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Glucuronoarabinoxylan structure in the walls of *Aechmea* leaf chlorenchyma cells is related to wall strength

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ABSTRACT

In CAM-plants rising levels of malic acid in the early morning cause elevated turgor pressures in leaf chlorenchyma cells. Under specific conditions this process is lethal for sensitive plants resulting in chlorenchyma cell burst while other species can cope with these high pressures and do not show cell burst under comparable conditions. The non-cellulosic polysaccharide composition of chlorenchyma cell walls was investigated and compared in three cultivars of *Aechmea* with high sensitivity for chlorenchyma cell burst and three cultivars with low sensitivity. Chlorenchyma layers were cut from the leaf and the non-cellulosic carbohydrate fraction of the cell wall fraction was analyzed by gas–liquid chromatography. Glucuronoarabinoxylans (GAXs) were the major non-cellulosic polysaccharides in *Aechmea*. The fine structure of these GAXs was strongly related to chlorenchyma wall strength. Chlorenchyma cell walls from cultivars with low sensitivity to cell burst were characterized by an A/X ratio of ca. 0.13 while those from cultivars with high sensitivity showed an A/X ratio of ca. 0.23. Xylose chains from cultivars with high cell burst sensitivity were ca. 40% more substituted with arabinose compared to cultivars with low sensitivity for cell burst. The results indicate a relationship *in vivo* between glucuronoarabinoxylan fine structure and chlorenchyma cell wall strength in *Aechmea*. The evidence obtained supports the hypothesis that GAXs with low degrees of substitution cross-link cellulose microfibrils, while GAXs with high degrees of substitution do not. A lower degree of arabinose substitution on the xylose backbone implies stronger cell walls and the possibility of withstanding higher internal turgor pressures without cell bursting.

1. Introduction

For commercial purposes many monocot plants with crassulacean acid metabolism (CAM) (e.g. *Aechmea*, *Phalaenopsis*) are grown under greenhouse conditions that are often not adapted to their specific needs; e.g. the occurrence of high relative humidity at night. One potential problem in these cultures is necrotic spot formation on the foliage of sensitive plants by rupture of chlorenchyma cells (Londers et al., 2005a). The physiological process behind this phenomenon is accumulation of malic acid in the early morning, causing high turgor pressures in the chlorenchyma cells of CAM-plants by elevating the osmotic pressure (Londers et al., 2005b; De Proft et al., 2007; Ceusters et al., 2008). *Aechmea* cultivars vary in their sensitivity to elevated turgor pressures and chlorenchyma cells of some cultivars have been observed to rupture while those from other cultivars seemed to be stronger and showed no cell burst under similar conditions (Londers 2001; Londers et al., 2005b). These observations prompt an investigation into the composition of the chlorenchyma cell walls of these monocot CAM-plants.

In primary plant cell walls, cellulosic microfibrils are embedded in a matrix of interwoven non-cellulosic polysaccharides and proteins (Talbott and Ray, 1992; Carpita and Gibeaut, 1993). The polysaccharide composition of cell walls of monocotyledons has been particularly studied in members of the economically important family of Poaceae (grasses and cereals). Poales, which belong to the group of commelinids, share common features with Zingiberales, Cyperales and Commelinales with respect to cell wall composition (Carpita, 1996; Smith and Harris, 1999). Although palms (Arecales) also belong to the commelinid monocotyledons, they do not contain large amounts of glucuronoarabinoxylans (GAXs) as the major non-cellulosic polysaccharides. The primary cell walls of palms resemble those of non-commelinid monocotyledons and eudicotyledons containing xyloglucans to interlock the microfibrils (Carpita, 1996; Harris et al., 1997; Carnachan and Harris, 2000; Harris, 2006).

GAXs consist of a backbone of xylose (Xyl) residues substituted with mostly single arabinose (Ara) and glucuronic acid (GlcA) (or 4-*O*-MeGlcA) residues linked at C(O)3 and C(O)2, respectively to

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the Xyl residues. Arabinoxylans (AXs) have been extensively studied in cereals, because of their impact in biotechnological processes such as bread making. The structure of AXs is analogous to GAXs except for the occurring glucuronic acid in the latter. AX are either water-extractable (WE-AXs) or water-unextractable (WU-AXs) (Mares and Stone, 1973). In a number of cereal-based processes, WE-AXs form highly viscous aqueous solutions, whereas WU-AXs have strong water-binding capacity (Izydorczyk and Biliaderis, 1995; Meuser and Suckow, 1986; Rouau and Moreau, 1993). Varying levels of the AX building blocks lead to varying ratios of arabinose over xylose (A/X) in AX molecules. This ratio can differ substantially from very lowly substituted (0.2) to highly substituted (>1.0) in wheat bran (Schooneveld-Bergmans et al., 1999; Maes and Delcour, 2002). In oat plants Reid and Wilkie (1969) showed that through plant development the ratio in leaves and stems decreases. Similar results were obtained during coleoptile elongation in maize where a transition occurred from GAXs whose Xyl units are nearly all branched to GAXs with an A/X ratio of less than 0.1 (Carpita, 1984). The side-groups greatly affect the ability of GAXs to bind to one another and to cellulose by means of hydrogen bounds. Consequently they determine porosity and surface charge (Carpita, 1996). Cell walls should be constructed in such a way that they can withstand osmotically generated turgor pressures that may reach values between 0.3 and 1.0 MPa in meristematic and differentiating cells (Carpita and Gibeaut, 1993; Cosgrove, 1993). The aggregate strength of non-covalent forces between wall polymers (e.g. between arabinoxylans themselves or arabinoxylan chains and cellulose fibers) appears to make this possible (McNeil et al., 1975; Iiyama et al., 1994). The degree of substitution of the xylan backbone is likely to control these arabinoxylan–cellulose interactions (Carpita, 1996), while ferulic and *p*-coumaric acid esters are known to cross-link arabinoxylans (Mueller-Harvey et al., 1986; Hatfield and Ralph, 1999) as well as lignin (Ralet et al., 1994; Grabber et al., 1995) and potentially protein (Fry, 1986; Oudgenoeg et al., 2001).

To date, there have been few studies to prove the existence *in vivo* of a clear relationship between cell wall composition and cell wall strength. Moreover, additional information is needed about cell wall composition in species out with those belonging to the Poaceae. In this report the authors investigated the relationship between the composition of chlorenchyma cell walls and susceptibility to damage by high turgor pressures in six cultivars of *Aechmea* that are known to have different sensitivities for chlorenchyma cell burst (Londers, 2001). The investigation will increase existing knowledge on cell wall composition of members of the Bromeliaceae with, pineapple the only other species of this family that has been investigated in this respect (Smith and Harris, 1995, 2001).

2. Results

2.1. Chemical properties

The cell wall non-cellulosic monosaccharide composition from leaf chlorenchyma cells of 6 *Aechmea* cultivars was determined by chromatographic analysis of the water-unextractable part (Table 1). Xylose content was the major neutral monosaccharide for all six investigated cultivars, accounting for approximately 19% of the cell walls. The second most abundant monosaccharide compound was glucose with an average value of 5%. No distinction could be made between these constituents in cultivars with high and low sensitiveness to cell burst. The remaining constituents i.e. arabinose, galactose and mannose were present in smaller amounts but showed significant differences between the cultivars with high and low sensitivity to cell burst. Chlorenchyma cell walls of sensitive cultivars contained higher amounts of arabinose and lower amounts of mannose and galactose compared to those from low sensitive cultivars.

Table 1
Neutral monosaccharide composition of the chlorenchyma cell walls (%) from leaves of three *Aechmea* cultivars with low sensitivity to chlorenchyma cell burst and three cultivars with high sensitivity ($n = 15$ plants)

Cultivar[a]	Ara (%)	Xyl (%)	Man (%)	Gal (%)	Glc (%)
AF (0)	2.49 ± 0.17	19.83 ± 1.08	2.09 ± 0.99	3.83 ± 0.66	5.49 ± 2.58
AC (5)	2.56 ± 0.09	18.15 ± 1.58	1.22 ± 0.40	3.90 ± 0.27	6.02 ± 2.16
AP (10)	2.45 ± 0.12	18.74 ± 1.03	1.56 ± 0.39	3.69 ± 0.34	5.32 ± 2.14
AH (76)	4.51 ± 0.19	22.01 ± 1.58	0.77 ± 0.25	2.53 ± 0.18	4.57 ± 2.71
AM (81)	4.02 ± 0.19	18.05 ± 1.30	0.65 ± 0.05	2.98 ± 0.18	5.23 ± 2.09
AV (92)	5.10 ± 0.62	19.26 ± 1.84	0.70 ± 0.11	3.28 ± 0.40	6.64 ± 2.80
p-Value	<0.05	>0.05	<0.05	<0.05	>0.05

Means of high versus low sensitive cultivars are tested for difference by two sample *t*-test ($\alpha = 0.05\%$).
[a] For each cultivar the percentage of plants showing ruptured chlorenchyma cells after natural induction is shown between brackets; these data have been imported from Londers (2001).

Table 2
Calculated amount of arabinoxylan (AX = 0.88 × (Ara + Xyl)) (%) and ratio between arabinose and xylose based on monosaccharide results of the chlorenchyma cell walls from leaves of three *Aechmea* cultivars with low sensitivity to chlorenchyma cell burst and three cultivars with high sensitivity ($n = 15$ plants)

Cultivar[a]	AX (%)	A/X
AF (0)	19.63 ± 0.93	0.13 ± 0.02
AC (5)	18.22 ± 1.43	0.14 ± 0.01
AP (10)	18.64 ± 1.09	0.13 ± 0.02
AH (76)	23.34 ± 1.52	0.20 ± 0.01
AM (81)	19.42 ± 0.43	0.23 ± 0.01
AV (92)	21.43 ± 2.14	0.26 ± 0.01
p-Value	<0.05	<0.05

Means of high versus low sensitive cultivars are tested for difference by two sample *t*-test ($\alpha = 0.05\%$).
[a] For each cultivar the percentage of plants showing ruptured chlorenchyma cells after natural induction is shown between brackets; these data have been imported from Londers (2001).

Due to their specific properties and their potential role in cell wall strength, the concentrations of AXs and the A/X ratio were calculated (Table 2). Cultivars with high sensitivity for cell burst contained more AX in their chlorenchyma cell walls and showed higher A/X ratios (ca. 0.23) than cultivars with low sensitivity for cell burst (A/X = ca. 0.13). Xylose chains from cultivars with high sensitivity for cell burst were ca. 40% more substituted with arabinose compared to cultivars with low sensitivity for cell burst. Moreover, a positive linear relationship ($p < 0.05$) was found between percentage of plants showing chlorenchyma cell burst after natural induction and A/X ratio.

2.2. Physiological measurements

To show the relationship *in vivo* between GAX fine structure of the chlorenchyma cell walls and their strength, the impact of the factor that induced chlorenchyma cell burst i.e. leaf turgor pressure needed to be evaluated. Therefore an experiment to examine physiological leaf damage in response to elevated turgor was set up with a high (AH) and low cell burst sensitive (AF) cultivar according to Londers et al. (2005b). During incubation the uptake of water into leaf discs of both plants was monitored by weighing them on specific time intervals (Fig. 1).

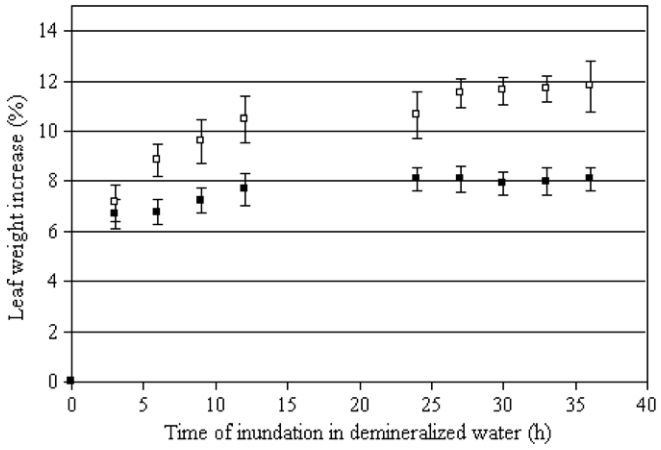

Fig. 1. Pattern of weight increase (%) of leaf discs from a cultivar (AF) with low sensitivity to chlorenchyma cell burst (■) and a cultivar (AH) with high sensitivity to cell burst (□) after immersion of leaves in demineralized water. Data are represented as means ± standard deviation (n = 30 plants).

The values for maximal water uptake stabilized after 30 h of incubation, therefore leaf turgor pressure was considered to be maximal at this time. The maximal leaf turgor pressure exerted on the cell walls before cell burst occurred, turned out to be significantly higher for AF compared to AH ($p < 0.05$). Turgor pressures of 1.03 ± 0.10 MPa and 0.94 ± 0.12 MPa were measured for AF and AH, respectively, and maximal leaf turgor pressures were about 0.3 MPa higher than the average leaf turgor pressures measured for these plants under normal culture conditions (Londers et al., 2005a, b).

3. Discussion

Studies on arabinoxylans have mainly focussed on members of the Poaceae, specifically wheat, rye and maize (Obel et al., 2002). The physicochemical properties of arabinoxylans in the bread making process are already extensively studied in wheat and rye grains (Courtin and Delcour, 2001; Cyran and Saulnier, 2007; Hartmann et al., 2005) and arabinoxylan content and composition have been related to hardness and consequently the milling behavior of wheat grains (Grefeuille et al., 2007; Peyron et al., 2002). Maize has been examined in a more fundamental way where the coleoptile serves as a convenient model to examine dynamic changes in polymer composition and concomitant cellular architecture (Carpita, 1983; Carpita et al., 2001). However, the impact of arabinoxylans with respect to the physiological functioning of plants may be even more far reaching than the examples described above.

Londers et al. (2004, 2005a,b) described a potential physiological problem occurring in the vegetative tissues of CAM-plants which leads to high turgor pressures in chlorenchyma cells. The finding that some cultivars could cope with pressures which were lethal for others prompted the current investigation into the composition of the chlorenchyma cell walls in six cultivars of *Aechmea*, three of which showed high sensitivity and three low sensitivity to cell wall bursting. No anatomical differences were found in the chlorenchyma layer concerning cell dimensions (unpublished results). Chemical analyses of the chlorenchyma cell walls confirmed the supposed presence of GAXs as the major non-cellulosic polysaccharides in *Aechmea*. The fine structure of these GAXs was strongly related to chlorenchyma wall strength. Chlorenchyma cell walls from cultivars with low sensitivity to cell burst were characterized by an A/X ratio of ca. 0.13 while those from cultivars with high sensitivity showed an A/X ratio of ca. 0.23. Xylose chains from cultivars with high sensitivity to cell burst were ca. 40% more substituted with arabinose compared to cultivars with low sensitivity to cell burst.

Andrewartha et al. (1979) showed a clear positive relationship between the degree of arabinose substitution and solubility of AX in water. In their view the solubilising effect of arabinose substituents was due to their ability to prevent intermolecular aggregation of unsubstituted xylose residues. In this way arabinose substituents can influence non-covalent forces between cell wall polymers. Harris (2005, 2006) postulated that GAXs with low degrees of substitution cross-link cellulose microfibrils, while GAXs with high degrees of substitution do not. The occurrence of highly substituted GAXs in unlignified walls and low-branched xylans in lignified walls in tissues of maize seems to confirm this statement (Suzuki et al., 2000). Moreover, Zimmermann et al. (2007) recently stated that even slight changes in cell wall composition could influence fracture mechanics and ultrastructural appearance of wood cell walls. In order to establish a relationship between A/X ratio and the strength of the *Aechmea* chlorenchyma cell walls, maximal turgor pressures were recorded in leaf tissues of the different *Aechmea* cultivars. Significant higher turgor pressures could build up in cultivars with low sensitivity to cell wall burst compared to cultivars with high sensitivity. These measurements confirmed that glucuronoarabinoxylan fine structure and not the magnitude of turgor pressure determined whether or not chlorenchyma cell walls will burst.

4. Concluding remarks

Our results have demonstrated the relationship *in vivo* between glucuronarabinoxylan fine structure and chlorenchyma cell wall strength in *Aechmea*. More precisely, the degree of substitution of the xylose backbone with arabinose is strongly correlated with chlorenchyma cell wall strength. Lower degree of substitution implies stronger chlorenchyma cell walls and the possibility to withstand higher internal turgor pressures without cell bursting as a consequence.

5. Experimental

5.1. Plant material

Six *Aechmea* cultivars, belonging to the family Bromeliaceae, were selected. Earlier studies by Londers (2001) revealed that cultivars AF, AC and AP show low sensitivity to cell burst in the chlorenchyma, while cultivars AH, AM and AV show high sensitivity to cell burst. Natural induction of cell burst in intact plants resulted in 0%, 5% and 10% of plants of AF, AC and AP, respectively showing ruptured chlorenchyma cells compared to 76%, 81% and 92% of plants of AH, AM and AV respectively (Londers, 2001). All plants were in vegetative status and grown in a greenhouse at the Catholic University of Leuven (Belgium). During daytime a minimum temperature of 21 °C was maintained while at night a minimum of 19.5 °C was achieved. Between 6:00 a.m. and 10:00 p.m. artificial lighting provided PAR of 30 μmol m^{-2} s^{-1}.

All leaf samples were taken from the top part of young fully developed leaves at 5:00 a.m. and were directly used for physiological measurements (n = 30) or frozen using liquid nitrogen for further chemical processing (n = 15).

5.2. Chemical analyses

Since the walls of different cell types within a leaf can differ greatly in composition (Gordon et al., 1985), the chlorenchyma layer was separated from the epidermis and hydrenchyma with a razor blade. The accuracy of this technique was confirmed by

microscopic analyses of the separated layers. Non-cellulosic carbohydrate composition and content of the chlorenchyma layer was determined in triplicate by gas–liquid chromatography. Prior to chromatographic detection, the water soluble and insoluble parts of the chlorenchyma layer were separated by aqueous extraction. Ground, dry samples were incubated with demineralized water at 40 °C (10 min) followed by 3 min sonication. After centrifugation the water soluble and insoluble portions were separated and the procedure was repeated twice using the insoluble portion. Afterwards the water insoluble portion was lyophilized followed by hydrolysis of the different fractions (10–15 mg) with 2.0 M trifluoroacetic acid (TFA) (5.0 ml) for 60 min at 110 °C. Reduction with sodium borohydride and acetylation with acetic acid anhydride of the obtained monosaccharides were executed according to the procedure of Englyst and Cummings (1984). The formed alditol acetates (1.0 μl) were separated on a Supelco SP-2380 polar column (30 m length, 0.32 mm internal diameter, 0.2 μm film thickness) (Supelco, Bellefonte, PA, USA) in an Agilent chromatograph (Agilent 6890 series, Wilmington, DE, USA) equipped with an autosampler, a splitter injection port (split ratio 1:20) and a flame ionization detector. The carrier gas was helium. Separation was at 225 °C, while the injection and detection were at 270 °C. AX content was calculated as 0.88 times the sum of xylose and arabinose contents.

5.3. Physiological measurements

Physiological leaf damage was induced on separate leaf discs that were punched out from the top of the leaf and placed in separate tubes with 10 mL demineralized water. The tubes were sealed with parafilm, shaken gently and kept at room temperature. After 2 days of incubation, leaf discs were visually screened for leaf damage, occurring as brown spots (Londers et al., 2005b).

Measurements of leaf water potential were conducted using a Psypro coupled to C-52 sample chambers (Wescor Inc). Leaf discs were punched out with a cork-bore of 0.6 cm diameter, placed in the chambers and equilibrated for 45 min resulting in the water potential reading. For further osmotic potential measurements the same discs were sealed in plastic cups and frozen at −20 °C for at least 24 h. After thawing, the discs were placed again in the chambers for equilibration resulting in the osmotic potential reading. Turgor pressure was calculated by subtraction of the osmotic potential from the water potential, assuming the matric and gravitational potentials to be zero.

5.4. Data analysis

Data were analyzed using the statistical software package SAS Enterprise Guide 4.0. Before carrying out statistical tests normality of the datasets was checked by means of the Kolmogorov–Smirnoff statistic ($p > 0.05$). Means are compared by two sample t-test ($\alpha = 0.05$).

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