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Original Article

Remodeling of pectin and hemicelluloses in tomato pericarp during fruit growth

Fabienne Guillon, Adeline Moïse, Bernard Quemener, Brigitte Bouchet, Marie-Françoise Devaux, Camille Alvarado, Marc Lahaye*

INRA, UR1268 Biopolymères, Interactions et Assemblages, BP 71627, F-44316 Nantes, France

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ABSTRACT

Tomato fruit texture depends on histology and cell wall architecture, both under genetic and developmental controls. If ripening related cell wall modifications have been well documented with regard to softening, little is known about cell wall construction during early fruit development. Identification of key events and their kinetics with regard to tissue architecture and cell wall development can provide new insights on early phases of texture elaboration.

In this study, changes in pectin and hemicellulose chemical characteristics and location were investigated in the pericarp tissue of tomato (*Solanum lycopersicon* var Levovil) at four stages of development (7, 14 and 21 day after anthesis (DPA) and mature green stages). Analysis of cell wall composition and polysaccharide structure revealed that both are continuously modified during fruit development. At early stages, the relative high rhamnose content in cell walls indicates a high synthesis of rhamnogalacturonan I next to homogalacturonan. Fine tuning of rhamnogalacturonan I side chains appears to occur from the cell expansion phase until prior to the mature green stage. Cell wall polysaccharide remodelling also concerns xyloglucans and (galacto)glucomannans, the major hemicelluloses in tomato cell walls. *In situ* localization of cell wall polysaccharides in pericarp tissue revealed non-ramified RG-I rich pectin and XyG at cellular junctions and in the middle lamella of young fruit. Blocks of non-methyl esterified homogalacturonan are detected as soon as 14 DPA in the mesocarp and remained restricted to cell corner and middle lamella whatever the stages. These results point to new questions about the role of pectin RGI and XyG in cell adhesion and its maintenance during cell expansion.

1. Introduction

Cell wall of fleshy fruits plays important biological functions and determines crop quality. This complex macromolecular assembly is mostly composed of cellulose, hemicelluloses and pectin with a minor amount of enzymes, structural proteins and phenolics [1]. It provides support and protection during seed development and its disassembly during fruit ripening facilitates seed dispersal. Control of the latter process represents a major issue with regard to fruit texture quality [2–4]. But its management is complex as it depends on a combination of several other factors at different scales regulated by genetics and developmental aspects [5].

Tomato pectin and hemicelluloses resemble those of dicots primary walls. Pectin comprises acidic polysaccharides made of variable proportions of three structural domains: homogalacturonan (HG), rhamnogalacturonan I (RG-I) and rhamnogalacturonan II (RG-II) [6]. HG is built on linearly linked 1,4-α-D-galacturonic acid while 1,2-α-L-rhamnose and 1,4-α-D-galacturonic acid alternate in the backbone of RG-I. RG-II is a HG substituted by complex side chains, some of which containing rhamnose. Further substitutions of HG by methyl and acetyl esters regulate the ability of pectins to form calcium mediated chain dimerization [7]. Both HG and RG-II domains are key features controlling cell-cell adhesion [8] and wall porosity [9,10]. RG-I can be covalently linked to hemicelluloses [11–13], partly esterified by acetic acid and are often ramified by long side chains predominantly made on 1,5-α-L-arabinose or 1,4-β-D-galactose or arabinogalactans that can interact with cellulose [14,15]. During tomato ripening, HG methyl esterification

Abbreviations: AIM, alcohol insoluble material; DM, degree of methylesterification; DPA, day post-anthesis; EndoPG, endo-polygalacturonase; GM, glucomannan; GgM, galactoglucomannan; HG, homogalacturonan; IgG, immunoglobulin G; PBS, phosphate buffered saline; PME, Pectin Methylesterase; RG-I, Rhamnogalacturonan I; RG-II, Rhamnogalacturonan II; XyG, Xyloglucan.

* Corresponding author.
E-mail address: marc.lahaye@inra.fr (M. Lahaye).

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decreases markedly and RG-I side chains are heavily metabolized as partial cell separation and cell wall swelling occur [2,16].

Like dicot primary wall, tomato hemicelluloses are mainly composed of xyloglucan (XyG) and lower amount or traces of galactoglucomannan (GgM) and glucuronoxylan [17,18]. Tomato, XyG side chains consist of α-(1 → 6)-D-xylose more or less extended by various sugars and short oligomers including galactose and arabinose residues. According to the letter code nomenclature introduced to designate XyG structures [19], individual 1 → 4 linked β-D-glucose residues are designated by the letter G while when branched by a α-D-xylosyl residue, they are refer to X. Tomato XyG extension of the branch include one β-D-galactosyl, that can be further extended by one α-L-arabinosyl residue or by the disaccharide β-L-arabinosyl (1 → 3) α-L-arabinoside linked on xylose. These structures are then referred to by the letters L, S, and T, respectively. Side chain distribution in tomato XyG leads to glucanase degradation products generally ending by two consecutive G units at the reducing end (XXGG-type) [20]. Tomato XyG can be further substituted by acetyl ester groups on unramified glucose residues, on terminal galactose and terminal arabinose residues in the side chains [21]. XyG structure is subject to genetically defined remodelling during ripening [22,23] in part due to xyloglucan endotransglycosylase/hydrolase activities [24] and likely to osidases hydrolysis [25,26] and/or transesterification [27]. XyG in plants is more or less accessible to exogeneous enzymes [28]. A small fraction intertwinded within cellulose fibrils is believed to be a major player in the control of the cell wall mechanical properties and cell expansion [29]. This small XyG fraction would be the target for the hydrogen bond breaking protein expansin, which plays a particularly key role in the ripening induced fruit softening [2].

Considering related solaneaceous species, *Nicotania tabacum* or *N. plumbaginifolia*, tomato GgM is thought to be made of alternating glucose and mannose residues with possible branching on mannose residues by α-D-galactopyranosyl or 2-*O*-β-D-galactopyranosyl-α-D-galactopyranosyl side-chains. Substitutions by small amounts of terminal arabinose or xylose residue are also possible and GgM can be partially acetyl esterified on glucose and galactose [30,31]. In tomato, GgM occurs as loosly and tightly interacting chains in the cell wall and is linked to glucuronoxylan [17]. They distribute in different populations of chains with regard to arabinose or xylose substitutions, some being nearly free of them [18]. The fine structure and/or accessibility of GgM to degrading enzymes is genetically defined and like XyG, are modified during ripening [22] likely due in part to mannan endotransglycosylase/hydrolase activities [32].

In contrast to studies focused on the chemical evolution of cell wall polysaccharides during ripening, little is known on their spatio-temporal deposition and modifications in the early developing tomato fruit. Thanks to the development of several cell wall polysaccharides specific antibodies, HG has been particularly located in middle lamella of tomato cell wall and its demethylesterification was shown to occur in patches on ripening [33]. RG-I was found restricted to the cell wall with peculiar galactan side chain concentrations close to the cell membrane, near pit connections [34]. Although widespread within the cell wall, the available antibodies to date located XyG at the cell surface and at the edge of adhesion planes of green tomato but not in the ripe fruit [35]. In the same study, GgM was located at the cell surface of tomato and shown to be involved in cell adhesion. XyG was also observed in the cell wall of very early developing tomato fruit (1 day pre-antesis, 3 and 5 days post-anthesis—DPA) without impact of the developmental stage on the intensity of the labeling [36].

In the present study, pectin and hemicellulose chemical characteristics and location were investigated in the expanding tomato (*Solanum lycopersicon* var Levovil) pericarp tissue between 7 DPA and mature green (MG) stages. In particular, the deposition and evolution of these polysaccharides during the late division cell division stages and the main fruit growth period revealed the presence of non-ramified RG-I rich pectin and XyG in cellular junction areas and in the middle lamella of young fruit that questions the role of these polysaccharides in cell adhesion.

2. Materials and methods

2.1. Material

2.1.1. Tomato fruits

The large fruited tomato line Levovil (S. lycopersicon Mill. referred to as LEV), was grown in CTIFL greenhouses (Centre Technique Interprofessionnel des Fruits et Légumes; 44000, Carquefou). Fruits were tagged from the day of anthesis.

2.1.2. Enzymes

AIM enzymatic degradation: 1,4-β-glucanase from *Trichoderma* sp., 1,4-β-galactanase, 1,5-α-arabinanase and 1,4-β-endopolygalacturonase from *Aspergillus niger* (EndoPG) were from Megazyme (Bray, Ireland). The pectin methylesterase (PME) was from *A. aculeatus* provided by Novozymes (Copenhagen, Denmark). Galactanase and PME pre-treatments of plant section prior to immunolabeling used the same enzymes as for AIM enzymatic degradation. The monocomponent preparation of *Aspergillus niger* endo-PGII was provided by Novozyme (Copenhagen, Denmark) and purified according to [37].

2.1.3. Antibodies

Rat monoclonal antibodies, LM20, LM19, LM5, LM6, LM21 were obtained from Dr J.P. Knox (Centre for Plant Science, School of Biochemistry and Molecular Biology, Leeds University, England). Mouse monoclonal antibodies 2F4 and CCRCM86 (M86) were by Pr P.Van Cutsem (Research unit in Plant Cell Biology, Faculté Universitaire Notre Dame de la Paix, Namur, Belgium) and Pr M. Hahn (CARBOSOURCE Services, Complex Carbohydrate Research, University of Georgia, Athens, USA), respectively. The mouse monoclonal antibody INRA RU1 (RU1) was produced in our laboratory. Specificities of these antibodies are summarized in Table 1.

2.1.4. Secondary antibodies

Goat anti-rat, anti-mouse and anti-rabbit-IgG conjugated with ALEXA Fluor 546 were obtained from Molecular Probe, Oregon (USA). Nanogold conjugates and silver enhancement kit were obtained from Aurion (NL).

2.2. Methods

2.2.1. Cell wall material

Three lots of two fruits were harvested at 3 stages of development (14, 21, 30–35: mature green (MG) days post-anthesis (DPA)). The un-peeled pericarp was isolated, immediately frozen in liquid nitrogen, freeze-dried, combined per lots of two fruits and crushed in powder prior to cell wall preparation. Cell walls were prepared as alcohol insoluble material (AIM) as described [38].

2.2.2. Chemical analyses

2.2.2.1. Sugar analyses. All measurements were performed in duplicate on dry samples. Neutral sugars were identified and quantified by gas–liquid chromatography (GC) on a DB 225 capillary column (J&W Scientific, Folsorn, CA, USA; temperature 205 °C, carrier gas H_2) after sulfuric acid degradation of AIM [39] and conversion of the monomers to alditol acetates [40]. Standard sugars solution and inositol as internal standard were used for calibration. Uronic acids in acid hydrolyzates were quantified using the metahydroxydiphenyl colorimetric method [41]. Starch glucose