Phytochemistry xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Phytochemistry

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

Structural alteration of cell wall pectins accompanies pea development in response to cold

Laëtitia Baldwin [a,1], Jean-Marc Domon [a], John F. Klimek [b], Françoise Fournet [a], Hélène Sellier [c], Françoise Gillet [a], Jérôme Pelloux [a], Isabelle Lejeune-Hénaut [c], Nicholas C. Carpita [b], Catherine Rayon [a,*]

[a] EA 3900-BIOPI, Biologie des Plantes et Innovation, Université de Picardie Jules Verne, 80039 Amiens, France
[b] Department of Botany & Plant Pathology, Purdue University, 915 West State Street, West Lafayette, IN 47907-2054, United States
[c] INRA USTL UMR 1281, Laboratoire de Génétique et d'Amélioration des Plantes, Estrées-Mons BP50136, 80203 Péronne, France

ARTICLE INFO

Article history:
Received 5 August 2013
Received in revised form 11 April 2014
Available online xxxx

Keywords:
Cell-wall
Cold
Pea
Pectin
Pectin acetylesterase
Pectin methylesterase
Pectic epitopes
Polygalacturonase

ABSTRACT

Pea (*Pisum sativum*) cell wall metabolism in response to chilling was investigated in a frost-sensitive genotype 'Terese' and a frost-tolerant genotype 'Champagne'. Cell walls isolated from stipules of cold acclimated and non-acclimated plants showed that cold temperatures induce changes in polymers containing xylose, arabinose, galactose and galacturonic acid residues. In the tolerant cultivar Champagne, acclimation is accompanied by increases in homogalacturonan, xylogalacturonan and highly branched Rhamnogalacturonan I with branched and unbranched (1→5)-α-arabinans and (1→4)-β-galactans. In contrast, the sensitive cultivar Terese accumulates substantial amounts of (1→4)-β-xylans and glucuronoxylan, but not the pectins. Greater JIM7 labeling was observed in Champagne compared to Terese, indicating that cold acclimation also induces an increase in the degree of methylesterification of pectins. Significant decrease in polygalacturonase activities in both genotypes were observed at the end of cold acclimation. These data indicate a role for esterified pectins in cold tolerance. The possible functions for pectins and their associated arabinans and galactans in cold acclimation are discussed.

1. Introduction

Pea seeds have high protein content (ca. 24%) and constitute an alternative to soybean as a rich protein feedstock for Western Europe. However, the pea crop, represented mainly by spring peas, has limited development prospects as a result of unstable yields. Stabilizing and improving the productivity of the crop could be achieved through the release of winter varieties, which have a longer life cycle than spring varieties associated with a higher biomass production. Deciphering the physiological mechanisms and the genetic determinants of the frost tolerance and cold acclimation of pea would assist selection of chilling tolerant varieties.

Plants differ in their tolerance to the physiological distinct chilling (0–15 °C) and freezing (<0 °C) temperatures (Pearce, 2001; Kreps et al., 2002; Chinnusamy et al., 2007; Chen et al., 2011). Chilling and freezing injuries can slow down enzyme activities and modify plant metabolism with deleterious effects on most biological functions (Stitt and Hurry, 2002). Temperate plants have developed mechanisms of cold acclimation to increase their ability to withstand freezing temperatures following a period of low but non-freezing temperatures (Kume et al., 2005; Levitt, 1980).

Cold acclimation is a complex mechanism that involves physiological and biochemical modifications, including changes in plant membrane composition, in cell wall structure, in establishment of ROS scavenger systems and in the accumulation of osmoregulants, sugars and proteins (for review, Winfield et al., 2010; Barros et al., 2012; Li et al., 2012; Rohloff et al., 2012; Sasidharan et al., 2011; Theocharis et al., 2012; Crosatti et al., 2013). Freeze-induced cell dehydration results in the collapse of the cell wall and, depending on the properties of cell components, may lead to different degrees of frost injuries (Levitt, 1980). Plant resistance to cold stress often depends on tissue morphology and mechanical

Abbreviations: HG, homogalacturonan; RG I, Rhamnogalacturonan I; RG II, Rhamnogalacturonan II; XHG, xylogalacturonan.

* Corresponding author. Address: Université de Picardie Jules Verne, EA 3900-BIOPI, 33 rue Saint Leu, 80039 Amiens cedex, France. Tel.: +33 322 827 536.

E-mail addresses: lbal@plen.ku.dk (L. Baldwin), jean-marc.domon@u-picardie.fr (J.-M. Domon), jfklimek@purdue.edu (J.F. Klimek), francoise.fournet@u-picardie.fr (F. Fournet), sellier@mons.inra.fr (H. Sellier), francoise.gillet@u-picardie.fr (F. Gillet), jerome.pelloux@u-picardie.fr (J. Pelloux), lejeune@mons.inra.fr (I. Lejeune-Hénaut), carpita@purdue.edu (N.C. Carpita), catherine.rayon@u-picardie.fr (C. Rayon).

[1] Present address: Department of Agriculture and Ecology, Plant and Soil Science Section, Faculty of Sciences, University of Copenhagen, Denmark.

http://dx.doi.org/10.1016/j.phytochem.2014.04.011
0031-9422/

properties of the cell-wall interactions with the plasma membrane (Hoson, 1998; Stefanowska et al., 1999; Fujikawa and Kuroda, 2000; Pearce and Fuller, 2001; Yamada et al., 2002; Evered et al., 2007). The plant cell wall is composed of cellulose microfibrils interlaced with non-cellulosic cross-linking polysaccharides, and embedded in a physiologically active pectin matrix and cross-linked with structural proteins (McCann and Roberts, 1991; Carpita and Gibeaut, 1993). Pectins contribute to the mechanical strength, porosity, adhesion and stiffness of the cell wall (Willats et al., 2001; Solecka et al., 2008). Structural changes in cell wall components are mediated through the activity of cell wall-modifying enzymes that play a major role in controlling cell wall plasticity/rheology.

Cold temperature was previously shown to reduce mung bean and sweet potato plant cell wall elongation (Yun et al., 2007; Noh et al., 2009). This could partly be due to the increase in cell wall thickness and rigidity observed for instance in oilseed rape plants, grape stem, oak and cranberry leaves in response to low temperatures (Stefanowska et al., 1999; Rajashekar and Burke, 1996). In suspension culture of grape cells and in apple, cold acclimation increases cell wall strength and decreases pore-size of cell wall (Rajashekar and Lafta, 1996). In oilseed rape leaves, the cold response was associated with an increase in pectins and PME activity (Kubacka-Zebalska and Kacperska, 1999; Solecka et al., 2008). Such increase in pectin was also observed in pea epicotyls during cold acclimation, with a higher level of arabinosyl residues (Weiser et al., 1990). This modification in pectin structure is often accompanied by changes in neutral sugar composition in vegetative tissues (Weiser et al., 1990; Stefanowska et al., 1999; Kubacka-Zebalska and Kacperska, 1999).

Transcriptomic and proteomic analyses have shown that most cell wall related genes or proteins identified in response to cold stress correspond to pectin remodeling enzymes, including pectin methylesterase (PME, EC 3.1.1.11), polygalacturonase (PG, EC 3.2.1.15), pectin acetylesterase (PAE, EC 3.1.1.6) and pectate lyase (PL, EC 4.2.2.2) (Kreps et al., 2002; Seki et al., 2002; Lee and Lee, 2003; Thonar et al., 2006; Nilo et al., 2010; Lucau-Danila et al., 2012; Mworia et al., 2012). Under cold stress, alteration in PME, PG and PAE enzyme activities is well observed in vegetative tissue of chicory roots, where lower PME and PAE activities are detected (Thonar et al., 2006), and of winter oilseed rape leaves, where PME activity is increased (Solecka et al., 2008).

In this work, a multidisciplinary approach was undertaken to understand the effects of cold acclimation and freezing on cell wall metabolism of two pea (*Pisum sativum*) genotypes with contrasted frost tolerances. After a cold-acclimation period, the cultivar Champagne becomes freeze-tolerant while Terese does not. The aim of this work was to determine changes in the cell walls associated with successful acclimation to freezing tolerance. We showed that cold acclimation in the frost-tolerant Champagne cultivar resulted in increased abundance of pectic polymers, particularly in arabinan side chains of RG I, whereas in the frost-sensitive Terese, a substantial decrease in pectic polymers was observed. Instead, a strong increase in xylans and glucuronoxylans is shown in Terese, indicating a premature end to cell growth and initiation of vascularization. Our results indicate that at least one determinant of frost-tolerance is the modification of pectin side-group substitution. These biochemical indicators of chilling tolerance provide new quantitative traits to assist in the breeding of frost tolerant cultivars.

2. Results

Two pea genotypes, frost-tolerant Champagne, a winter forage line, and frost-sensitive Terese, a spring dry genotype, were chosen for their contrasting behavior towards frost. In addition, Champagne and Terese differ in their leaf structure: Champagne is considered the wild-type phenotype, whereas Terese is an *afila*-type pea (Fig. 1A). Considering these morphological differences, all subsequent analyzes were performed on stipules, the common aerial organ of these two cultivars. This should provide biochemical and physiological data to support genetic, transcriptomic and proteomic studies performed on the stipules of these two genotypes (Lejeune-Hénaut et al., 2008; Dumont et al., 2009; Lucau-Danila et al., 2012). Both genotypes were submitted to cold acclimation (CA) and compared to controls without cold acclimation (NA). During the time course of the experiments, first-stage stipules were harvested at different sampling dates (N, A5, A10, A20, F). In control experiments, the first step (N) was grown longer to reach the same developmental stage prior to frost (F), compared with cold-acclimated plants (Fig. 1B and C). Considering the wealth of data generated, the emphasis will be on the differences between cold-acclimated (CA) plants and non-cold-acclimated (NA) plants for each harvest time point.

2.1. Evaluation of frost damage

Frost injury in the aerial portions of the plants ranged from 0 (uninjured) to 5 (dead plants). For both genotypes, plants submitted to frost without cold acclimation (NA experiment) died during the frost-recovery period. In contrast, in the cold-acclimated (CA) experiment, the Champagne genotype was shown to be more frost-tolerant (score: 2 ± 0.01) compared to the Terese genotype (score: 3.7 ± 0.4) (Fig. 2).

2.2. Monosaccharide composition and linkage structure of cell walls

2.2.1. Pectic polymers

During cold acclimation the levels of arabinose increased in both genotypes compared to non-cold acclimated plants, but with distinct levels: At the end of the cold acclimation period (A20), arabinose content was increased by 2.5-fold and 1.3-fold in Champagne and Terese respectively (Table 1). The increase in Ara content was mostly in 5-Ara in both genotypes, indicating unbranched (1→5)-L-arabinans (Tables 2 and 3). During cold acclimation 5-Ara increased by 77% above non-acclimated controls in Champagne, whereas this linkage group increased only 26% above controls in Terese. Although lower in abundance, similar trends were observed in other Ara linkage groups, including the branch-point residues 2,5-Ara and non-reducing *t*-Ara residues. The increase in total Ara accompanied an increase in branched residues of Rhamnogalacturonan I (RG I) during cold acclimation. The degree of branching of 2,4-Rha increased 2-fold in Champagne during cold acclimation compared to control, whereas it remained steady in Terese. These data indicate an increase in arabinan side-chains of RG I in the frost-tolerant genotype specifically accompanies frost tolerance in the Champagne cultivar.

Cold-acclimated Champagne plants also showed a 50% increase in Gal content compared to non-cold-acclimated plants (Table 1, A20), whereas no change was observed in Terese. A higher level of 4-linked Gal residues in Champagne upon cold acclimation accounted for much of this change, indicating an increase in (1→4)-β-D-galactans on RG I during cold acclimation in Champagne, but not in Terese (Tables 2 and 3). Cold acclimation was also accompanied by increases in *t*-Gal, 6-Gal, and 3,6-Gal residues, indicators of type II arabinogalactan-proteins, in the tolerant Champagne cultivar. In contrast, these residues either remained unchanged or decreased in the sensitive Terese cultivar upon cold-acclimation.

Levels of 4-linked GalA, a measure of both HG and RG I, also changed during acclimation but were not overall consistent with the changes in pectic arabinan and galactan content. In

Please cite this article in press as: Baldwin, L., et al. Structural alteration of cell wall pectins accompanies pea development in response to cold. Phytochemistry (2014), http://dx.doi.org/10.1016/j.phytochem.2014.04.011