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Structural characterization of cell wall polysaccharides from two plant species endemic to central Africa, *Fleurya aestuans* and *Phragmenthera capitata*

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ABSTRACT

Fleurya aestuans (Linnaeus) Miquel and *Phragmenthera capitata* (Spreng) are two plants endemic to central Africa that are used in traditional medicine. However, information on their molecular constituents is lacking. In the present study and as part of our research on the structure/bioactivity relationship of plant cell wall molecules, we investigated the structure of polysaccharides isolated from leaf cell walls of both plant species. To this end, we used sequential extraction of polysaccharides, gas chromatography, matrix assisted laser desorption ionisation-time of flight mass spectrometry (MALDI-TOF MS) and immuno-dot assays. Our data indicate the presence of both pectin and hemicellulosic polysaccharides in the cell walls of both plants. In particular, cell wall of *F. aestuans* leaves appears to contain much more pectin than those of *P. capitata*. Structural analysis of hemicellulosic polysaccharides revealed differences in the structure of xyloglucan isolated from both species. While only the XXXG-type was found in *P. capitata*, both XXXG and XXGG types were detected in *F. aestuans*. No arabinosylated subunits were found in any of the xyloglucan isolated from both plant species. In addition, xylan structure with non methylated-α-D-glucuronic acid on side chains was only detected in *F. aestuans* leaf cell walls. Finally, structural analysis of rhamnogalacturonan-I (RG-I) and rhamnogalacturonan-II (RG-II) shows that unlike RG-II, RG-I is qualitatively different between *F. aestuans* and *P. capitata* leaves.

1. Introduction

Fleurya aestuans (Linnaeus) Miquel and *Phragmenthera capitata* (Spreng) are two Gabonese medicinal plants. *Fleurya aestuans*, an herb that grows abounding in Ogooue basin, is used as antipyretics, antirheumatics and diuretics and for wound-healing (Walker & Sillans, 1961). *Phragmenthera capitata* (Loranthaceae) is a parasite shrub which invades *Pentaclethra macrophylla*. It is endemic to Central Africa. The *P. capitata* leaves are also used in traditional medicine for wound-healing and headache relief (Walker & Sillans, 1961). Despite the importance of *F. aestuans* and *P. capitata* in traditional medicine, no information is available on the compounds or metabolites involved in their pharmacological activities. Indeed, a very few studies have been devoted to these plants and none of them has investigated the structure of their cell wall polysaccharides. Examination of the composition of *F. aestuans* leaves showed that they contain mostly fat, fiber, carbohydrates, tannins and oxalate and low amount of proteins (Akpan & Udoh, 2004). Furthermore, introduction of *F. aestuans* leaves during cocoyam corm cooking resulted on a marked decrease of anti-nutrients and an increase in phosphorus content (Akpan & Udoh, 2004).

Plant cell walls vary greatly in their composition and physical properties depending on the cell type and plant species (Harris & Smith, 2006). Nevertheless, all plant cell walls have a similar basic structure. They consist of a fibrillar phase made up of cellulose microfibrils set in a matrix of non-cellulosic polysaccharides including pectins and hemicelluloses, and glycoproteins (Lerouxel, Cavalier, Liepman, & Keegstra, 2006). Pectic polysaccharides consist of three major classes namely, homogalacturonan, rhamnogalacturonan-I and rhamnogalacturonan-II (Ridley, O'Neill, & Mohnen, 2001; Scheller, Jensen, Sorensen, Harholt, & Geshi, 2007). The principle hemicellulosic polysaccharides are xyloglucan (XyG) and xylan (Lerouxel et al., 2006). While XyG is mainly found in the primary cell wall of dicotyledonous plants, xylan is a major component of secondary walls (McCartney, Marcus, & Knox, 2005).

In traditional medicine, extracts of polysaccharide-containing plants are widely used for skin treatment, epithelium wounds and mucous membrane irritation (Bedi & Shenefelt, 2002). Moreover, several studies have indicated that polysaccharides from many plants are responsible for wound-healing (Diallo, Berit, Torun, & Terje, 2003).

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Some of these polysaccharides have an influence on the immune system and are often called immunomodulators when the complement system is involved (Diallo, Berit, Torun, & Terje, 2001; Ebringerová, Kardošová, Hromádková, Malovíková, & Hříbalová, 2002; Yamada, 1996). To date, a limited number of studies have been carried out on the structure and function of polysaccharides isolated from African traditional medicine plants (Diallo et al., 2001; Inngjerdingen et al., 2005; Nergard et al., 2004). Thus, identification, characterization and evaluation of new polysaccharides from African medicinal plants, as new safe compounds for medicine, have become a challenge. It is essential to gain detailed information on the chemical structure of such molecules prior to any investigation of their biological activities.

In this study, we report on the structural characterization of cell wall polysaccharides including XyG, xylan, RG-I and RG-II isolated from leaves of *F. aestuans* and *P. capitata*. Our findings indicate that the structure of XyG, xylan and RG-I is qualitatively different between the two plant species.

2. Experimental

2.1. Plant materials

Fleurya aestuans and *P. capitata* leaves were collected in the forest near Libreville (Gabon, Central Africa). The identification of the voucher specimens of *F. aestuans* and *P. capitata* was confirmed by the National Herbarium of Gabon. Leaves were dried and ground in the *Institut de Pharmacopée et de médecine traditionnelle* (IPHAMETRA) in Libreville, then used to extract cell wall material and characterize cell wall polysaccharides at the University of Rouen.

2.2. Cell wall extraction, isolation of hemicellulosic fractions and enzymatic digestion with endo-glucanase and xylanase

Dry *F. aestuans* and *P. capitata* leaves (8 g) were ground to a fine powder (under liquid N_2) and resuspended in 100 mL of boiling 80% ethanol (v/v) for 30 min. The pellet was extracted overnight at room temperature with 100 mL of 90% DMSO first, then 24 h with MeOH–$CHCl_3$ (1:1; v/v), 24 h with MeOH–acetone (1:1; v/v), and finally with acetone–water (4:1; v/v). The residue was air dried at 80 °C and the cell wall material (CWM) obtained was resuspended in 50 mM acetate, pH 5.4, and destarched at 80 °C using a thermostable α-amylase and amyloglucosidase (EC3.2.1.1; Megazyme International). To extract polysaccharide fractions, CWM (1 g) was extracted twice with boiled ammonium oxalate at 0.5% for 1 h. This fraction was designated as a pectic fraction (PF). The residue was then incubated in 4 M KOH overnight at room temperature as described by Ray, Loutier-Bourhis, Condamine, Driouich, and Lerouge (2004) to extract hemicelluloses. The 4 M KOH hemicellulosic fraction was separated into a soluble (SF) and an insoluble fraction (InF). Alkaline extracts were acidified to pH 5 with acetic acid prior to dialysis. XyG and xylan oligomers were generated from 4 M KOH hemicellulosic fractions after enzymatic digestion with endo-β-D-(1,4)-glucanase (Megazyme International Ireland, EC 3.2.1.4), or with endo-β-(1,4)-xylanase (Megazyme International Ireland, xylanase M6) as described previously (Lerouxel et al., 2002; Ray et al., 2004).

2.3. Immuno-dot assays

Hemicellulosic and pectic fractions from *Fleurya* and *Phragmenthera* leaves were analyzed by immuno-dot assays (Willats, McCartney, Mackie, & Knox, 2001). One to 5 μL aliquots of extracts were applied to nitrocellulose membranes. The membranes were then air-dried at room temperature and blocked in TBS buffer (20 mM Tris–HCl, 500 mM NaCl, pH 7.5) containing 5% (w/v) no-fat-dried milk (TBS-M) for 1 h prior to the incubation with the primary antibody [anti-pectins, LM5 and JIM7 (Willats et al., 2001); anti-xyloglucan, CCRCM1 and CCRCM86 (Hahn, 2007; Puhlmann et al., 1994); and anti-xylan LM10 (McCartney et al., 2005)] diluted 1/10 in TBS-M for 1.5 h. After incubation with the primary antibody, membranes were washed extensively in TBS containing 1% (v/v) Tween 20 prior to incubation for 1.5 h with the secondary antibody diluted 1/50 in TBS-M. For the LM5, JIM7, anti-xylan epitopes detection, the secondary antibody was anti-rat horseradish peroxidase conjugate (Sigma) while for detection of anti-xyloglucan binding, the secondary antibody was anti-mouse horseradish peroxidase conjugate (Sigma). Membranes were revealed in 50 mL TBS, 10 mL methanol containing 30 mg 4-chloro-1-naphthol and 30 μL 30% H_2O_2.

2.4. Monosaccharide composition analysis

Monosaccharide composition was performed according to Ray et al. (2004) analyzed after trifluoroacetic acid hydrolysis of the fractions. The generated methyl glycosides were converted into their TMS derivatives and separated by gas chromatography using inositol as an internal standard (Fitchette, Dinh, Faye, & Bardor, 2007).

2.5. Digestion and isolation of RG-I and RG-II

To investigate the structure of the pectic polysaccharides RG-I and RG-II, the CWR (200 mg) was treated with endo-polygalacturonase (EPG) as described previously in Ishii, Matsunaga, and Hayashi (2001). RG-I and RG-II polysaccharides were purified from the EPG-solubilized material by elution from size-exclusion chromatography (SEC) on a Superdex 75 prep grad and a column (Amersham Pharmacia Biotech 1.6 × 35 cm). SEC/RI were performed with a Shodex RI-71 system with a refractive index detector connected to a Superdex-75 column (Amersham Pharmacia Biotech) eluted at 1 mL min^{-1} with 50 mM ammonium formate, pH 5.3. The fractions of RG-I and RG-II were collected by a collector (Pharmacia Biotech).

2.6. MALDI TOF mass spectrometry

Mass spectra of the xyloglucan and xylan fragments obtained by endo-glucanase and endo-xylanase were acquired on a Voyager DE-Pro Matrix Assisted Laser Desorption Ionization-Time of Flight (MALDI-TOF) instrument (Applied Biosystems, USA) equipped with a 337-nm nitrogen laser. Mass spectra were performed in the reflector mode, delayed extraction mode using 2.5-dihydroxybenzoic acid (Sigma-Aldrich) as matrix. The acquisition and external calibration were made according to Nguema-Ona et al. (2006). Xyloglucan-derived oligosaccharides are named according to Fry et al. (1993).

3. Results

3.1. Monosaccharide composition of cell wall, pectic and hemicellulosic fractions

Table 1 shows the monosaccharide composition of cell wall material (CWM) isolated from leaves of *F. aestuans* and *P. capitata*. The three major sugars in cell wall of *F. aestuans* are Glc, Gal and GalA accounting for about 60% of the total sugars, whereas in *P. capitata* cell wall, the main sugars are Glc, Xyl and Ara. This data suggests the presence of both pectin and hemicellulosic polysaccharides in the cell walls of both plants. In *P. capitata*, the cell walls seem to contain more hemicellulosic polysaccharides than pectins.

Table 1
Monosaccharide composition (μg/mg of dry material) of the cell wall fractions isolated from the leaves of *F. aestuans* (*F.a*) and of *P. capitata* (*P.c*)

Glycosyl residues	CWM		SF		InF		PF		Residue after KOH 4 N extraction	
	F.a	*P.c*	*F.a*	*P.c*	*F.a*	*P.c*	*F.a*	*P.c*	*F.a*	*P.c*
Yield (w/w)	66.3	56.3	10	11	3	6	41	15	36	58
Ara	2.9 ± 0.2	7.5 ± 0.3	1.8 ± 0.5	4.2 ± 0.2	2.0 ± 0.2	4.0 ± 1.8	1.0 ± 0.1	6.1 ± 0.4	0.4 ± 0.4	0.9 ± 0.3
Rha	3.0 ± 0.2	1.1 ± 0.01	0.7 ± 0.1	1.6 ± 0.0	0.3 ± 0.05	0.6 ± 0.3	3.2 ± 0.2	1.7 ± 0.1	0.4 ± 0.4	0.5 ± 0.05
Fuc	0.6 ± 0.03	0.5 ± 0.01	0.7 ± 0.1	1.5 ± 0.1	0.1 ± 0.02	0.1 ± 0.1	0.3 ± 0.0	0.4 ± 0.1	0.5 ± 0.5	0.1 ± 0.05
Xyl	3.1 ± 0.4	6.9 ± 0.0	11.8 ± 1.9	19.1 ± 1.2	0.8 ± 0.1	2.4 ± 0.5	0.4 ± 0.0	0.8 ± 0.04	0.3 ± 0.2	0.5 ± 0.05
GlcA	0.8 ± 0.1	0.2 ± 0.1	0.6 ± 0.1	1.5 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.3 ± 0.0	0.3 ± 0.04	0.3 ± 0.1	0.2 ± 0.6
GalA	5.9 ± 0.8	2.1 ± 0.1	0.6 ± 0.1	0.1 ± 0.0	0.3 ± 0.03	0.2 ± 0.0	6.5 ± 0.2	6.4 ± 1.2	0.2 ± 0.2	0.1 ± 0.01
Man	0.4 ± 0.0	0.3 ± 0.04	0.0 ± 0.0	0.1 ± 0.01	0.2 ± 0.02	0.1 ± 0.1	0.1 ± 0.01	0.3 ± 0.03	0.3 ± 0.05	0.0 ± 0.0
Gal	3.9 ± 0.4	0.6 ± 0.0	1.0 ± 0.7	0.5 ± 0.1	0.7 ± 0.04	1.0 ± 0.1	0.4 ± 0.0	0.6 ± 0.1	0.8 ± 0.8	0.5 ± 0.20
Glc	4.8 ± 0.3	6.0 ± 1.0	13.4 ± 0.7	27.7 ± 1.1	7.3 ± 0.4	9.9 ± 1.4	1.5 ± 0.1	8.0 ± 0.1	16.5 ± 1.3	16.6 ± 2.5

CWM, cell wall material; PF, pectic fraction; SF, hemicellulosic soluble fraction; InF, hemicellulosic insoluble fraction. The isolation procedure of each fraction is described in Section 2.

The cell wall materiel from the leaves of both plants was fractionated in order to obtain two hemicellulosic fractions (soluble and insoluble) and a pectic fraction. Hemicellulosic soluble fraction (SF) from both plants contains mainly Glc and Xyl (Table 1). It is interesting to note that the content of Xyl in the SF from *P. capitata* leaves is higher than the one found in the SF from *F. aestuans*. In contrast, the content of Glc in the same fraction is twice lower in *P. capitata* than in *F. aestuans* SF. Such a composition suggests that the SF fraction is enriched in XyG and xylan. The hemicellulose insoluble fraction (InF) contains high levels of Glc in both species, although appreciable amounts of Gal, Xyl and Ara are also found in *P. capitata*. The pectic fraction (PF) isolated from the cell wall of *F. aestuans* is enriched in GalA (47%) and Rha (23%) indicative of the occurrence of the polysaccharide RG-I (Table 1). In contrast, the same fraction isolated from the cell wall of *P. capitata* leaves contains predominantly GalA (45%) suggesting that homogalacturonan is abundant (Table 1). The residue after KOH 4 N extraction seems to be similar in two plants.

3.2. Immuno-dot assays using anti-polysaccharide antibodies

To investigate the presence of particular carbohydrate epitopes indicative of the occurrence of certain type of cell wall polysaccharides, we used immuno-dot assays on different cell wall fractions isolated from *F. aestuans* and *P. capitata* leaves with antibodies recognizing specific carbohydrate epitopes associated with plant cell wall polysaccharides (Hahn, 2007; McCartney et al., 2005; Puhlmann et al., 1994; Willats et al., 2001). The data show that the antibodies directed against hemicellulosic epitopes including CCRCM1, CCRCM86 and anti-xylan LM10 reacted quite strongly with the hemicellulosic soluble fraction but not with the pectic fraction in both plants (Table 2). This is consistent with the presence of xylan and XyG in the soluble hemicellulosic fraction. Conversely, the antibodies JIM7 and LM5, both directed against pectin epitopes, labelled intensely the pectic fraction (Table 2) but not the hemicellulosic fraction indicating the presence of homogalacturonan and rhamnogalacturonan-I. To extend on these observations, we performed detailed structural analysis of the same fractions (see below).

3.3. Structural analysis of xyloglucan

To determine the structure of XyG in the hemicellulosic soluble fraction, we employed the enzymatic oligosaccharide fingerprinting technique (Lerouxel et al., 2002) using the enzyme endo-β-D-(1,4)-glucanase that cleaves β-(1,4)-glucosidic linkages of XyG backbone next to an unbranched glucose residue. The resulting fragments were then analyzed by MALDI-TOF MS. Such an analysis revealed differences in the structure of XyG-derived oligosaccharides between both species (Fig. 1). The XyG-rich fraction of *F. aestuans* showed the presence of eight major $[M+Na]^+$ ions at, respectively, m/z = 953, 1085, 1115, 1247, 1277, 1393, 1409 and 1555. The dominant XyG fragments in this fraction is XXFG (ion at m/z = 1393), followed by four equally distributed fragments namely XXGG, XXXG, XXLG/XLXG and XLFG/XFLG (Fig. 1A and Table 3) corresponding to both non-fucosylated and fucosylated types of XyG. Analysis of XyG-derived oligosaccharides from *P. capitata* cell walls revealed two highly represented oligosaccharides namely XXXG (ion at m/z = 1085) and XLFG/XFLG (ion at m/z = 1555). It is worth noting that more fucosylated XyG oligosaccharides are found in the cell walls of *P. capitata* than in the cell walls of *F. aestuans*, although these are structurally different. In addition, the following oligosaccharides GXXGG and/or XLGG and LLGG and/or GXLGG were detected in *F. aestuans* but not in *P. capitata*. Structures of these oligosaccharides were determined and proposed based on the mass of generated ions and usual XyG subunits nomenclature described by Fry et al. (1993) (Table 3). Other fragments were assigned to xylan or mannan-derived oligosaccharides resulting from the hydrolysis of hemicellulosic fraction by contaminating endo-glycanase activities.

3.4. Structural analysis of xylan

To gain information on the structure of xylan present in the hemicellulosic fraction, we used endo-β-(1,4)-xylanase treatment and analyzed the generated fragments by MALDI-TOF MS as previously described (Ray et al., 2004). As shown in Fig. 2, three major oligosaccharide fragments were detected in *F. aestuans* cell walls. On the basis of the molecular weight, the main $[M+Na]^+$ ion at m/z = 759 was assigned to Xyl_4-4-*O*-methyl-α-D-glucuronic acid whereas ions at m/z = 891 and 1023 were assigned to Xyl_5-4-*O*-methyl-α-D-glucuronic acid and Xyl_6-4-*O*-methyl-α-D-glucu-

Table 2
Immuno-dot-blot of the binding of anti-polysaccharide antibodies to different cell wall fractions isolated from the leaves of *F. aestuans* (*F.a*) and of *P. capitata* (*F.c*)

Antibody	SF		PF		Epitopes	References
	F.a	*P.c*	*F.a*	*P.c*		
LM5	–	–	+ +	+ +	(1, 4)-β-D Galactan	Willats et al. (2001)
JIM7	–	–	+ ++	+ ++	HG (+/– esterified)	Willats et al. (2001)
CCRCM86	+++	+++	–	–	(1, 4)-β-D Glucan	Hahn (2007)
Anti-xylan (LM10)	++	++	–	–	(1,4)-β-D-Xylose	McCartney et al. (2005)
CCRCM1	++	++	–	–	α-Fuc-(1,2)-β-gal	Puhlmann et al. (1994)

SF, hemicellulosic soluble fraction; PF, pectic fraction; the + sign indicates the intensity of the reaction. The – sign indicates no reaction.

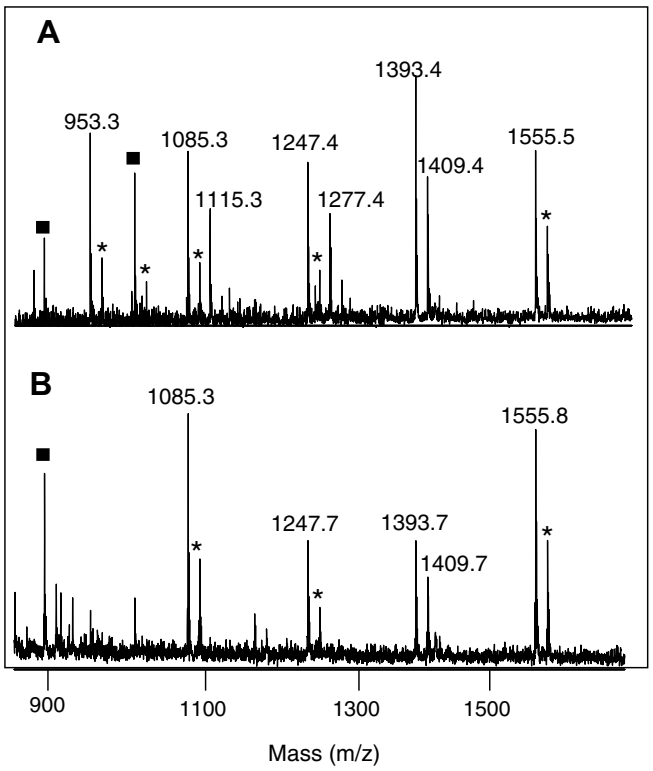

Fig. 1. MALDI-TOF mass spectra of endoglucanase-generated xyloglucan oligosaccharides from the cell wall of *Fleurya aestuans* (A) and *Phragmenthera capitata* leaves (B). *: $[M+K]^+$ ions. ■: contaminating oligosaccharide fragments.

ronic acid, respectively. Ions at $m/z = 745$ and 1009 correspond to fragments composed of non-methylesterified α-D-glucuronic acid units linked to four and six Xyl residues (Fig. 2A). In *P. capitata* cell walls, xylan fragments were identified as exclusively bearing 4-*O*-methyl-α-D-glucuronic acid (Fig. 2B). Thus, unlike in *F. aestuans* xylan, non methylated α-D-glucuronic acid residues do not occur in the xylan structure of *P. capitata* cell walls.

3.5. Structural analysis of RG-I and RG-II

Pectic polysaccharides were extracted from the cell wall residues using endo-polygalacturonase treatment (Ishii et al., 2001). The purified endo-polygalacturonase-soluble fraction was then fractionated into RG-I and RG-II polysaccharides by size-exclusion chromatography (Ishii et al., 2001). The chromatograms shown in Fig. 3 indicate that RG-I is the main polysaccharide present and that RG-II occurs mostly, if not exclusively, under its dimer form. In addition, it shows that the content of RG-I from *F. aestuans* cell wall is much higher than that of RG-I from *P. capitata* cell wall. The level of RG-II seems also to be slightly higher in *F. aestuans* than in *P. capitata* cell walls. Analysis of the monosaccharide composition of RG-I revealed significant differences in the content of major sugars composing this polysaccharide (Table 4). Rha and GalA residues constitutive of the RG-I backbone represent 46% of total sugars of this polysaccharide in *F. aestuans*, while they represent 19% only in *P. capitata* RG-I. Ara and Gal residues, two major sugars of the side chains of RG-I (McNeil, Darvill, & Albersheim, 1982), represent 32% and 46% of total sugars of RG-I from *F. aestuans* and *P. capitata*, respectively. Together these data indicate that the length of RG-I backbone is possibly higher in *F. aestuans* than in *P. capitata* cell walls, whereas Ara/Gal-containing side chains of this polysaccharide are longer or more frequent in *P. capitata* than in *F. aestuans* cell walls. In contrast to RG-I, the content of different sugars of the polysaccharide RG-II is similar in both plants (Table 4).

4. Discussion

The goal of the present study was to characterize cell wall matrix polysaccharides of two plants endemic to central Africa that are used in traditional medicine. We found that hemicellulosic and pectic polysaccharides are present in the cell walls of both species. However, it appears that pectin is more abundantly present in *F. aestuans* cell walls than in *P. capitata* cell walls. Also different polysaccharide classes, including XyG, xylan, HG and rhamnogalacturonans are all detected. XyG is the main hemicellulosic polysaccharide in cell walls in the primary cell wall of most vascular plants and has a diversity of structures. It is classified based upon the types of oligosaccharides released after hydrolysis by endo-glucanase (Vincken, York, Beldman, & Voragen, 1997). Structural analysis of this polysaccharide in the present study revealed differences in its structure between the two plant species examined. *Phragmenthera capitata* cell walls have a XXXG-type of XyG that is predominantly found in a wide range of dicotyledonous and monocotyledonous species (Hoffman et al., 2005; Vincken et al., 1997). However, the dominant fucosylated side chains subunit is rather XLFG/XFLG than XXFG as usually found. The structure of xyloglucan subunits of *F. aestuans* suggests the presence of two types of this polysaccharide. A XXXG-type in which side chains are substituted with Xyl-Gal-Fuc residues and a XXGG type whose side chains contain terminal Gal residues such as in tobacco and tomato cell walls (Hayashi, 1989; Vincken et al., 1997; York, Kumar Kolli, Orlando, Albersheim, & Darvill,1996). Such an atypical XXGG-type of branching pattern is not common but has also been previously found in the cell walls of tobacco leaves and tomato suspension cultures, two solanaceous species (Hoffman et al., 2005; Jia, Qin, Darvill, & York, 2003; York et al., 1996). Nevertheless, unlike in tobacco and tomato, *F. aestuans* XyG does not seem to contain arabinosylated subunits. It is also interesting to note that XyG galactosylation is much higher in *F. aestuans* than in *P. capitata* species which is probably associated with an important activity of galactosyltransferases involved in XyG biosynthesis within leaf cells. The functional significance of the presence of substantial

Table 3
Relative quantification of the xyloglucan type oligosaccharides prepared from the hemicellulosic soluble fraction isolated from the cell wall of *F. aestuans* (*F.a*) and *P. capitata* (*P.c*) leaves

m/z	Assignment	Compound	Relative intensities in *F.a*	Relative intensities in *P.c*
953	$[M+Na]^+$	XXGG and/or XLG	14.5	5.3
1085	$[M+Na]^+$	XXXG	13.1	29.4
1115	$[M+Na]^+$	GXXGG and/or XLGG, LXGG	8.7	–
1247	$[M+Na]^+$	XXLG and/or XLXG	12.3	13.9
1277	$[M+Na]^+$	LLGG and/or GXLGG	8.2	–
1393	$[M+Na]^+$	XXFG	19	13.9
1409	$[M+Na]^+$	XLLG	11.1	9.5
1555	$[M+Na]^+$	XLFG or XFLG	13.1	27.9
			Total = 100	Total = 100

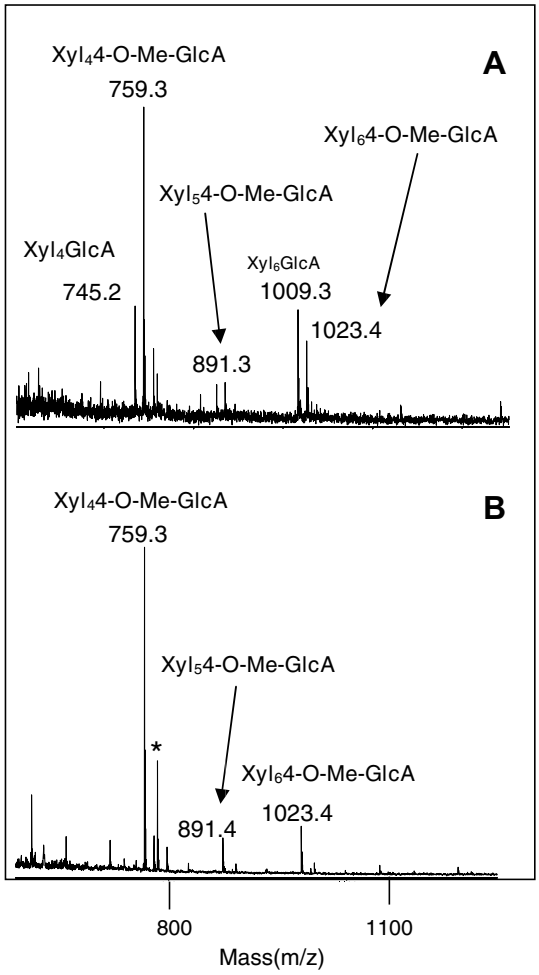

Fig. 2. MALDI-TOF mass spectra of endoxylanase-generated xylan fragments from the cell wall of *Fleurya aestuans* (A) and *Phragmenthera capitata* leaves (B). ∗: $[M+2Na-H]^+$ ions.

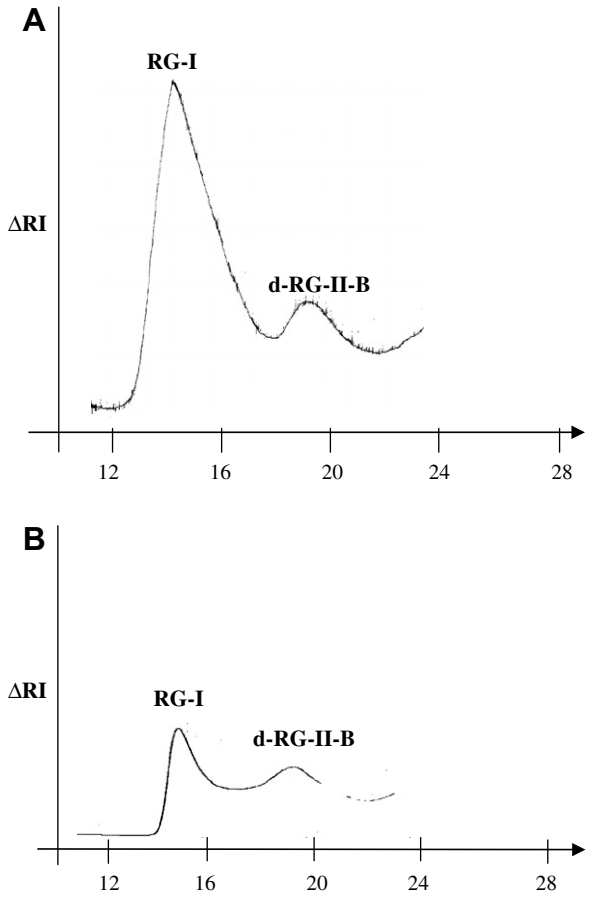

Fig. 3. Size exclusion chromatography profile of the material released after EPG treatment of treated cell walls of *Fleurya aestuans* and *Phragmenthera capitata* leaves. d-RG-II-B: Borate ester-dimerized RG-II.

Table 4
Monosaccharide composition (mol%) of RG-I and RG-II isolated from *F. aestuans* (*F.a*) and *P. capitata* (*P.c*) leaves

Glycosyl residues	RG-I		RG-II	
	F.a	*P.c*	*F.a*	*P.c*
2-O-Me-Xyl	–	–	1	tr
Kdo	–	–	3	2
Ara	13	22	19	21
Rha	24	10	14	16
Fuc	tr	tr	tr	2
Xyl	4	6	16	17
Glc A	7	2	1	1
Gal A	22	9	9	9
Man	7	2	4	4
Gal	19	36	20	18
Glc	4	13	13	10

tr, Trace.

amounts of Gal in XyG from *F. aestuans* is unknown, but this may possibly impact the functional properties of the cell wall in relation to cell development. In this context, studies on two Arabidopsis mutants including *murus 3* and *root epidermal bulger 1* have pointed to a link between XyG galactosylation and the regulation of cell expansion (Madson et al., 2003; Nguema-ona et al., 2006).

In addition to XyG, xylan is also present in the cell walls of both plant species. Our data suggest that this polysaccharide is composed of a linear backbone of β-(1,4)-linked D-xylosyl residues with side chains containing 4-*O*-methyl-α-D-glucuronic acid and non-methylated-α-D-glucuronic acid in *F. aestuans*. In *P. capitata* only methylated-α-D-glucuronic acid was found associated with xylan. It is known that xylose residues can also be substituted with arabinosyl residues in some species (Ebringerová & Heinze, 2000). No such residues were detected in xylan isolated from the cell walls of both African species. Thus, xylan from *P. capitata* and *F. aestuans* is typically a (methyl)-glucuronoxylan that is mainly present in dicot wood (Ebringerová & Heinze, 2000).

It is worth mentioning that although no arabinosylated structures were found in xylan and xyloglucan, arabinose residues were present in the SF and InF fraction (see Table 1). This is most likely due to contamination by arabinogalactan-proteins as both fractions reacted, although weakly, with beta-glucosyl Yariv reagent known to bind such proteoglycans (data not shown).

Structural analysis of pectin-containing fraction suggests that HG and RG-I are present in the cell walls of both species and that *F. aestuans* cell walls are richer in RG-I than *P. capitata* cell walls. This is confirmed by subsequent isolation and sugar analysis of RG-I from both species. Comparison of the content of GalA and Rha residues suggests that RG-I backbone is longer in *F. aestuans* than in *P. capitata* cell walls. Also, proportion of Ara and Gal, suggests that RG-I in *P. capitata* is more substituted than RG-I isolated from *F. aestuans* cell walls. The Ara/Gal-containing side chains of *P. capitata* RG-I might be either high in length or more frequent but shorter. It is known that 20–80% of the rhamnosyl residues in RG-I are substituted depending on the plant source (O'Neill et al.,

1996). Thus, it is likely that the proportion of substituted rhamnosyl residues of RG-I isolated from the two species is different, but the number of such rhamnosyl residues remains to be determined for both species. Unlike RG-I, the structure of RG-II isolated from both African plants seems to be similar. This polysaccharide is predominantly present under its dimer form as found in all higher plant walls analyzed to date (Ridley, O'Neill, & Mohnen, 2001). However, the content of GalA, the principal sugar in RG-II is very low (9%) in the two species as compared to other higher plants including *Arabidopsis thaliana* (44%), sycamore (31%), pea (51%) and grape (37%) (O'Neill et al., 1996; Zablackis, Huang, Müllerz, Darvill, & Albersheim, 1995).

To summarize, the study presents detailed structural characterization of various cell wall polysaccharides from *F. aestuans* and *P. capitata* and introduce these species as valuable sources for galactosylated XyG, acidic (methyl)glucuronoxylan, as well as RG-I. It is known that certain cell wall polysaccharides of hemicellulosic and/or pectin nature has antibacterial, antiviral, antitumor and immune activities (Aydin et al., 2006; Yamada, 1996). Whether cell wall-derived polysaccharides and/or oligosaccharides of *F. aestuans* and *P. capitata* play a role in their pharmacological activities is not known as yet. Studies are currently conducted to investigate these possibilities.

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