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PLANT PHYSIOLOGY

Effect of Nitrogen Deficiency on the Ion-Exchange Properties of Cell Wall Polymers from Wheat Roots

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Abstract—The ion-exchange properties of cell wall polymers isolated from the roots of wheat (*Triticum aestivum* L.) plants grown on either nitrate-free (N-deficient) or nitrate-containing (+N) hydroponic nutrient medium have been investigated. Irrespective of the nitrogen nutrition regimen, the studied cell walls contained four types of ion-exchange groups: primary amino groups of structural proteins ($pK_a < 3$), carboxyl groups of polygalacturonic acid in pectin (pK_a ~4.7), carboxyl groups of hydroxycinnamic acids (pK_a ~7.3), and phenolic OH-groups of lignin (pK_a ~10.2). The quantitative ratio between these types of ion-exchange groups, the mass fraction of cell walls in the dry weight of roots, and the swelling coefficient of cell walls depended on the nitrate presence in the growing medium. Compared to the +N variant, the N-deficient variant was characterized by a 2.4 times higher content of phenolic OH-groups in cell walls and 1.24 times higher mass fraction of cell walls; at the same time, the swelling coefficient for this variant was lower by 10%. The obtained data indicate that nitrogen deficiency results in a formation of thicker root cell walls with a higher degree of polymer cross-linking that may be caused by the increased lignin content.

Keywords: cell wall, nitrogen, wheat, carboxyl groups, mineral nutrition, ion exchange.

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INTRODUCTION

The level of nitrogen supply represents one of the key factors determining plant growth and productivity. Soil nitrogen is available to plants as either nitrate (NO_3^-) or ammonium (NH_4^+) ions. The form of available nitrogen influences on such integral physiological parameters of plants as their photosynthetic productivity [1], adsorption and transportation of other macro- and microelements [2], and the growth of the main and lateral roots [3].

At the same time, fine mechanisms of plant adaptation to the varying content and form of soil nitrogen, especially those connected with the cell wall (CW) functioning, still remain poorly studied. Ion-exchange properties of CW polymers from root cells play an important role at the initial stages of mineral uptake from soil [4]. Four types of ion-exchange groups were found in plant CWs [5]: (1) carboxyl groups of polygalacturonic acid (PGA; pK_a 4–5) in pectin, (2) carboxyl groups of hydroxycinnamic acids (HCA; pK_a 6–7), (3) phenolic OH-groups (pK_a ~10) in lignin, and (4) primary amino groups ($pK_a < 3$) in structural CW proteins. At physiological pH values (4–8), only carboxyl groups are involved in ion-exchange reactions, since the ionization constants of the two other groups are beyond these pH limits. The total amount of ion-exchange groups in plant CWs varies within wide limits and makes 700–1500 μmol/g CW DW [6]. The highest content of PGA carboxyl groups was revealed in the root CWs of legumes (500–700 μmol/g CW DW), whereas root CWs of cereals and goosefoot plants contain the highest amount of HCA carboxyl groups (400–500 μmol/g CW DW) [6]. These values correlate with the content of the corresponding CW polymers. In both dicotyledons and monocotyledons (excepting cereals), pectins compose 20–50% of CWs; in cereals, their content in CWs does not exceed 10% [7]. HCA (mainly ferulic acid) are involved in ion-exchange reactions together with PGA [6] and play an important role in the formation of cross-links between polymeric chains in the CWs of cereals and goosefoot plants [7].

The chemical composition of plant CWs depends on the growth conditions, particularly on the character of mineral nutrition [7–10]. However, the number of studies devoted to the influence of nitrogen nutrition on the characteristics of plant root CWs is very low, and such studies are devoted to the transcriptome analysis of genes responsible for the CW synthesis and modification rather than to the detailed description of its chemical composition [9]. In most cases, changes in the CW composition in response to abiotic stresses, including nitrogen deficiency, occur via three main mechanisms [10]: (1) increase in the content of enzymes, which loosen the primary CW (xyloglucan

endotransglycolases and expansins); (2) increase in the branching degree of rhamnogalacturonan I resulting in the increased CW plasticity; (3) thickening of the secondary CW caused by the deposition of hemicelluloses and lignin. In the case of the use of NH_4^+ as a sole nitrogen source, a decrease in the content of pectins and hemicelluloses accompanied by the reduction of the cation-exchange capacity of polymers was observed in CWs of rice roots [8].

Thus, the analysis of published data shows that the information about the influence of various nitrogen nutrition types on the composition and properties of the CW polymer matrix is very limited. The purpose of this study was to compare ion-exchange properties of CW polymers from roots of wheat plants grown hydroponically on nitrate-containing and nitrate-free medium.

MATERIALS AND METHODS

The objects of our study were 19-day-old wheat plants (*Triticum aestivum* L., cv. Inna). Plants were grown hydroponically under constant aeration in modified Pryanishnikov medium of the following composition: (1) nitrate-containing (+N) variant: 3 mM $Ca(NO_3)_2$, 1 mM $CaHPO_4$, 0.5 mM $MgSO_4$, 2 mM KCl, 0.15 mM $FeCl_3$; (2) N-deficient variant: 2 mM $CaSO_4$, 1 mM $CaHPO_4$, 0.5 mM $MgSO_4$, 2 mM KCl, 0.15 mM $FeCl_3$. Nutrient solutions were replaced every 4 days. Plants were grown under controlled environmental conditions at 24–26°C and 14-h photoperiod with the light intensity 110 μmol/m^2 s.

The root CWs were isolated as previously described [5]. Root samples (~5 g each) were rinsed with distilled water and then sequentially washed with 1% NaOH (~0.5 L, 24 h, at a constant stirring), distilled water (~2 L, 2–3 h), and 1% HCl (~0.5 L, 24 h, at a constant stirring). Finally, the samples were washed with distilled water until a complete removal of chloride ions from the washing water that was determined by the titration with $Hg(NO_3)_2$. Then the CW preparations were dried at 55–60°C until constant weight. This standardization method, i.e., the conversion of all cation-exchange groups into the H^+-form and all anion-exchange groups (amino groups) into the free amine form ($-NH_2$) makes it possible to compare sorption properties of samples with different content of functional groups [11]. Earlier we showed that the use of this technique allowed us to obtain CW samples that were free of DNA and cytoplasmic proteins and contained the amount of PGA and structural proteins of the same order as in CW samples isolated by other methods [12].

A potentiometric titration was carried out by a separate sample method [6]. Samples of dried and ground CWs (0.0400 ± 0.0001 g) were placed into glass flasks with glass stoppers and mixed with 12.5 mL of KOH or HCl solutions of different concentrations, which had a constant ionic strength (100 mM) provided by the addition of the corresponding NaCl solutions. The concentration range of alkaline and acid solutions was 0–10 mM. After a 48-h incubation, CWs were separated from the solution; pH of the solution was measured with a pH-meter and the final concentrations of KOH or HCl were determined by titration with bromthymol blue. The sorption capacity of CWs at the corresponding pH value (pH_i) was calculated using the following formula:

$$S_i^{cat,an} = \frac{|(C_o - C_i)| \times V}{g}, \quad (1)$$

where $S_i^{cat,an}$ is the cation- or anion-exchange capacity of CWs at the corresponding pH_i (2.8–12), μmol/g CW DW; C_o and C_i are initial and final KOH (HCl) concentrations in the solution, mM; V is the volume of the solution, mL; g is the dry weight of a sample, g.

The number of types of ion-exchange groups (j) and their content (ΔS^j) in CWs were determined using differential curves $(dS_i/dpH_i) = f(pH_i)$ as described earlier [6]. For each group, the degree of ionization (α) was calculated using the following formula:

$$\alpha = S^j/\Delta S^j, \quad (2)$$

where S^j is the content of dissociated groups of the j-type at the corresponding pH value.

The ionization constant for each ion-exchange group was calculated using the modified Henderson–Hasselbalch equation [13]:

$$pH = pK_a + n\log(\alpha/(1-\alpha)), \quad (3)$$

where pK_a is an apparent ionization constant of the ion-exchange group of a polymer; α is its degree of ionization; n is a constant determined by the structure of the polymer matrix and the nature of a counterion [14].

Using the determined parameter values (ΔS^j, pK_a^j, n^j), the S_i^{cal} values were calculated with the following summary equation [6]:

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

where S_i^{cal} is the calculated value of the CW ion-exchange capacity at the corresponding pH_i value; S_o^{cat} is the maximum CW cation-exchange capacity; ΔS^j is the content of the j-type ion-exchange groups; pK_a^j is the apparent ionization constant of the j-type ion-exchange groups; n^j is the constant of the equation (3) for the j-type ion-exchange groups; k is the number of points on the potentiometric curve; m is the number of types of ion-exchange groups. S_o^{cat}, ΔS^j, and S_i^{cal} are expressed in μmol/g CW DW.

The adequacy of the applied approach to the description of the acid-base equilibrium was estimated by the regression analysis method via the determination of parameters of the following equation:

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

where S_i^{cal} and S_i^{exp} are the calculated (using the equation (4)) and experimental (equation (1)) ion-exchange capacity of CWs at the corresponding pH value (μmol/g CW DW), respectively, whereas A and B are regression parameters.

The CW swelling coefficient (K_{CW}, g H_2O/g CW DW) was determined in the solutions with 100 mM ionic strength and pH varying between 2.8 and 12, and calculated according to [6] using the following formula:

$$K_{CW} = (G_F - G_D)/G_D, \quad (6)$$

where G_F and G_D are fresh and dry weight of CW samples (g), respectively.

The percentage of a CW dry weight (G_{CW}) in the dry weight of roots was determined using the following formula:

$$G_{CW} = (G_{CW}/G_r) \cdot 100, \quad (7)$$

where G_r and G_{CW} are the dry weights (g) of roots and isolated cell walls, respectively.

All experiments and analytical measurements were arranged in 3 or 6–10 replications, respectively. The statistical treatment of obtained data was carried out using Microsoft Excel and IBM SPSS Statistics software. Significance was determined by independent two-sample t-test, with $p < 0.05$ deemed significant. The normality of data distribution was assessed by the Kolmogorov–Smirnov test.

RESULTS AND DISCUSSION

The experimental curves $S_i = f(pH_i)$, which were obtained by a potentiometric titration of CWs isolated from wheat roots, had several inflection points that indicated the presence of several types of ion-exchange groups in a CW polymer matrix. At pH > 10.8 and pH < 3, the sorption capacity of CWs for NaOH (S_T^{cat}) and HCl (S_T^{an}), respectively, reached the maximum level and characterized the total content of both acid and basic groups in the CW structure, which were potentially able to participate in the ion-exchange reactions in the apoplast at the corresponding pH values. In both +N and N-deficient treatments, S_T^{cat} values significantly exceeded those of S_T^{an} (Fig. 1), i.e., irrespectively of nitrogen nutrition type, CWs of wheat roots were characterized mainly by cation-exchange properties. Root CWs in both treatments contained a small and approximately the same number of anion-exchange groups (Fig. 1, S_T^{an}), but they significantly differed in the content of cation-exchange groups (Fig. 1, S_T^{cat}).

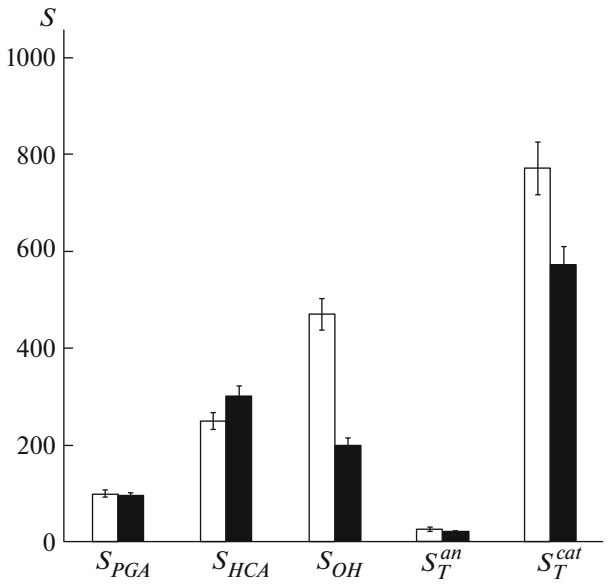

Fig. 1. Content of the carboxyl groups of polygalacturonic acid (S_{PGA}), carboxyl groups of hydroxycinnamic acids (S_{HCA}), phenolic OH-groups (S_{OH}), and the total content of anion-exchange (S_T^{an}) and cation-exchange (S_T^{cat}) groups in cell walls isolated from the roots of wheat plants grown on nitrogen-deficient (white rectangles) and nitrogen-containing (black rectangles) media (μmol/g CW DW).

The experimental curves were divided into monotonous nonlinear segments according to differential curves $dS/dpH = f(pH)$, which had a series of minimums corresponding to the beginning ($\alpha = 0$) and the end ($\alpha = 1$) of ionization of a j-type functional group. The evaluation of the approach that was used to describe acid-base equilibrium by the regression analysis showed that the calculated and experimental values of the CW ion-exchange capacity coincided within the measurement inaccuracy limits. This was confirmed by the values of regression parameters (A, B) and correlation coefficient (r) for the $S_i^{cal} = f(S_i^{exp})$ function (equation (5)): in the case of the +N treatment, $B = 0.95$, $A = 12.5$, $r = 0.984$, whereas $B = 0.97$, $A = 15.9$, $r = 0.982$ for the N-deficient variant. We found that CWs in both treatments contained three types of cation-exchange groups and one type of anion-exchange group. The ionization constants of anion-exchange groups (amino groups of CW structural proteins [5]), were not calculated because the content of these groups in CWs (20–25 μmol/g CW DW). In both treatments, CWs of wheat roots had the same qualitative composition of cation-exchange groups, which was confirmed by the similar pK_a values of corresponding groups (see table). According to the

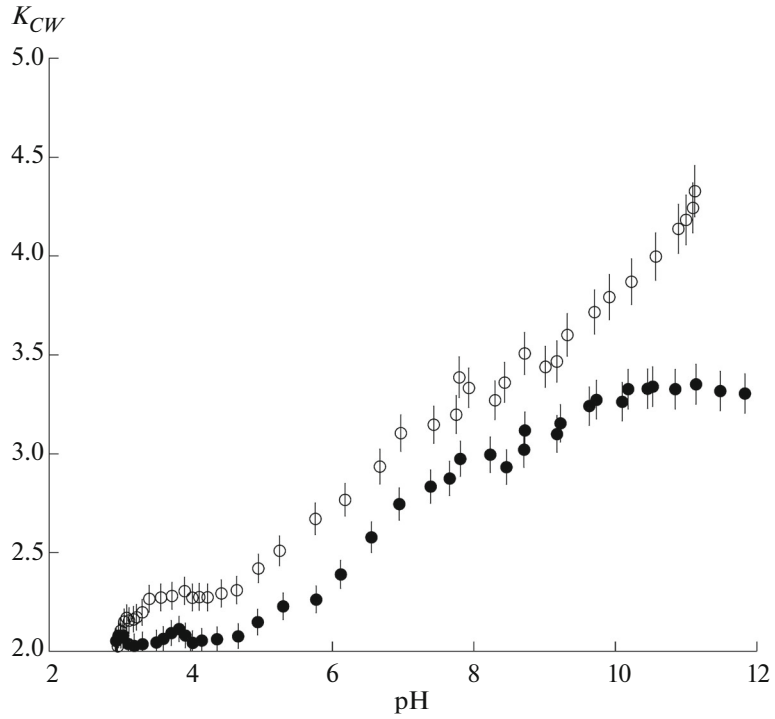

Fig. 2. The relationship between the swelling coefficient of cell walls isolated from wheat roots (K_{CW}, g H_2O/CW DW), the pH level and nitrogen nutrition regimen. Black and white dots indicate nitrogen-containing and nitrogen-deficient growth media, respectively.

previously obtained results [6], cation-exchange groups with pK_a ~4.7 represented PGA carboxyl groups in pectins, groups with pK_a ~7.3 represented HCA carboxyl groups, and groups with pK_a ~10.2 represented phenolic OH-groups of lignin.

Root CWs of nitrate-supplied and N-deficient plants did not differ significantly in the content of PGA and HCA carboxyl groups (Fig. 1, S_{PGA} and S_{HCA}). They differed only in the content of phenolic OH-groups (Fig. 1, S_{OH}): in the case of a nitrogen deficiency, their number was 2.4 times higher. One can suppose that the content of pectins and HCA in CWs was equal in both treatments, whereas the content of lignin sharply increased in the case of a nitrogen deficiency. An increased content of phenolic compounds in root tissues was also shown for N-deficient chamomile (*Matricaria chamomilla*) plants [3]. An intensive lignification of the secondary CW was observed under the influence of other adverse external factors, such as drought, low temperature, and salinity [10].

Parameters of equation (3) for the cell walls isolated from roots of wheat plants grown under various nitrogen nutrition regimens. *j,* group type (*1*, carboxyl groups of polygalacturonic acid; *2*, carboxyl groups of hydroxycinnamic acids; *3,* phenolic OH-groups); pK_a^j, ionization constant of the *j*-type group; n^j, constant of equation (3) for the *j*-type group; r^j, correlation coefficient for the *j*-type group; *k*, number of points on the potentiometric curve

Growth medium	*j*	pK_a^j	n^j	r^j	*k*
+N	*1*	4.72 ± 0.06	0.75 ± 0.08	0.965	8
	2	7.31 ± 0.04	1.47 ± 0.09	0.993	6
	3	10.06 ± 0.16	2.04 ± 0.41	0.926	6
N-deficient	*1*	4.65 ± 0.05	1.08 ± 0.10	0.980	7
	2	7.35 ± 0.04	1.15 ± 0.06	0.992	8
	3	10.36 ± 0.06	1.48 ± 0.14	0.978	7

The CW percentage in the dry weight of roots strongly depended on the nitrate presence in growth medium. In the case of N-deficient medium, the value of this parameter was 57 ± 2.1%, whereas it was equal to 46 ± 3.5% in the +N medium. It is possible that the increase in the CW weight occurs due to lignin accumulation. In addition, nitrogen deficiency results in the decreased consumption of carbohydrates in the processes of nitrate reduction and ammonium assimilation in roots [15]. This may result in intensified synthesis of cellulose and cross-linking glycans, which do not have ion-exchange groups in their structure.

The swelling coefficient of a synthetic ion-exchange material is determined by the cross-linking degree of its polymeric chains, the total number of ion-exchange groups and the level of their dissociation, and the concentration of external solution; it also depends on the radius of hydrated ions that fill the sor-

bent. Among the above-listed factors, the cross-linking degree of polymeric chains is the main parameter that determines the swelling level [14]. One may suppose that the experimental evaluation of the CW swelling would make it possible to assess the rigidity of the CW three-dimensional structure and the ability of the CW to change its volume under the influence of various external factors. The measurement of the CW swelling coefficient (K_{CW}) showed that, in the whole pH range tested, the value of this parameter for the N-deficient variant was 10–30% lower than in the +N variant. The difference was pH-dependent; moreover, the K_{CW} value increased with increasing pH (Fig. 2). This result indicates that the nitrogen deficiency causes an increase in the cross-linking degree of CW polymers, probably due to an intensive CW lignification.

Thus, the stress caused by the nitrogen deficiency in a growth medium may result in the activation of the lignin synthesis in the secondary CWs of root cells that, in turn, increases the CW mass fraction and reduces the CW swelling capacity.

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