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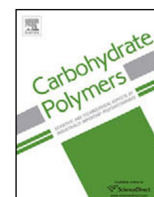

Profiling the main cell wall polysaccharides of grapevine leaves using high-throughput and fractionation methods

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ABSTRACT

Vitis species include *Vitis vinifera*, the domesticated grapevine, used for wine and grape agricultural production and considered the world's most important fruit crop. A cell wall preparation, isolated from fully expanded photosynthetically active leaves, was fractionated via chemical and enzymatic reagents; and the various extracts obtained were assayed using high-throughput cell wall profiling tools according to a previously optimized and validated workflow. The bulk of the homogalacturonan-rich pectin present was efficiently extracted using CDTA treatment, whereas over half of the grapevine leaf cell wall consisted of vascular veins, comprised of xylans and cellulose. The main hemicellulose component was found to be xyloglucan and an enzymatic oligosaccharide fingerprinting approach was used to analyze the grapevine leaf xyloglucan fraction. When *Paenibacillus* sp. xyloglucanase was applied the main subunits released were XXFG and XLFG; whereas the less-specific *Trichoderma reesei* EGII was also able to release the XXXG motif as well as other oligomers likely of mannan and xylan origin. This latter enzyme would thus be useful to screen for xyloglucan, xylan and mannan-linked cell wall alterations in laboratory and field grapevine populations. This methodology is well-suited for high-throughput cell wall profiling of grapevine mutant and transgenic plants for investigating the range of biological processes, specifically plant disease studies and plant–pathogen interactions, where the cell wall plays a crucial role.

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1. Introduction

Plant polysaccharides constitute some of the most abundant and structurally complex biopolymers found in the natural world (Albersheim, Darvill, Roberts, Sederoff, & Staehelin, 2011). Commonly found in the form of storage polymers (e.g. starch) and structural components (e.g. cell walls) they play a fundamental role in the growth and development of plants. Cell wall polysaccharides are of critical importance in a number of agriculturally and industrially relevant processes; e.g. fruit ripening, plant–pathogen interactions, substrates for biofuels (e.g. wood, coal and oil), paper and pulp industries, and are therefore of considerable economic interest (Albersheim et al., 2011). In relation to agriculture, significant research efforts have been focused on understanding how cell walls mediate the softening and texture properties of fruit during ripening (Albersheim et al., 2011; Cantu, Vicente, Labavitch, Bennett, & Powell, 2008a), and how these polysaccharide components are involved in the dynamic interplay between the host plant and fungal pathogen (e.g. necrotrophs such as *Botrytis cinerea*) in plant disease processes (Albersheim et al., 2011; Cantu et al., 2008b). Considering the importance of cell walls in agricultural research, it is surprising that relatively few crop species have benefited from the tools available to rapidly profile their wall polysaccharides (Nguema-Ona et al., 2012).

Although domesticated grapevine (*Vitis vinifera*) is considered the world's most important fruit crop (OIV, 2009), there is no comprehensive cell wall composition data for grapevine leaves. Weber, Hoesch, & Rast (1995) analyzed leaf-bound phenol components but not carbohydrates) while limited information is available on grape berries (summarized in Moore & Divol, 2011). As no information is available for leaves it is only possible to summarize what

Abbreviations: AIR, alcohol insoluble residue; CoMPP, comprehensive microarray polymer profiling; CBM, carbohydrate binding module; mAb, monoclonal antibody; EPG, endopolygalacturonase; ESI-MS, electrospray ionization-mass spectrometry; FT-IR, Fourier transform-infrared spectroscopy; HPAEC-PAD, high performance anion exchange chromatography-pulse amperometric detection; XEG, xyloglucan-specific endoglucanase; RG, rhamnogalacturonan; HG, homogalacturonan; XyG, xyloglucan; *Psp*XEG5, *Paenibacillus sp* xyloglucan specific endoglucanase 5; *Tr*EGII, *Tricshoderma reesei* endoglucanase II.

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is known regarding grape berries where ripening-related cell wall changes have been surveyed (Moore & Divol, 2011). Earlier studies focused on the changes associated with ripening berries (Davies & Robinson, 2000). Red grapes undergo a change from 'small, green and acidic' berries through a stage termed 'véraison' to 'large, red and sugary' ripe berries just prior to harvest in commercial wine vineyards (Davies & Robinson, 2000). Ripening did not alter the composition of the cellulose and hemicellulosic components (e.g. xyloglucan) to any detectable degree (Nunan, Sims, Bacic, Robinson, & Fincher 1998). However, the pectin components underwent significant alteration (Doco, Vuchot, Cheynier, & Moutounet, 2003; Nunan et al., 1998; Vidal, Williams, Doco, Moutounet, & Pellerin, 2003); particularly the arabinogalactan-II polysaccharides which decreased in abundance (Nunan et al., 1998). A shift in the solubility of the pectin components during ripening appeared to imply a subtle remodelling of the wall (Nunan et al., 1998). Arabinogalactan proteins (AGPs) and rhamnogalacturonan-II (RGII) components of the berry pectin matrix are commonly found in red wine after processing (Doco et al., 2003), whereas the other components are removed (e.g. as skins) or degraded by pectinases (see Arnous & Meyer, 2009 for data on polysaccharide turnover in wine). The importance of cell wall remodelling, biosynthesis and turnover in ripening berries has been further emphasized in recent years through transcriptomic studies (see Goulao, Fernandes, Lopes, & Amâncio, 2012). These studies have highlighted numerous cell wall genes (e.g. expansins, xyloglucanases, pectinases etc.) which correlate with distinct ripening phases (Lücker, Laszczak, Smith, & Lund, 2009; Pilati et al., 2007; Schlosser et al., 2008) indicating the critical contribution polysaccharide organization plays in grape berry development.

Ripening berries are subject to fungal diseases such as grey rot (*B. cinerea*) and mildew (e.g. *Uncinula necator* (powdery mildew) and *Plasmopara viticola* (downey mildew)) which cause significant crop damage worldwide with severe economic consequences for the international grape and wine industries (reviewed in Creasy & Creasy, 2009; Gomès & Coutos-Thévenot, 2009). These pathogenic fungi usually first infect leaves (with younger leaves more vulnerable) damaging vine health and reducing photosynthetic efficiency during the season (Creasy & Creasy, 2009). Thereafter the secondary spread of the fungi to berries occurs, resulting in fruit rot and reducing berry yield/quality. Most preventative control mechanisms are leaf-based, in order to control fungal inoculum sources and overwintering of the pathogen in the permanent structures of the vine. A necrotroph such as *B. cinerea* kills and destroys green tissue while enzymatically degrading the pectin network, whereas a biotroph, such as *Erysiphe necator*, feeds off the living tissue parasitically without causing significant cell death in susceptible hosts (Gomès & Coutos-Thévenot, 2009). Understanding how these fungal diseases progress, given the cell wall is the first barrier to infection and is in effect the battleground (Albersheim et al., 2011), is severely impaired by the lack of information on grapevine leaf cell wall structure and composition. Understanding disease resistance mechanisms, as well as processes relating to leaf ageing and senescence, is dependent on knowledge of the molecular structure and composition of cell walls (Albersheim et al., 2011), conspicuously absent in the scientific literature. Numerous tools are now available to rapidly profile cell wall polysaccharides, including gas liquid chromatography for the determination of cell wall monosaccharide composition (Reiter, Chapple, & Somerville, 1993; Reiter, Chapple, & Somerville, 1997); Fourier transformed infrared spectroscopy (FT-IR; Kacurakova, Capek, Sasinkova, Wellner, & Ebringerova, 2000); immunocytcochemistry (Hervé, Marcus, & Knox, 2011; Knox, 1997; Moller et al., 2007); and cell wall degrading enzymes combined with different analytical techniques (Lerouxel et al., 2002; Nguema-Ona et al., 2006; Persson, Sorensen, Moller, Willats, & Pauly, 2011).

This study builds on the profiling approach that was recently optimized and implemented to rapidly analyze the cell wall composition and structure of fully expanded mature tobacco leaves (Nguema-Ona et al., 2012, 2013). The same approach was used here to develop a baseline study of the cell wall polymers of grapevine leaves. A combination of high-throughput techniques were used; including monosaccharide compositional analysis, FT-IR spectroscopy, comprehensive microarray polymer profiling (CoMPP) analysis and oligosaccharide mass fingerprinting as well as more in-depth approaches using chemical and enzymatic fractionation methods were employed. This combination of tools provide a convenient and useful approach to profile the different wall polymers present in developing and mature grapevine leaves. The baseline datasets obtained, and tools evaluated, are essential for screening laboratory plants (for developmental and plant-pathogen studies) and field-grown grapevines (for plant disease monitoring during the season).

2. Experimental

2.1. Plant material

Samples were collected from the Department of Viticulture and Oenology's (Stellenbosch University) experimental vineyards at Welgevallen during October 2010; leaves were collected from a Shiraz vineyard (*V. vinifera* cv. Shiraz) at berry set. Fully expanded photosynthetically active leaves were harvested (samples were taken from five vines and two primary shoots per vine) and flash-frozen using liquid nitrogen and stored at −80 °C until further use. For statistical purposes four biological samples (i.e. leaves) per analyses were utilized with two technical repeats per sample.

2.2. Cell wall isolation and fractionation

Cell wall materials were extracted from frozen leaves and fractionated (using chemical reagents and enzymes) according to the protocol used in Nguema-Ona et al. (2012). Briefly, frozen leaves were ground, under liquid nitrogen using a mortar and pestle, to a fine powder. After successive washes in ethanol, methanol, chloroform the material was de-starched. The fractionation process was performed as described in Nguema-Ona et al. (2012).

2.3. Monosaccharide composition analysis by gas chromatography

A gas liquid chromatography method (York, Darvill, McNeil, Stevenson, & Albersheim, 1985) was used to determine the monosaccharide content of cell wall residues and fractions as described in Nguema-Ona et al. (2012). After hydrolysis (2 M TFA, 110 °C, 2 h), the liberated monosaccharides converted to methoxy sugars using 1 M methanolic HCl at 80 °C for 24 h. Silylation was performed at 80 °C (20 min) to produce trimethyl-silyl-glycosides which were dissolved in cyclohexane. The derivatives were separated and analyzed in a gas chromatograph (Hewlett Packard 5890 series II) coupled to a flame ionization detector, Error bars in the histograms represent the standard deviation (SD) of the mean of four biological samples with two technical replicates per biological sample.

2.4. High performance anion exchange chromatography

Enzyme-generated oligosaccharides were analyzed by high performance anion exchange (HPAE) chromatography Dionex Ultimate 3000 equipped with a CarboPac PA-1 column combined with pulsed amperometric detection (PAD; Coulochem 111 detector). This is as was performed in Nguema-Ona et al. (2012)

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