	0.977	31

Note: *B*, *A*) parameters of Eq. (4); r_{corr}^j) the correlation coefficient of the dependence $S_i^{calc} = f(S_i^{exp})$; *k*) the number of points on the titration curve; *I*) ionic strength of solutions at potentiometric titration, mM.

The capacity of the spinach cell wall polymeric matrix for swelling in solutions increased with increase in pH and decreased with increase in the ionic strength of the solution (Fig. 3).

DISCUSSION

The polymeric matrix structure of cell walls of both glycophyte and halophyte roots [8] includes four types of

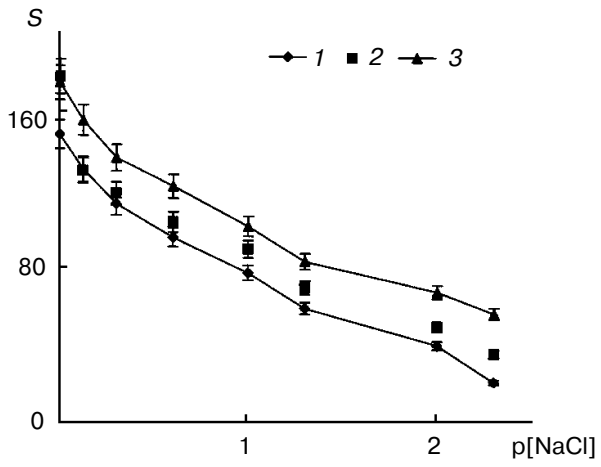

Fig. 2. Effect of NaCl concentration (p[NaCl]) on the ion-exchange capacity of root cell wall polymeric matrix of spinach plants (S, μmol/g dry weight of cell walls) grown at 0.5 (*1*), 150 (*2*), and 250 mM NaCl (*3*) in nutrient solutions. p[NaCl] = –log(C^{NaCl}), where C^{NaCl} is the NaCl concentration (M) in the experimental solutions.

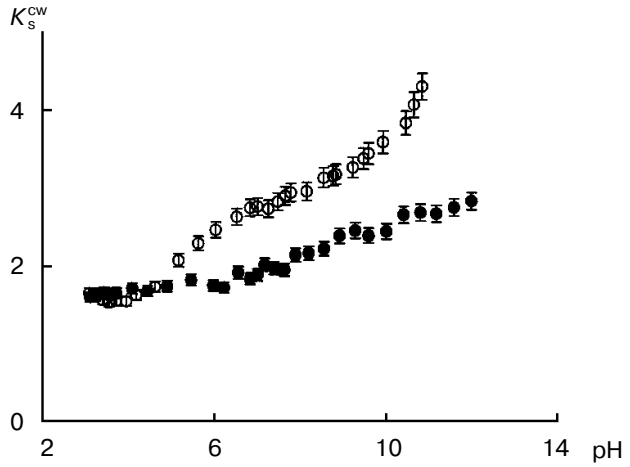

Fig. 3. Effects of pH and ionic strength of solution (I) on the swelling coefficient of polymeric matrix of cell walls (K_s^{cw}, g H_2O per g dry weight of cell walls) isolated from roots of spinach plants grown at 150 mM NaCl. I = 10 and 250 mM (open and closed circles, respectively).

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 amount of anion-exchange groups (S_t^{an}) varies from 620 to 840 and the total amount of cation-exchange groups (S_t^{cat}) varies from 1200 to 1800 μmol/g dry weight of cell walls (Table 1). In all cases, the value of the S_t^{cat} parameter was significantly higher than that of S_t^{an}. Thus, the cell walls of the glycophyte roots, similarly to those of the halophyte roots [8], are natural cation exchangers.

Root cell walls were earlier shown to be capable of anion exchange, and, depending on the plant species, the amount of amino groups with p$K_a \leq 3$ varied from 50 (lupine) to 180 (pea) μmol/g dry weight of cell walls [8, 11, 12, 17]. Note that in these studies anion-exchange groups were determined by titration with aqueous solutions of HCl. Later we established that this approach resulted in an incomplete determination of amino acid groups in the isolated cell walls. Therefore, in the present study we used non-aqueous titration (with solution of $HClO_4$ in glacial CH_3COOH), the approach successfully used for determination of the total amount of amino groups in low- and high-molecular-weight compounds, including analysis of amino acids generating zwitterions in aqueous solutions [13]. Depending on the environmental salt concentration, the amount of amino groups was 620-840 μmol/g dry weight of cell walls in the glycophyte (Table 1) and 520-650 μmol/g dry weight of cell walls in the halophyte (our unpublished data). With increase in the salt concentration in the nutrient solution, the amount of these groups in the walls of the plants under study decreased, on average, by 20%.

Elemental analysis performed with an automated CNH analyzer (Carlo-Erba, Italy) also revealed a decrease in the total nitrogen content in the isolated

Table 4. 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 spinach plants depending on the NaCl concentration in the nutrient solution (C^{NaCl}, mM)

C^{NaCl}	Spinach			Seablite*	
	Q	$K_w^{c.w}$	G	K_w^{cw}	G
0.5	9.8 ± 0.9	11.0 ± 0.8	24 ± 2	7.2 ± 0.5	56 ± 3
150	8.2 ± 1.0	10.7 ± 1.1	24 ± 2	—	—
250	8.3 ± 1.0	8.7 ± 0.4	28 ± 3	7.5 ± 0.6	47 ± 2

Note: K_w^{cw}, Q, and G values were determined by formulas (6)-(8). Q and K_w^{cw} are expressed in g H_2O per g dry weight of the root tissue and cell walls, respectively.

* Data from [8].

walls, from 4.6 to 3.4% in the glycophyte and from 2.3 to 2.0% in the halophyte. The standard recalculation of protein nitrogen (total nitrogen minus nitrogen of amino groups) onto the protein content has shown that, depending on the growth conditions, the structural proteins in the glycophyte cell walls are 20-15% and in the halophyte cell walls 8.8-8% of dry weight of the cell walls. It should be noted that the generally accepted method of protein calculation by nitrogen content can be inadequate in the case of isolated cell walls. Structural proteins of cell walls are known to be glycoproteins, with the carbohydrate component from 40 to 90%. Moreover, extensins contain large amounts of lysine and histidine residues [3], which have in the structure two and three nitrogen atoms, respectively.

The literature lacks information about direct quantitative determinations of total nitrogen and nitrogen-containing proteinaceous and non-proteinaceous compounds in the isolated cell wall. All information about the composition and quantities of structural proteins is obtained by chemical analysis of extracts or hydrolyzates prepared by treatment of cell walls with solutions of salts, alkalis, or acids at different temperatures, and these data mainly concern the primary cell wall [18-20]. At present, five groups of structural proteins are distinguished: extensins, arabinogalactans, proline- and glycine-enriched proteins, and lectins of Solanaceae. Extensins represent the majority of the cell wall structural proteins; they comprise 90% of all proteins of the cell wall [3]. These proteins are also known to be glycoproteins containing 50-65% carbohydrate bound to hydroxyl groups of oxyamino acid residues (e.g., oxyproline or tyrosine). The oxyproline content in cell wall hydrolyzates can reach 40 mole %.

The total amount of protein in cell walls strongly varies (from 0.1 to 20% of dry weight [3]), and its content is significantly higher in the walls of dicotyledons than in monocotyledons [18, 19]. Moreover, in addition to ~10% of protein, hydrolyzates of cell walls isolated from *Acer pseudoplatanus* were found to contain about 2% of hydroxyproline [20]. Thus, our data on the contents of amino groups represented, in particular, by oxyproline bound by the hydroxyl group to the glycoprotein carbohydrate moiety and on the protein amount in the cell walls of the halophyte and glycophyte of the Chenopodiaceae family are in agreement with data of other authors.

Based on the calculated values of pK_a^j (Table 2), data on the chemical composition of cell walls [21], and data on properties of cell walls of other plant roots [8, 11, 12], it is suggested that the groups with pK_a^2 are carboxyl groups of α-D-polygalacturonic acid, the groups with pK_a^3 are carboxyl groups of oxycinnamic acids, and the groups with pK_a^4 are phenolic groups.

It is reasonable to especially focus attention on cation-exchange groups with ionization constants of ~7 (Table 2). These groups were earlier shown to be carboxyl [8, 12], but no hypotheses were proposed concerning their nature. Based on the recent data on the cell wall structure [21], these carboxyl groups were concluded to be residues of oxycinnamic acids. This conclusion is based on the literature data on ionization constants of carboxyl groups in low- and high-molecular-weight compounds. The ionization constant of the carboxyl group of cinnamic acid, which, with some assumptions, can be considered a low-molecular-weight analog of oxycinnamic acids, is 4.44 [22]. However, pK_a values of low-molecular-weight and polymeric acids are significantly different. This can be illustrated by the following changes in pK_a value on turning from low-molecular-weight compounds to three-dimensional polymeric structures: for acrylic acid pK_a is 4.26 [22], for polyacrylic acid it is 4.8 [23], and for its three-dimensional analog pK_a is 5.0-7.5 [23], depending on the type and amount of the linking agent. Based on the above-presented data, it was suggested that in the three-dimensional structure of cell walls cation-exchange groups with $pK_a \sim 7$ should be carboxyl groups of oxycinnamic acids.

The polymeric matrix of the glycophyte root cell walls is composed of the same ionogenic groups as that of the halophyte root walls [8], and this is shown by values of dissociation constants of the corresponding groups (Table 5). 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 amount of polygalacturonic acid carboxyl groups in the cell walls of both glycophyte and halophyte changed in dependence on the salt concentration in the medium, and the change was more pronounced in the halophyte (Fig. 4). Such changes in the amount of polygalacturonic acid groups seemed to be a response reaction of the plants under study to salinity. With increase in the salt concentration, the contents of cation exchange groups of two other types also increased 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 4.8 to 3.7, whereas the pK_a of two other cation exchange groups slightly depended on osmotic pressure of the external solution (Table 5). In previous works [24-26] the dissociation constant of polygalacturonic acid carboxyl groups has been calculated by Gelferich's equation [16]:

$$pK_a = pK_a' + \log_{10}(C^{Na^+}) - \log_{10}(X/2),$$

where pK_a is the ionization constant of 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 5); C^{Na^+} is the sodium concentration in the

Table 5. Effect of ionic strength of solution (I, mM) on the mean value of dissociation constant (pK_a^j) of cation-exchange groups of the cell wall polymeric matrix of spinach and seablite roots

Plant	I	pK_a^j		
		$j = 2$	3	4
Spinach	10	4.79 ± 0.02	7.33 ± 0.04	10.00 ± 0.28
Seablite*	10	4.79 ± 0.13	7.49 ± 0.07	10.13 ± 0.12
Spinach	250	3.77 ± 0.15	6.88 ± 0.43	9.63 ± 0.45
Seablite*	250	3.73 ± 0.15	7.32 ± 0.10	9.31 ± 0.12

Note: j) Type of ionogenic group: 2) carboxyl groups of polygalacturonic acid; 3) carboxyl groups of oxycinnamic acids; 4) phenolic groups.
* Data from [8].

solution, M; X is the concentration of active groups in the ionite, M. Thus, the plot of the dependence

$$pK_a' = f[\log_{10}(C^{Na^+})]$$

has to intercept the ordinate axis at pK_a. Analysis of the dependence

$$pK_a^2 = f[\log_{10}(C^{Na^+})]$$

for polygalacturonic acid carboxyl groups of the glycophyte cell walls gives $pK_a = 3.3$. This value is close to values obtained by other researchers [24-26]. According to the conclusion from Gelferich's equation, this means that in both *S. oleracea* and *S. altissima* [8] the groups with pK_a^2 really are carboxyl groups of polygalacturonic acid in a three-dimensional polymeric structure, because the pK_a value of soluble polygalacturonic acid is 3.42 [17].

In the glycophyte, similarly to the halophyte [8], the ionization constant of polygalacturonic acid carboxyl groups decreased or their acidic properties strengthened with the increase in the NaCl content in the nutrient solution. Thus, in response to salinity, the ion-exchange capacity of the glycophyte cell walls had to increase. This statement was confirmed by the observed dependences of ion-exchange capacity of the isolated glycophyte cell walls on the NaCl concentration in the external solution (Fig. 3).

Under all growth conditions, the ion-exchange capacity of the cell walls sharply increased with the increase in C^{NaCl} in the glycophyte (the present work data) and in the halophyte [8]. Depending on the growth conditions, for the seablite roots this parameter varied from 50 to 150-250 and for the spinach roots from 30 to 200 μmol/g dry weight of cell walls in the interval of ionic strength changes from 5 to 1000 mM. Note that under all conditions the seablite cell walls had higher capacity of ion exchange than those of spinach.

The ionization degree (α) of weak acids and bases with ionogenic groups in the structure of the cell wall polymeric matrix depends only on two factors: values of pH and pK_a. Based on the data of the present work, the dissociation degree of each group can be calculated in dependence on pH and ionic strength of the solution. Because the qualitative composition of ionogenic groups is the same in the glycophyte and halophyte cell walls (Table 5), the presented curves characterize the state of ionogenic groups of the polymeric matrix of both *S. oleracea* and *S. altissima* at different ionic strength of the external solution (Fig. 5). Thus, at pH 5 about 60% of polygalacturonic acid carboxyl groups are ionized in the presence of 10 mM NaCl, whereas at 250 mM NaCl the dissociation degree of these groups is 80%. Under such conditions, all carboxyl groups of oxycinnamic acids are unavailable for exchange reactions with the environment ions. At pH 7, carboxyl groups of polygalacturonic acid

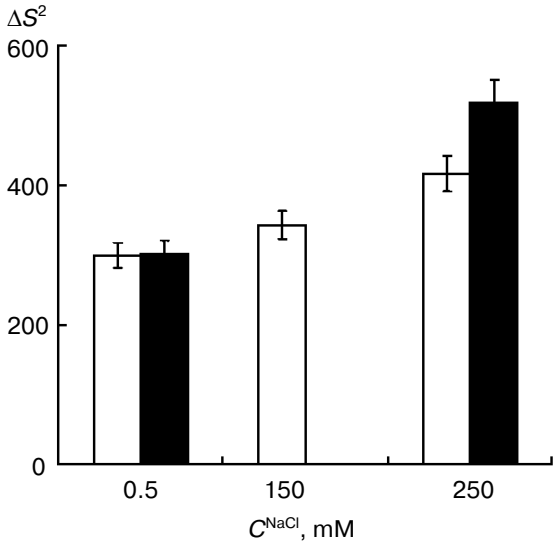

Fig. 4. Effect of salt concentration on the amount of polygalacturonic acid carboxyl groups (ΔS^2, μmol/g dry weight of cell walls) in the polymeric matrix of the cell walls of spinach and seablite roots (light and dark rectangles, respectively).

are completely dissociated, whereas α of carboxyl groups of oxycinnamic acids is 30%. It should be noted that under physiologic conditions (pH 5.0-8.0), phenolic and amino groups are always closed, i.e., non-ionized and, consequently, not involved in ion-exchange reactions.

The plant cell wall is a natural weakly cross-linked ion exchanger [8, 12]. Swelling is an important physicochemical feature of a polymer as an ion exchanger. This process is quantitatively characterized by the swelling coefficient (K_s^{sw}). 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 an ion exchanger depends on the ionite properties and 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 hydrated ion used for filling the sorbent [16].

Comparison of the present work findings and results of work [8] shows that the swelling coefficient of cell walls depends on the ionic strength of the external solution. The K_s^{cw} value increases with a decrease in the ionic strength of the solution and increase in pH (or α) (Fig. 3). In all cases, the cell wall swelling was minimal in the acidic region. This means that the cell walls compress or diminish in volume with an increase in pH in the apoplast or external medium. Thus, the volume of root cell walls of both the glycophyte and halophyte is not constant but depends on the ionic conditions and pH of the external solution and apoplast.

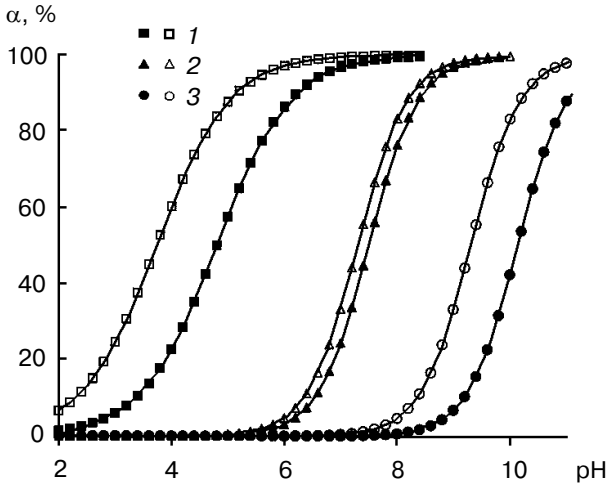

Fig. 5. The pH dependence of the dissociation degree (α) of cation exchange groups of spinach root cell walls in the presence of 10 (closed symbols) and 250 mM NaCl (open symbols). *1*, *2*) Carboxyl groups of α-D-polygalacturonic acid and oxycinnamic acids, respectively; *3*) phenolic groups.

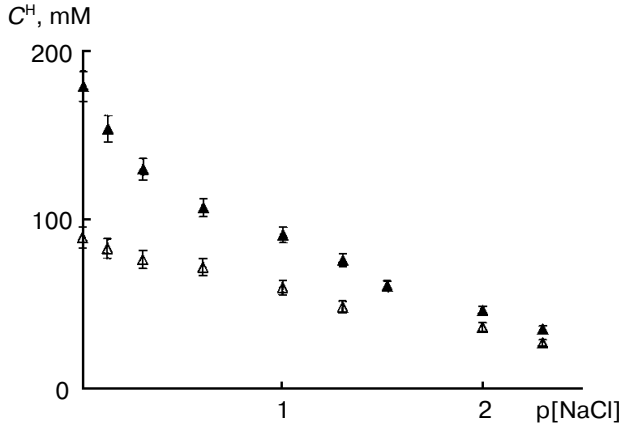

Fig. 6. Effect of salt concentration in solution (p[NaCl]) on the proton concentration in the water space of polymeric matrix of the cell walls isolated from spinach (open triangles) and seablite (closed triangles, data from [8]) grown at 250 mM NaCl in the medium. C^H is proton concentration in aqueous space of polymeric matrix of cell walls, mM; p[NaCl] = $-\log_{10}(C^{NaCl})$, where C^{NaCl} is NaCl concentration in the solution, M.

Considering physicochemical regularities of swelling of ion exchangers [16], it was suggested that the ability for swelling should be mainly determined by the cross-linking degree of polymeric chains in the structure of cell walls. There are no direct approaches for determination of this parameter, but it can be assessed indirectly. It is known that the higher is the cross-linking degree of polymeric chains, the lower is the swelling coefficient of the polymeric material in water [23, 27]. In correlation with the data on swelling of the halophyte cell walls [8] and results of the present work, it was supposed that the cross-linking degree of the polymeric chains in the root cell walls in the glycophyte *S. oleracea* should be lower than in the halophyte *S. altissima* (Table 4). Moreover, the cell wall fraction in the dry weight of the tissues (*G*) is lower in the glycophyte (Table 4). Thus, differences in the polymeric matrix structure of the halophyte and glycophyte cell walls are caused by the different cross-linking degree of the polymeric chains. Seablite has the more rigid structure of the polymeric matrix, which provides for the higher mechanical and chemical resistance of the cell walls under conditions of salt stress.

Decrease in pH of the solution and/or increase in its ionic strength led to reduced swelling of the cell walls (Fig. 3). On the other hand, acidification of the medium decreased the hydraulic conductance of cell walls [28]. It is also shown that at the 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 rapid water absorption by the plant roots [29]. Based on the literature data and our findings, we concluded that in both seablite and spinach the swelling of the polymeric

matrix of the root cell walls directly correlated with the water flow, and the important physiological function of the root cell walls in these plants could be associated with 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 the increase in the environmental salt concentration seem to be an essential factor in adaptation of both halophyte and glycophyte to salt stress.

Based on data of the present work and work [8], we can evaluate the proton concentration in the extracellular space, which is a result of exchange reactions between the environmental cations and protons of ionized carboxyl groups of cell walls in response to changes in the salt concentration of the environment (Fig. 6). Calculations show that an increase in the salt concentration in the external solution from 10 to 50 mM will increase the proton concentration in the extracellular space of the glycophyte and halophyte to 20 and 30 mM, respectively. Thus, a dramatic increase in the NaCl content in the environment will decrease 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. It should be emphasized that these reactions are caused only by properties of the extracellular space and do not depend on the condition within cells.

Thus, in response to an increase in salt concentration in the environment, the amount of active sites of cation binding increased in the root cell walls of both the halophyte and glycophyte of the Chenopodiaceae family. However, under conditions of salt stress, the cell walls of the halophyte protect the cell more efficiently due to the higher cation-exchange capacity of its cell walls, higher cross-linking degree of the polymeric matrix, and thicker cell walls.

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