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# Major changes in the cell wall during silique development in *Arabidopsis thaliana*

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ABSTRACT

Fruit development is a highly complex process, which involves major changes in plant metabolism leading to cell growth and differentiation. Changes in cell wall composition and structure play a major role in modulating cell growth. We investigated the changes in cell wall composition and the activities of associated enzymes during the dry fruit development of the model plant *Arabidopsis thaliana*. Silique development is characterized by several specific phases leading to fruit dehiscence and seed dispersal. We showed that early phases of silique growth were characterized by specific changes in non-cellulosic sugar content (rhamnose, arabinose, xylose, galactose and galacturonic acid). Xyloglucan oligosaccharide mass profiling further showed a strong increase in *O*-acetylated xyloglucans over the course of silique development, which could suggest a decreased capacity of xyloglucans to be associated with each other or to cellulose. The degree of methylesterification, mediated by the activity of pectin methylesterases (PMEs), decreased over the course of silique growth and dehiscence. The major changes in cell wall composition revealed by our analysis suggest that it could be major determinants in modulating cell wall rheology leading to growth or growth arrest.



## 1. Introduction

Plant primary cell wall is a highly complex structure composed of diverse polysaccharides and structural proteins. Plant cell shape and growth largely depend upon changes in cell wall composition and structure, which are mediated by specific pools of enzymes. Plant cell wall composition of leaves of the model plant *Arabidopsis thaliana* is typical of type I species (dicotyledonous, non-commelinoid monocots and gymnosperms) with cellulose, hemicelluloses, pectins and proteins accounting for 14%, 24%, 43% and 14%, respectively (Zablackis et al., 1995). Cellulose microfibrils, which confer rigidity to the cell wall, interact with hemicelluloses to structure a rigid and tensile network. In Arabidopsis, xyloglucan (XG), the major hemicellulosic compound (20%), is composed by hepta to decasaccharide units. The nomenclature of XG is based on the substitutions of the four D-glucose linked in β(1–4) that constitute the backbone of the unit (Fry et al., 1993). This backbone can be substituted in α(1–6) by up to three D-xylose residues (XXXG). Xylose residues can be further substituted by up to two D-galactose in β(1–2) (XLLG) and one terminal L-fucose linked in α(1–2) with the galactose (XLFG). Furthermore, XG units can be acetylated on galactose. In Arabidopsis, the major form of XG is XXXG, which accounts for about 40% of the total units (Mouille et al., 2006).

Pectin, the main component of the primary cell wall, is often described as a highly complex polysaccharide rich in galacturonic acids (GalA). Depending on the backbone and its composition, 5 classes of pectic polysaccharides are often distinguished in dicot species (Caffall and Mohnen, 2009). Homogalacturonan (HG), which is a linear α-1,4-linked D-GalA homopolymer with a typical degree of polymerization of ~100, can be acetylated and methylesterified on specific carbons (C2–C3 and C6, respectively). It can further be substituted by D-xylose, D-apiose or up to 12 different types of glycosyl residues to constitute xylogalacturonan (XGA), apiogalacturonan (AP) and rhamnogalacturonan II (RGII), respectively. Rhamnogalacturonan I (RGI) is the only pectin not built on a HG backbone, but rather on a polymer of galacturonic acid (D-GalA) and rhamnose (L-Rha) disaccharide subunits. RGI can be branched at rhamnose residues by arabinan, galactan or arabinogalactan side chains (Lau et al., 1985). In Arabidopsis leaves, HG

*Abbreviations:* AIR, alcohol insoluble residues; DAF, days after flowering; DM, degree of methylesterification; GalA, galacturonic acid; GlcA, glucuronic acid; HG, homogalacturonans; PG, polygalacturonase; PME, pectin methylesterase; MALDI-TOF-MS, matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry; XEG, xyloglucan endo-β-(1–4) glucanase; XyGOs, xyloglucan oligosaccharides.

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accounts for ~50% of total pectins while RGI and RGII account for ~19% and ~26%, respectively. The pectic network is principally structured by covalent interactions between HG, RGI and RGII, as well as ionic interactions between non methyl-esterified HG chains, forming so called egg-box motifs (Liners et al., 1989).

Plant development requires major changes in cell wall synthesis and refolding, which is mediated by enzymes that belong to multigenic families in Arabidopsis (Arabidopsis Genome Initiative, 2000). For instance, the cellulose–xyloglucan network can be modified by a wide range of proteins including glucanases, transglycosidases and expansins (Harpster et al., 2002). Enzyme mediated changes in the pectic network have been well documented during the course of pulpy fruit maturation, where a strong increase in enzymes involved in pectin degradation, such as pectin methylesterases (PMEs, EC 3.1.1.11), exo- and endo-polygalacturonases (PG, EC 3.2.1.67; EC 3.2.1.15) and pectate lyases (PL, EC 4.2.2.2) have been shown (Redgwell et al., 1992; Wakabayashi et al., 2003; Eriksson et al., 2004; Arancibia and Motsenbocker, 2006). The changes in cell wall composition and dynamics during dry fruit development have, however, not been well documented. In green bean pods, major changes were detected in the pectic compounds during development, with degradation of galactose-rich pectic polymers and accumulation of homogalacturonan (Stolle-Smits et al., 1999). At the pod dehiscence stage, ionically complexed pectins increased. A role for PG-mediated changes in the pectic network has been shown in the dehiscence process in numerous studies (Jenkins et al., 1999; Sander et al., 2001; González-Carranza et al., 2007). In *A. thaliana*, previous work showed distinct cell wall polysaccharide composition between organs (seeds, roots, leaves and stems) as well as during the course seed development (Baud et al., 2002; Zandleven et al., 2007; Macquet et al., 2007). However, no integrated investigation of cell wall modifications during the various stages of silique development was undertaken, despite data showing major changes in the expression of cell wall-related enzymes during this process (Jenkins et al., 1999; Louvet et al., 2006; Hruz et al., 2008).

In this study, changes in cell wall polysaccharides, as well as changes in selected enzyme activities were studied during silique development from the onset of growth through maturation and dehiscence.

## 2. Results and discussion

### 2.1. Silique developmental stages

Flower buds (FB) and siliques were harvested at different stages of development as described (Louvet et al., 2006). The seeds were not separated from the siliques. Silique development was classified into nine stages (1–2 DAF (Days after flowering), 3–4 DAF, 5–6 DAF, 7–8 DAF, 9–11 DAF, 12–14 DAF, 15–17 DAF and 18–20 DAF) as shown in Fig. 1. Development of siliques was preceded by floral bud initiation which was named FB, corresponding to 0 DAF. During the first stages (1–2 DAF, 3–4 DAF, 5–6 DAF and 7–8 DAF), siliques grew in length up to 1.4 cm. At 7–8 DAF, elongation stopped and lateral expansion started, suggesting that 7–8 DAF could be a shift point during silique development. Stages 15–17 DAF onwards corresponded to silique maturation. Stages 18–20 were clearly dehiscent with major seed loss upon harvesting.

### 2.2. Monosaccharide composition of AIR from sequential silique developmental stages

Alcohol insoluble residues (AIR) were prepared from siliques harvested at different developmental stages and from mature seeds. Cell wall material was hydrolysed by TFA (2 M), and monosaccharide composition of the non-cellulosic polysaccharides from AIR was performed by gas liquid chromatography of the trimethylsilyl methyl glycosides. Glucose residues from neutral sugars were not quantitatively analysed because starch was not removed from the AIR.

The main noncellulosic sugars in siliques were GalA, arabinose, xylose, rhamnose and galactose (Fig. 2). Amounts of fucose, mannose and glucuronic acid were relatively low throughout silique development. GalA was the most abundant monosaccharide with values between 23.6 μg/mg AIR (±3.37) and 100.5 μg/mg AIR (±0.67) (Supplementary data 1). It represented more than 90% of the uronic acid content. At 1–2 DAF (Fig. 2 and Supplementary data 1), neutral sugar content was 34.4 μg/mg (±1.94) of rhamnose, 80.1 μg/mg (±3.42) of arabinose, 23.5 μg/mg of xylose (±1.05), 12.8 μg/mg of mannose (±0.02) and 50.70 μg/mg of galactose (±2.31). Very few fucose residues (4.6 ± 0.25 μg/mg) were identified in Arabidopsis siliques.

Major changes in neutral sugars were detected for almost all monosaccharides present in the cell wall during the silique development. In particular, while the amount of xylose residues mainly increased during the silique development, arabinose and galactose contents showed the opposite trend. In contrast, the rhamnose and fucose contents remained relatively constant throughout the silique development. The decrease in arabinose content from 3–4 DAF to 9–11 DAF (80.1 (±3.42) to 28.7 (±0.79) μg/mg) was followed by a slight increase during the late developmental stages and in mature seeds. The amount of galactose increased from 42.6 (±1.71) to 64.3 (±7.54) μg/mg during the first three development stages then decreased from 5 to 6 DAF stage (45.3 ± 1.86 μg/mg) onwards (21.70 ± 0.77 μg/mg at 18–20 DAF stage). In contrast, the amount of galactose was high in mature seeds (46.4 ± 2.26 μg/mg).

The amount of xylose remained at a steady-state level at the beginning of silique development (20.9 (±0.37)–23.5 (±1.05) μg/mg) and was followed by an increase to a level of 115.6 μg/mg (±1.20) at stages 7–8 DAF, when the elongation diminished. Its amount decreased during maturation phase (9–11 DAF) to the onset of dehiscence (18–20 DAF, 45.2 ± 0.06 μg/mg). Xylose and arabinose were still the most abundant neutral monosaccharides at the last stages of silique development. At 18–20 DAF, siliques are mainly devoid of seeds when harvested and the high level of arabinose is likely to reflect the pericarp content. Indeed, the amount of arabinose at the dehiscence stage (18–20 DAF) was 40.9 μg/mg (±1.05) versus 53.2 (±6.93) μg/mg in the mature seeds, suggesting a tissue specificity of cell wall composition. Similarly to what we observed, the percentage of arabinose and galactose decreased during early stages of green pod development (Stolle-Smits et al., 1999). However, in this study, the rhamnose content remained relatively constant in the pericarp, compared to our results where a slight increase was observed during the maturation stage (i.e. 9–11 DAF to 15–17 DAF). In Arabidopsis, that increase of rhamnose could thus be more related to an accumulation of rhamnose in the seeds, rather than in the pericarp. The seed mucilage, which consists of RGI rhamnose-rich polysaccharide (Macquet et al., 2007), could contribute to the overall changes in the pectic content in the silique.

A decrease in galactose and arabinose content, which are the two major neutral sugars in pectins, has also been reported in *A. thaliana* roots, stems, leaves and fruits during development (Voragen et al., 1983; Dawson et al., 1992; Redgwell et al., 1992; Usadel et al., 2004; Zandleven et al., 2007).

In apple, galactose and arabinose levels decreased drastically during different stages of development (Peña and Carpita, 2004). In other pulpy fruits, the analysis of sugars after sequential extraction on AIR showed a decrease in the amounts of arabinose and