



Research Paper

The role of cell walls and pectins in cation exchange and surface area of plant roots



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ABSTRACT

We aimed to assess role of cell walls in formation of cation exchange capacity, surface charge, surface acidity, specific surface, water adsorption energy and surface charge density of plant roots, and to find the input of the cell wall pectins to the above properties. Whole roots, isolated cell walls and the residue after the extraction of pectins from the cell walls of two *Apiaceae* L. species (celeriac and parsnip) were studied using potentiometric titration curves and water vapor adsorption – desorption isotherms.

Total amount of surface charge, as well as the cation exchange capacity were markedly higher in roots than in their cell walls, suggesting large contribution of other cell organelles to the binding of cations by the whole root cells. Significantly lower charge of the residues after removal of pectins was noted indicating that pectins play the most important role in surface charge formation of cell walls. The specific surface was similar for all of the studied materials. For the separated cell walls it was around 10% smaller than of the whole roots, and it increased slightly after the removal of pectins. The surface charge density and water vapor adsorption energy were the highest for the whole roots and the lowest for the cell walls residues after removal of pectins.

The results indicate that the cell walls and plasma membranes are jointly involved in root ion exchange and surface characteristics and their contribution depends upon the plant species.

1. Introduction

Nutrients and water uptake by plants occur mainly at the interphase between the plant roots and the soil, therefore the properties of the root surface being in contact with soil solid and liquid phases govern the above processes. Among root surface properties the electric charge is most commonly studied to describe the root cation exchange capacity, its changes with soil pH, balance of plant cations of different valence, toxicity of heavy metals and aluminum, extra cellular transport of minerals (Grignon and Sentenac 1991; Marschner 1995) and many others. Electric charge of the root compartments is dominated by negatively charged groups, so positively charged cations accumulate near the roots surface (Clarkson 1993). The negative charge of the roots is considered to be carried mainly by the carboxyl groups of pectins and hemicelluloses of the cell walls (Carpita and McCann 2000), however, other cell walls compounds as phenols, proteins and amino acids may also contribute to the binding and accumulation of cations (Meychik and Yermakov, 2001; Krzeslowska 2011). The acidic polysaccharides (pectins) built mainly from galacturonic acid (GalA), are considered to play a key role in the binding of cations on the negatively charged surfaces

of the cell walls (Fritz 2007; Horst et al., 2010). Generally, the pectin family consists of three pectic polymers: homogalacturonan and rhamnogalacturonan I and II (Caffall and Mohnen, 2009). Krzeslowska (2011) stated that demethylated carboxylic groups of the homogalacturonan are of utmost importance in the process of binding cations by the cell wall. The cations binding capacity of roots comes also from the cytoplasmic membrane phospholipids and proteins (Postma et al., 2005; Kopitke et al., 2011; Wang et al., 2011). Since most of the information on the input of particular root components into the roots charge comes from correlations studies, we tried to estimate it more directly. To do this we estimated the negative charge of the whole roots, next in separated root cell walls and finally in the cell walls residue after removal of pectins.

The negative charge was evaluated by potentiometric titration (Nederlof et al., 1993; Szatanik-Kloc et al., 2009). This method evaluating the surface charge-pH dependence was applied to estimate the overall surface charge (Q_{tot}), CEC, distribution of surface groups of different strengths, as well as the average value of surface acidity. Along with the surface charge characteristics we estimated water vapor adsorption-desorption isotherms of the studied objects that allowed for

Abbreviations: CW, cell wall; CW-pect, cell wall without pectins; CEC, cation exchange capacity; Q_{tot} , total surface charge; pKapp, apparent dissociation constant; S, surface area; SCD, surface charge density

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calculation of surface areas and adsorption energies. Surface charge and area were used to estimate the surface charge density (SCD), which relates to the strength of cations binding by roots.

2. Materials and methods

2.1. Plant growth

Seeds of two species of *Apiaceae* L. family: celeriac (*Apium graveolens* L. var. Talar) and parsnip (*Pastinaca sativa* L. var. Hollow Crown) were seeded on a moist filter paper and then 40 germs of each species were transferred onto plastic liners positioned in black 5 dm³ polyethylene containers filled with a Hoagland medium (Marschner 1995) supplemented by a set of essential micronutrients, and aerated continuously. Every day the volume of the medium was supplemented by the distilled water and, if necessary, the pH of the medium was adjusted to a value of 7 ± 0.2 using either 1 M potassium hydroxide, or 1 M hydrochloric acid. The medium was renewed on the 7th day of the plants growth. The 16/8 h (day/night) regime at temperatures of 296 K (day) and 286 K (night) was maintained during the whole plant growth cycle. The day-lighting of the plants was provided with sodium lamps WLS 400 (photon flux density = $300 \mu\text{M m}^{-2} \text{s}^{-1}$ PAR (Photosynthetic Active Radiation)). After 14 days of the experiment, the plant roots were harvested, washed with distilled water, drained with filter paper, dried at 303 K and stored at liquid nitrogen temperature. Before the further experiments the roots were freeze dried. The experiment was performed in three independent replicates.

2.2. Isolation of roots cell walls

Some steps of isolation and storage of cell wall were performed at low temperatures because of the proven effect of inactivation of polygalacturanases and other hydrolases. Moreover, this can eliminate autolytic reduction of polysaccharides end-groups during the further extraction of pectins (Carrington et al., 1993; Gallego 1996). The cell walls were isolated according to a method of Renard (2005) modified by Cybulska et al. (2015). Briefly, the roots were ground in a cooled ball mill (Retsch MM400, Germany), mixed with 70% ethanol (1:7 roots: ethanol ratio), boiled for 20 min at a temperature of about 351 K, cooled in ice bath, filtered through a nylon 10 μm Millipore filter, transferred to a glass dish, quenched with cold 70% ethanol (1:3 filter cake: ethanol ratio), stirred by 20 min, dewatered by triple exchange of the solvent with 96% ethanol and acetone mixture in an ice bath, filtered again, finally dried under vacuum to completely remove ethanol/acetone, finally dried at 303 K and stored at 277 K. Since microscopic studies of the resulting material showed lack of intracellular structures, it was considered as cell walls (CW).

2.3. Removal of pectins from CW

Soluble pectins were removed from CW according to the method of Redgwell et al. (1988) modified by Cybulska et al. (2015). The procedure consisted from 3 steps: extraction of water soluble (WSP) pectins by deionized water, extraction of chelator soluble pectins (CSP) by 0.05 Mdm⁻³ CDTA and extraction of diluted alkali soluble pectins (DASP) by sodium carbonate solution. The residue after pectin-extraction procedure (CW-pect) was washed with deionized water, lyophilized and stored at 277 K.

The efficiency of the cell walls and the pectins extraction procedures was determined after drying subsamples of all materials at 105 °C during 24 h and it was expressed on dry mass basis. However, the further experiments were performed for samples obtained as described in previous sections, because we feared that 105 °C heating may cause some irreversible changes in the studied biological materials.

2.4. Potentiometric titration

The titration of roots, CW and CW-pect was performed automatically using the Titrino 702 MS device provided by Metrohm AG Switzerland. Since all details of the titration procedure along with mathematical elaboration of the titration data are easily available in J & zefaciuk et al. (2014), below we provide only a short outline of the method. A suspension of a given materials equilibrated overnight with 1 M dm⁻³ NaCl solution was adjusted to pH = 3.0 until the pH was stable over the next 5 min and titrated by 60 s increments of 1 μL 0.100 M dm⁻³ sodium hydroxide solution to pH = 10 under gaseous nitrogen flux. From the titration curve of a suspension, the titration curve of its equilibrium solution (assumed to be the titration curve of 1 M dm⁻³ NaCl) was subtracted to obtain the titration curve of the solid phase. The latter curve was taken as the surface charge-pH dependence. The surface charge at pH = 7 was taken as the material CEC, that is in accordance with a method proposed by Morvan et al. (1979) and the charge at pH = 10 was taken as the total surface charge, Q_{tot} . Under the assumption that surface acids dissociate stepwise (known as a condensation approximation) one can assume that the actual pH is equal to the $-\log$ of apparent surface dissociation constant, pK_{app} , and the first derivative of the surface charge vs. pK_{app} curve on pK_{app} gives the distribution function of apparent surface dissociation constants, $f(\text{pK}_{\text{app}})$, showing fractions of surface acids of given dissociation constants. Knowing these fractions, the average value of pK_{app} for the whole surface, $\text{pK}_{\text{app,av}}$, was calculated as $\sum f(\text{pK}_{\text{app},i}) \text{pK}_{\text{app},i}$. The measurements were performed in triplicate with differences not exceeding 6.3%.

2.5. Adsorption – desorption isotherms

Water vapor adsorption-desorption isotherms were measured and elaborated mathematically according to the procedure described in details by J & zefaciuk et al. (2013) and Szatanik-Kloc (2014). Summarizing: first the experimental desorption isotherms were measured by weighing the materials samples equilibrated in a vacuum chamber over sulphuric acid of stepwise increasing concentrations (decrease of the relative water vapor pressure p/p_0) and next the adsorption isotherms were measured at stepwise decreasing concentrations of the sulphuric acid. From the sample masses measured at various p/p_0 , the dry mass of the sample (measured at the end of the experiment after 24 h heating of the sample at 378.2 K) was subtracted to find the amount of the water vapor adsorbed by the samples at given p/p_0 values. The desorption data expressed as the amount of adsorbed water per unit mass of the material, $a(p/p_0)$ (kg kg^{-1}) were fitted to the standard Brunauer-Emmett-Teller – BET model (Brunauer et al., 1938), from which the surface areas, S ($\text{m}^2 \text{g}^{-1}$), were estimated. Since Chiou et al. (1990) suggested that for organic materials where processes of absorption and adsorption are difficult to distinguish, the surface area should be understood as apparent surface area, so we keep in mind this meaning further. Following the BET model the water vapor adsorption energy, E , was defined as $E = -\log(p_0/p) - 1$ and the amount of water vapor adsorbed (adsorption branch) in monolayer at a given E was calculated as $a_m(E) = a(p/p_0) \cdot (1 - p/p_0)$. The E values are defined as dimensionless, negative adsorption energies showing the excess of adsorption energy, E_a (J Mol^{-1}), over water vapor condensation energy, E_c (J Mol^{-1}), in units of RT (where R ($\text{J M}^{-1} \text{K}^{-1}$) is universal gas constant and T (K) is the temperature of the measurement). The first derivative of $a_m(E)$ on E gave the adsorption energy distribution functions, $f(E)$, from which the average adsorption energies were calculated as $\sum f(E_i) E_i$. The measurements were performed in triplicate with differences not exceeding 4.3%.

From the ratio of the total surface charge to the surface area, the surface charge density, SCD (C m^{-2}), was calculated.

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