Plant Physiology and Biochemistry 120 (2017) 194–201

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

journal homepage: www.elsevier.com/locate/plaphy

Research article

Input of different kinds of soluble pectin to cation binding properties of roots cell walls

A. Szatanik-Kloc, J. Szerement, J. Cybulska, G. Jozefaciuk*

Institute of Agrophysics Polish Academy of Sciences, Doswiadczalna 4, 20-290 Lublin, Poland

ARTICLE INFO

Keywords:
Degree of methylation
Pectin methylesterase
Cation exchange
Surface charge
Surface acidity

ABSTRACT

It is widely believed that pectin are responsible for the vast majority of cation binding positions in the root cell walls. To estimate the role of particular kinds of pectin, we studied the cell wall material isolated from the roots of monocots (wheat and rye) and dicots (clover and lupine) before and after removal of different fractions of soluble pectin. Simultaneously PME activity and degree of pectin methylation were determined. From potentiometric titration curves cation exchange capacity, total surface charge and acidic strength of surface functional groups responsible for surface charging were determined. Monocots had smaller cation exchange capacity and lower pectin content than dicots. Removal of pectin induced up to 50% reduction in the cell walls surface charge. Pectin seem to have more acidic character than the other roots components that is seen from an increase in very weakly acidic groups fraction and significant decrease in the average dissociation constant of the cell walls material after pectin removal. Water soluble pectin and non-pectic soluble compounds had the dominant role in surface charging, while chelator and diluted alkali soluble pectin contributed to surface charge only at high pH's.

1. Introduction

From the point of view of mineral nutrition, the cell walls (CW) forms a route for nutrients transport to the plasma membrane and a place for adsorption and accumulation of ions (Ae and Otani, 1997). The root CW, next to plasma membranes, are considered as a major factor contributing to ions binding by plant roots (Sattelmacher, 2001; Kinraide, 2004; Guigues et al., 2014). Since cell walls (CW) are negatively charged, the ions bound by CW are positively charged cations (Clarkson, 1993). Various functional groups such as carboxyl (COOH), hydroxyl (OH), and thiol (SH) present in polysaccharides, proteins, amino acids, and phenolic compounds are responsible for negative charge of the CW (Meychik and Yermakov, 2001; Pelloux et al., 2007). The dominant input to CW surface charging is usually attributed to the presence of pectin. Pectin are polysaccharides containing α-D galacturonic acid (GalA) that is observed in three pectic domains: homogalacturonan (HGA) and rhamnogalacturonan I and II (RGI and RGII), which differ both in the macromolecular structure and the presence and variety of side chains (Caffall and Mohnen, 2009). The CW cation binding properties are mainly related to demethylated carboxylic groups of HGA, which are generally considered to be responsible for 70–90% of the CW cation binding properties (Haynes, 1980; Krzeslowska, 2011). Demethylated pectin are more soluble than the methylated ones and their mobility depends on charge and molecular mass, therefore standard manners of pectin characterization involve solubilisation of different fractions of pectin in a range of chemical agents (Redgwell et al., 1988). We hypothesize that different fractions of soluble pectin have different inputs to the CW negative charge. To check this hypothesis we determined the roots CW charge before and after extraction of different pectin fractions and tried to correlate the charge characteristics with their amounts. The cation exchange capacity (CEC), total surface charge (Q_{tot}) and the acidic character of charge generating surface functional groups, defined by their apparent dissociation constants (pK_{app}) were determined by back titration method (Nederlof et al., 1993; Szatanik-Kloc et al., 2009). The CW isolated from the roots of monocots and dicots plants were used.

2. Materials and methods

2.1. Plant growth

Two monocot plants: wheat (*Triticum vulgare* L.) cv. Tonacja and rye (*Secale cereale* L.) cv. Rostockie and two dicot plants: clover (*Trifolium pretense* L.) cv. Jubilatka and lupine (*Lupinus angustifolius* L.) cv. Zeus were cultivated in hydroponic conditions under strictly controlled mineral composition and pH of the medium. The medium was prepared

* Corresponding author.
E-mail address: jozefaci@ipan.lublin.pl (G. Jozefaciuk).

http://dx.doi.org/10.1016/j.plaphy.2017.10.011
Received 4 August 2017; Received in revised form 11 October 2017; Accepted 13 October 2017
Available online 16 October 2017

Abbreviations

GalA	1- 4 – α - D galacturonic acid
CW	cell wall
CW-pect	cell wall material without soluble pectin
WSP	water-soluble pectin
CSP	chelator-soluble pectin
DASP	diluted alkali (sodium carbonate)-soluble pectin
DM	degree of methylation
CEC	cation exchange capacity
Q_{tot}	total surface charge
K_{app}	apparent surface dissociation constant
PME	pectin methylesterase activity

according to the modified Hoagland method (Marschner, 1995), continuously aerated and renewed every 7 d. The plants were grown at 16 h, 296 K, 75% RH (day) and 8 h, 289 K 70% RH (night) regime. The photon flux density of 300 μmol· m^{2}·s^{-1} PAR was maintained during the day. Every day the solution pH was adjusted to 7 ± 0.2 either with 0.1 M hydrochloric acid or 0.1 M potassium hydroxide. The plant roots were collected 4 weeks after germination, rinsed three times with distilled water and blotted with paper towels. Fresh root samples were instantaneously frozen in liquid nitrogen and stored in plastic tubes at 193 K. The experiment was performed in three independent replicates.

2.2. Preparation of cell walls

The CW was isolated from the whole root material according to the method described by Renard (2005) and Cybulska et al. (2015). The root samples were carefully homogenized using a cutting edge mechanical homogenizer (HO 4/A Buehler Germany), cooled with liquid nitrogen and crushed in a ball mill (Retsch MM400, Germany). Approximately 10 g of the roots were mixed with 70 mL of 70% ice cooled ethanol, stirred, filtered through a Millipore nylon membrane in an ice bath, washed tree times with 10 mL cold 96% ethanol and 50 mL acetone, centrifuged, washed with deionized water, centrifuged again, dried for 24 h at 303 K and weighed. A half of CW material was stored at 277 K for further analysis, and the remaining half was used to remove pectin. The CW material remaining on the nylon filter was examined with a light microscope showing an absence of intracellular structures. Isolation of the cell wall was performed at low temperatures to inactivate polygalacturanases and other hydrolases thus eliminating autolytic reduction of polysaccharide end-groups during pectin extraction (Carrington et al., 1993; Gallego, 1996).

2.3. Determination of pectin content

Pectin were isolated from the CW according to the method described by Redgwell et al. (1988) and Cybulska et al. (2015). Ten mL of deionized water was added to 0.5 g of a dry CW sample, stirred by 6 h at 289 K and centrifuged at 10 000 *g* by 15 min. The supernatant containing the fraction of water-soluble pectin (WSP) was collected. Next, the sediment was added with 10 mL of 0.1 M trans-1,2-diaminocyclohexane-N,N,N′,N′-tetraacetic acid (CDTA solution), stirred by 2 h at 298 K, and centrifuged. The supernatant containing the fraction of chelator soluble pectin (CSP) was collected. The sediment was then treated with 10 mL of 0.05 M sodium carbonate (Na_2CO_3) supplemented with 20 mM sodium borohydride ($NaBH_4$) by 20 h at 274 K and next by 2 h at 298 K, and centrifuged. The supernatant containing the dilute alkali-soluble pectin fraction (DASP) was collected. The temperature regime during DASP extraction and addition of $NaBH_4$ reduced the degradation of pectin (Fisher and Amado, 1994).

The content of pectin was determined in the subsequent supernatants with the automatic flow chemistry analyser (CFA) SanPlus (Scalar, the Netherlands) according to the colorimetric method of Blumenkrantz and Asboe-Hansen (1973). The samples were decomposed in 96% sulphuric acid (H_2SO_4)/disodium tetraborate $Na_2B_4O_7$ x $10H_2O$ mixture and added with 3-phenyl-phenol forming a coloured dye. The concentration of the dye was measured at a wavelength of 530 nm. The calibration curve was constructed for mono-galacturonic acid solutions (Szymanska-Chargot and Zdunek, 2013). The pectin content in CW was thus expressed as galacturonic acid equivalent. The total soluble pectin content was expressed as a sum of the individual fractions.

2.4. Determination of the degree of methylation (DM)

FTIR spectra of the lyophilized CW were scanned within 4.000–500 cm^{-1} range using FTIR spectrometer Nicolet 6700 (Thermo Scientific, Waltham, MA, USA) using the attenuated total reflectance (ATR) method in 10 replicates. All spectra were normalized and baseline-corrected with Omnic Software (Thermo Scientific). The data was analysed using OriginPro 8.5.0 software (OriginLab Corporation, Northampton, MA, USA). The DM was determined from FTIR spectra intensities at 1750 cm^{-1} (esterified carbonyl groups), and at 1630 cm^{-1} (carboxylic anions). The DM value was determined from the ratio of esterified carbonyls to the sum of the esterified carbonyls and carboxylic anions (Fellah et al., 2009).

2.5. Determination of the pectin methylesterase activity (PME)

Pectin methylesterase (PME) activity was determined according to Wei et al. (2010). The fresh roots sample (3g) was stirred with 6 ml of cold 12% polyethyleneglycol containing 0.2% sodium bisulphate, centrifuged (10 min, 6000 × g) and washed at 277K with 0.2% sodium bisulphate. The sediment was then reacted with 6 mL of extraction buffer (1M sodium acetate at pH 5.2, 1 M NaCl, 2% (v/v) mercaptoethanol and 5% (w/v) polyvinylpyrrolidone) at 277K for 1 h and the resulting homogenate was centrifuged for 10 min at 6000 × *g*. The supernatant was used to determine pectin methylesterase activity. One mL of the supernatant was mixed with 4 mL of 1% (w/v) citrus pectin and titrated at pH-stat (pH = 7.4) mode at 310 K with 0.01 M NaOH for 1 h. The PME activity was calculated from the amount of the consumed base taking one unit of activity as 1 mMol NaOH consumed by 1 g of fresh roots in 10 min.

2.6. Potentiometric titration

Potentiometric titration curves for CW before and after pectin extraction were taken using an auto-titrator SM Titrino 702 (Metrohm, AG-Switzerland). Titration measurements were performed under nitrogen atmosphere in three replicates with the deviation not exceeding 5.6%. The titration data were used to estimate the variable surface charge vs. pH dependencies and apparent surface dissociation constants distribution functions according to Nederlof et al. (1993), Jozefaciuk and Szatanik-Kloc (2004), and Łukowska and Józefaciuk (2016). The suspension of 0.05 g of the studied material in 20 mL 1 mol·dm^{-3} sodium chloride NaCl solution was adjusted to pH = 2.95 (not changing within 5 min) and slowly titrated (1 min/1 μL increments) to pH = 10 with 0.1 mol·dm^{-3} sodium hydroxide (NaOH). The amount of the base consumed by the whole suspension (N_{susp}) is used for neutralization of acidic groups of the solid surface (NS) and the acids present in the supernatant (N_{sol}). The calculated NS value at any pH (NS = N_{susp} - N_{sol}) is equivalent to variable surface charge. The variable charge at pH = 7 was taken as the cell wall CEC (according to the method described by Morvan et al., 1979) and the charge developed in the whole