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Dynamic changes in cell wall polysaccharides during wheat seedling development

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Abstract

Changes in arabinoxylan content and composition during development of wheat seedlings were investigated. The cell walls isolated from the seedlings showed an increasing content of arabinoxylan during development, which could be correlated to increased activity of xylan synthase and arabinoxylan arabinosyltransferase. Arabinoxylan changed from initially having a high degree of arabinose substitution to a much lower degree of substitution. β-Glucan was present in the walls at the early stages of development, but was actively degraded after day 4. Increased deposition of arabinoxylan did not take place until β-glucan had been fully degraded. Ferulic and *p*-coumaric acid esters were present at all points but increased significantly from day 3 to 6, where lignification began. Ferulic acid dimers did not appear in the cell wall until day three and the different ferulic acid dimers varied in the course of accumulation. The ratio of ferulic acid dimers to free ferulic acid was maximal at the time when the wall had been depleted for β-glucan, which had not yet been fully replaced by arabinoxylan. This pattern suggests a role for ferulic acid dimers in stabilizing the wall during the transition from a flexible to a more rigid structure. To investigate if the same changes could be observed within a single seedling, 7 day old seedlings were divided into four sections and the walls were analyzed. Some of the changes observed during the seedling development could also be observed within a single seedling, when analyzing the segments from the elongation zone at the base to the top of the leaf. However, the expanding region of older seedlings was much richer in hydroxycinnamates than the expanding region of younger seedlings. Diferulic acids are stabilizing the wall in the transition phase from an expanding to a mature wall. This transition can take place in different manners depending on the cell and tissue type.

Keywords: Arabinoxylan; *Triticum aestivum*; Poales; Ferulic acid; *p*-Coumaric acid; Hydroxycinnamate dimers

1. Introduction

Plant cell walls provide the mechanical support that allows the plant to stand upright and they provide a barrier against a great variety of organisms and against environmental factors. However, the wall does not simply provide a strong and rigid structure but must also be flexible and allow the young cells to expand. From a practical viewpoint, cell walls have many uses and the wall properties have important implications for industrial processes and products, e.g. paper pulping and dough quality. Therefore, cell walls are obvious targets for molecular breeding, but the basic understanding of cell wall synthesis is to a great extent still lacking. Wheat seedlings are providing a good model system for investigating wall biosynthesis since large amount of wall material is produced within a short period and changes in the walls can be observed as an extensible wall is replaced by a more rigid structure.

Cell walls consist of polysaccharides, protein and sometimes lignin, and can be divided into primary and secondary walls. In primary walls, cellulose microfibrils are making up a rigid structure of the wall and a less rigid matrix, consisting of pectin, arabinoxylan, xyloglucan and β-glucan surrounds the microfibrils. Depending on the polysaccharide composition, the primary wall can be designated type I or II. Type I is the most common while type II is typical for grasses (Bacic et al., 1988). Type II walls are distinct in having a low content of pectin and xyloglucan and a high content of arabinoxylan (Carpita, 1996). Type II walls also contains mixed linked β-glucan which is not found in type I walls. Ester-linked hydroxycinnamates, especially

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ferulic acid, are a distinct feature of the xylans in type II walls. A number of proteins are located in the wall such as hydroxyproline-rich glycoproteins, proline-rich proteins, and glycine-rich proteins. Furthermore, many enzymes including cell wall modifying enzymes like *endo*- and *exo*-glycosidases and peroxidases are secreted by the cell to the apoplastic space (Bolwell, 1993). Secondary walls are often deposited after cessation of cell expansion. These walls usually contain lignin, which in grasses is mainly build from coniferyl and sinapyl alcohols with some *p*-hydroxy-coumaryl alcohol (Carpita, 1996).

According to the cell wall model proposed by Carpita and Gibeaut (1993) the cellulose microfibrils in type II walls are cross-linked through hydrogen-bonds between the cellulose and stretches of unsubstituted arabinoxylan. β-Glucan is thought to be related to growth, acting as a temporary cross-linker of cellulose during cell wall expansion and ultimately replaced by arabinoxylan, which can form a more rigid network through hydroxy-cinnamate cross-links (Carpita and Gibeaut, 1993). The arabinoxylan-cellulose interaction is likely to be controlled by the degree of substitution of the xylan backbone (Carpita, 1996). The ferulic and *p*-coumaric acid esters are known to cross-link arabinoxylans (Mueller-Harvey and Hartley, 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). Furthermore, it has been demonstrated that ferulic acid can act as an initiation site for lignin deposition (Ralph et al., 1995).

Arabinoxylan in grasses has a backbone consisting of β-(1→4) linked D-xylopyranose residues, which can be single or double substituted with α-(1→2) or α-(1→3) linked arabinofuranose. The xylan backbone can also be substituted to some extent with glucuronic acid at the O-2 position or acetyl groups at the O-2 or O-3 position (Carpita, 1996). The arabinose can have ferulic or *p*-coumaric acid ester-linked at the 5′ position (Smith and Hartley, 1983) and can also be part of small arabinan sidechains (Schooneveld-Bergmans et al., 1999).

Most studies of arabinoxylan in grasses have focused on the grains of cereals. To improve the understanding of how arabinoxylan acts in the cell walls of vegetative tissues and how arabinoxylan biosynthesis relates to the changes in the wall, we have characterized the arabinoxylan in young wheat seedlings. By isolating cell walls from 2 to 7 day old seedlings and characterizing the arabinoxylan concomitantly with assaying for xylosyl and arabinosyl transferase activity we have been able to correlate modifications of the hemicelluloses with the changing requirement of the seedling.

2. Results

2.1. Growth and cell wall content

Wheat seedlings maintained an almost linear growth for the 7 day period investigated with a lag for the first 2 days. The average fresh weight of the seedlings

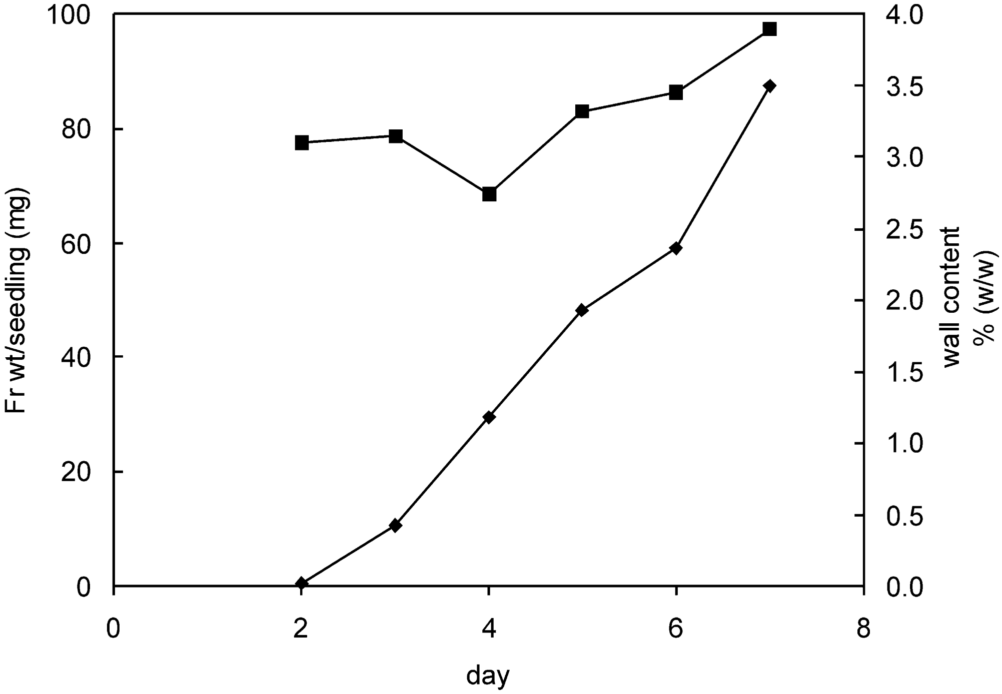

Fig. 1. Growth and wall content of wheat seedlings. The fresh weight accumulation (◆) and corresponding wall content (■) of 2–7 days old wheat seedlings.

Table 1
The monosaccharide composition of cell walls isolated from wheat seedlings. Arabinoxylan was calculated as the combined amounts of arabinose and xylose, while ara:xyl is the ratio of arabinose to xylose. Mannose was found in trace amounts in some samples while rhamnose was not detected in any sample. Numbers represent means±95% interval of confidence

Day	Dry weight content in cell walls.% (w/w)					
	Arabinose	Galactose	Glucose	Xylose	Arabinoxylan	Ara:xyl
2	4.5±0.7	3.0±1.3	3.7±1.4	5.9±1.04	10.4±1.7	0.80±0.08
3	3.0±0.5	0.7–0.3	3.1±0.6	4.4±1.0	7.4±1.4	0.64±0.05
4	4.1±0.4	0.3–0.1	3.4±0.1	8.6±0.9	12.7±1.3	0.48±0.01
5	3.4±0.3	0.6±0.2	1.0±0.2	9.4±1.2	12.8±1.5	0.36±0.02
6	2.8±0.2	0.6±0.1	1.2±0.3	10.0±0.8	12.7±0.7	0.28±0.04
7	3.8±0.4	0.7±0.2	1.7±0.2	13.0±0.7	16.8±1.0	0.29±0.02
Segments of 7 day old seedlings						
Upper 1/3	3.5±0.6	1.1±0.6	1.3±1.0	14.3±1.3	17.8±0.8	0.25±0.06
Middle 1/3	2.4±0.2	0.4±0.2	2.0±1.2	9.3±2.3	12.6±1.9	0.27±0.09
Lower 1/3	2.3±0.1	0.0±0.1	2.5±0.7	5.9±0.9	8.2±1.0	0.40±0.06
Coleoptile	2.2±0.2	0.1±0.1	1.2±0.2	8.7±1.0	10.9±0.9	0.25±0.04

increased from 0.4 mg at day 2 to almost 90 mg (Fig. 1). In this period the seedlings grew from a height of 4 mm to 20 cm and at the latest stage, i.e. 7 days, no elongation was taking place in the upper 2/3 of the plant. The relative wall content decreased initially from day 3 to 4, and increased thereafter from 2.7 to 3.8% (w/w) of the fresh weight (fr wt), (Fig. 1). Seven day old seedlings were divided into four parts corresponding to the upper 1/3, middle 1/3 and lower 1/3 of the plant and the remaining coleoptile. The wall content was only 1.6% (w/w) in the upper 1/3 of the plant while the coleoptile had a high content of almost 8% (w/w).

2.2. Monosaccharide composition

An estimate of the arabinoxylan content was based on the combined amounts of arabinose and xylose. Arabinoxylan content varied significantly during development, with an initial decrease from 10.4% of the walls (w/w) at day 2 to 7.4% (w/w) at day 3, then an increase to a stable level of 12.7% (w/w) from day 4 to 6, followed with an increase to 16.6% (w/w) at day 7 (Table 1). The degree of arabinose substitution was expressed through the ratio of arabinose to xylose, ara:xyl, and decreased by 0.8 at day 2 to 0.29 at day 7 (Table 1). The glucose content essentially representing β-glucan was at a stable level from day 2 to 4 and then rapidly decreased with 70% within 24 h. Galactose was at a high level at day 2, but decreased by more than 75% between days 2 and 3 and remained at a low level of approximately 0.6% (w/w) for the remaining period (Table 1).

When investigating the different segments of 7 day old plants the same pattern appeared with the highest arabinoxylan content and lowest substitution in the oldest tissue, i.e. the upper 1/3 (Table 1). Glucose increased towards the base of the seedling and a low level of glucose was observed in the coleoptile. With respect to arabinoxylan and glucan the lower segment of older seedlings thus resembled younger seedlings. In contrast galactose was abundant only in younger seedlings and upper segments (Table 1).

2.3. Hydroxycinnamates

The relative content of ester linked hydroxy-cinnamates in the wall increased 7–8-fold over the period, with ferulic acid being the predominant hydroxy-cinnamate. Ferulic acid was at a stable level for the first two time points (days 2 and 3), and then increased from 0.01 to 0.07 mg (g dry wt)$^{-1}$ within the following 3 days [Fig. 2 (a)]. *p*-Coumaric acid showed a similar dramatic increase over time but was much less abundant than ferulic acid [Fig. 2(a)]. Three different ferulic acid dimers could be identified: 8-*O*-4′-diferulic acid, 5,5′-diferulic acid and 8,5′-diferulic acid. No diferulic acids could be detected before day 3, and at all subsequent time points 8,5′-diferulic acid was the most abundant. However, the pattern of accumulation varied among the diferulic acids. The content of 8,5′-diferulic acid was at a maximum level at day 5 and then decreased while 8-*O*-4′-diferulic acid increased until day 5 and 5,5′-diferulic acid increased until day 6 [Fig. 2(b)].

Seven day old seedlings had the highest levels of ferulic acid in the lower 1/3 and the coleoptile, and the amount of ferulic acid decreased towards the top of the plant. No differences in the levels of *p*-coumaric acid and ferulic acid dimers could be detected (Fig. 3).

2.4. Substitution pattern of the arabinoxylan

The type of xylan substitution was estimated by ^{1}H NMR as described by Roels et al. (1999) and revealed that virtually all arabinose in all the fractions was linked to O-3 of the xylopyranosyl residue, since only the shift at

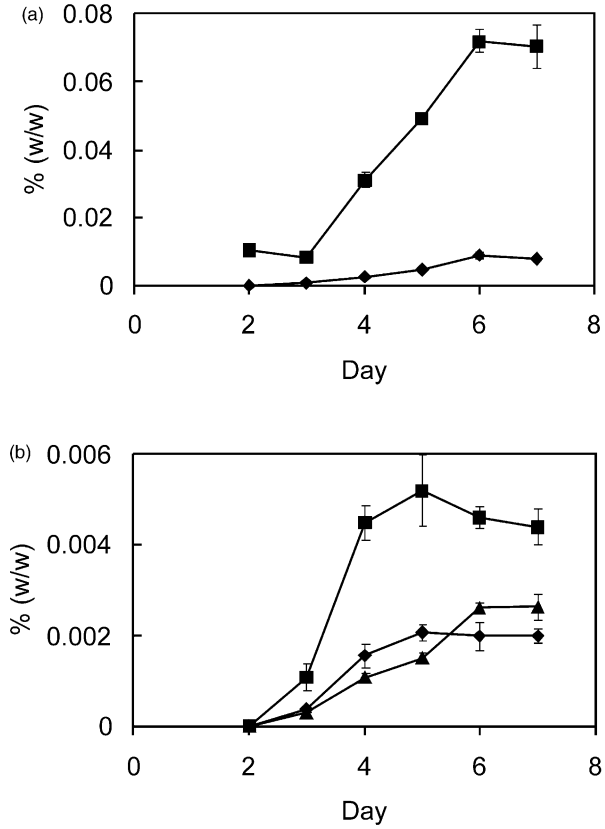

Fig. 2. Hydroxy-cinnamic acids and ferulic acid dimers of the cell wall. (a) The wall content of ferulic acid (■) and *p*-coumaric acid (◆) from 2–7 days after germination. (b) The 8,5′-diferulic acid (■), 5,5′-diferulic acid (◆) and 8-*O*-4′-diferulic acid (▲) for the same period. Values shown are per cent of wall (w/w) ± the 95% interval of confidence.

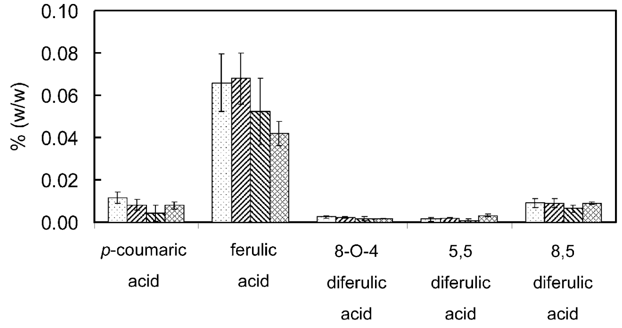

Fig. 3. Hydroxy-cinnamic acids and ferulic acid dimers in different segments of the 7 day old wheat seedling. The upper 1/3 segment (□), the middle 1/3 segment (▨), the lower 1/3 segment (▧) and the remaining coleoptile (▩). Values shown are percent of wall (w/w) ± the 95% interval of confidence.

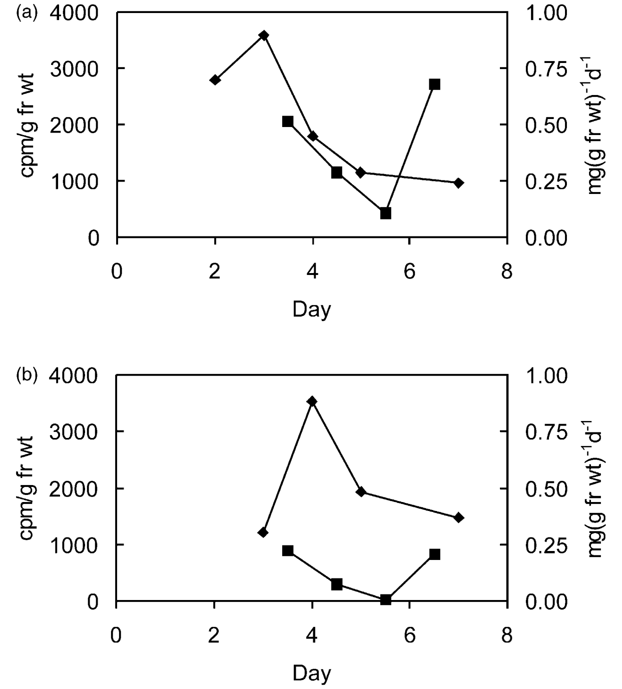

Fig. 4. Incorporation of UDP-xylose and UDP-arabinose and corresponding net deposition of these sugars in the wall. (a) The incorporation of UDP-xylose (◆) by microsomes prepared from differently aged wheat seedlings shown on the left axis, while the net daily deposition per day of xylose (■) is given on the right axis. (b) The corresponding figures for UDP-arabinose (◆) and net daily deposition of arabinose (■).

5.4 ppm corresponding to the anomeric arabinofuranose proton linked to the O-3 position of xylopyranose was detected. No double peak at 5.23 and 5.30 ppm corresponding to the anomeric proton from arabinofuranose linked O-2 and O-3 on double substituted xylopyranose residues were detected. The anomeric proton of arabinofuranose O-2 linked to xylopyranose has a shift of 5.28 ppm (Vietor et al., 1994) and a peak representing less than 2% of the 5.4 ppm peak was detected indicating a very low presence of O-2 arabinose substitution. No peak at 5.26 ppm was observed demonstrating a low content of arabinogalactan (Roels et al., 1999). The relative intensities of the arabinose anomeric protons remained unchanged in the different wall materials but the intensities compared to the xylose protons correlated with the changing degree of arabinose substitution.

2.5. Arabinofuranosyl- and xylosyl transferase activity

The activities of the arabinosyl transferase and xylosyl transferase showed maximum activity of xylosyl transferase at day 3 as reported by Porchia and Scheller (2000), while the arabinoxylan arabinofuranosyl transferase activity had the maximum at day 4 [Fig. 4(b)].

3. Discussion

Over the 7 days development of the seedlings, the fr wt increased in a linear manner while the cell wall content increased from 3.1 to 3.9% (w/w). During this period the majority of the cells went from the stage of cell division and expansion to a mature stage with no further expansion. Major changes in the composition and rate of accumulation in the matrix polysaccharides took place during this development.

Arabinoxylan content was estimated from the combined amounts of arabinose and xylose. Obviously this is not strictly correct since some xylose has originated from xyloglucan and some arabinose has originated from glycoproteins. However, the xyloglucan content is very low in grasses (Bacic et al., 1988) and the NMR-spectra indicated a low content of arabinogalactan-proteins. In the same manner it is not correct, when assigning all non-cellulose glucose to originate from β-glucan. A more rigorous determination of the arabinoxylan and β-glucan using different extractions and enzymatic solubilizations is not really an alternative for complete quantification since extractability of arabinoxylan varies during development. We have therefore found that the quantification based on monosaccharide composition was a reasonable method.

The combined amounts of β-glucan and arabinoxylan are 15–20% of the cell wall and the remaining 80–85% is likely to consist of mainly cellulose and some protein. The content of hemicellulose is thus somewhat lower than what has been observed in maize coleoptiles (Carpita et al., 2001).

Glucose, representing mainly β-glucan, showed a very large decrease after the first 4 days. This decrease is so large that it can not be explained by cessation of biosynthesis but most involve the active break down of wall polymer as also observed in maize coleoptiles (Luttenegger and Nevins, 1985). Elongation in a large part of the seedling stopped around day 4 and therefore the decrease in β-glucan is in good agreement with its proposed role as a cross-linker of the cellulose microfibrils in developing walls, while arabinoxylan replaces β-glucan in mature wall (Carpita and Gibeaut, 1993). Surprisingly, no sharp increase in arabinoxylan was observed until two days after the decrease in β-glucan content. However, the hydroxy-cinnamates accumulated dramatically simultaneously with the disappearance of β-glucan.

The arabinoxylan changed character over the period of investigation from a highly substituted polymer with an ara:xyl ratio of 0.8 at day 2 to a much less substituted and less soluble polymer at days 6 and 7 where the ara:xyl ratio was below 0.3. While the levels of β-glucan and arabinoxylan changed abruptly, the degree of arabinose substitution changed gradually. This gradual change is probably reflecting the complex manner in which high and low substituted arabinoxylan is deposited in primary and secondary walls and in different cell typers (Suzuki et al., 2000).

The ester-linked hydroxy-cinnamates increased from day 3 to 6. The constant level of ferulic and *p*-coumaric acid from day 6 to 7 might not reflect a decreased rate of synthesis but could reflect an increased involvement of hydroxy-cinnamates in ether-linkages. Since lignin cross-links to ferulic acid (Iiyama et al., 1994; Jacquet et al., 1995), the ferulic acid content may have been underestimated from day 6 when lignification began. The level of ferulic and *p*-coumaric acid at the different time points reported here is similar to what has been reported for barley seedlings by Yamamoto and Towers (1985). The predominant diferulic acid was the 8,5′-diferulic acid, while 8-*O*-4′- and 5,5′-diferulic acid were found in smaller quantities. This distribution of ferulic acid dimers is different to what has been observed in wheat straw by Waldron et al. (1996), where more 8-*O*-4′-diferulic acid and 8,5′-diferulic acid in the benzofuran form than the 8,5′- and 5,5′-diferulic acid was reported. A different distribution of ferulic acid dimers in wheat seedlings and straw could be explained if some dimers are formed intracellularly as suggested by Fry et al. (2000), while others are formed in muro by peroxidases and laccases (Fry, 1986; Figueroa-Espinoza et al., 1999) since this would lead to a change in the ferulic acid dimer profile over time. Interestingly, the ratio of diferulic to ferulic acid over the 7 day period increased from 0 to 0.23 between days 2 and 4 and subsequently decreased to a stable level of 0.13 at day 6 (Fig. 5). The diferulic acid is perceived as a cross-link of the cell wall (Fry, 1986; Iiyama et al., 1994), but the decrease from day 4 to 6, which is prior to the major lignin deposition, suggest a role for diferulic acids in the enforcement of the cell wall while it undergoes the transformation from a developing immature cell wall to the more rigid structure of the mature cell wall. The maximum ratio of diferulic acid to ferulic acid coincides with the decrease in β-glucan content. Hence, the data suggest a sequence of events where β-glucan is actively degraded while the remaining xylan is reinforced with diferulate cross-links, and no deposition of additional arabinoxylan takes place until β-glucan has been completely removed.

To investigate if changes over time could be observed within the 7 day old seedling as well, where young elongating tissue is located at the base, while a mature non- elongating tissue is present at the top, we divided 7

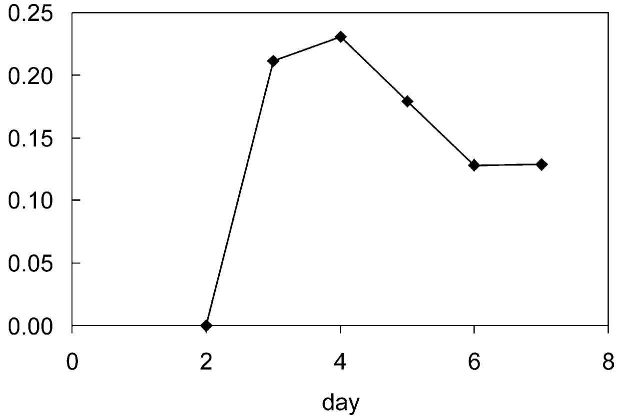

Fig. 5. Ratio of ferulic acid dimers to ferulic acid.

day old seedlings into four sections. As expected the arabinoxylan increased from the lower and elongating tissue to the more mature upper sections (Table 1). The ara:xyl ratio decreased in the same direction reflecting an increasingly more insoluble arabinoxylan towards the mature tissue at the top, β-glucan also repeated the pattern observed over time with a decrease in β-glucan content toward the top of the seedlings (Table 1). Galactose increased towards the top of the seedling corresponding to an increase in galactose observed from day 4 to 7, but while a high galactose content was observed in the very young seedlings only traces of galactose were observed at the base of the 7 day old seedling. Hydroxy-cinnamates also behaved in an unexpected manner. If the time pattern observed in the seedling should have been repeated, low levels should have been observed in the lower section followed by an increase toward the top of the seedling. In reality the inverse was observed with the highest amount of ferulic acid at the base, while only low levels of hydroxy-cinnamates were found in the mature tissue at the top. This unexpected result may partly reflect an extensive cross-linking of ferulic acid to lignin as discussed above and observed by Iiyama et al. (1990 and 1994). However, the hydroxy-cinnamate content in the expanding cells of the 7 day old seedlings was much higher than in the expanding cells of young seedlings. Another distinct difference between maturing tissue over time and the maturing gradient within a single seedling was the variations in *p*-coumaric acid and ferulic acid dimers where no significant variations could be determined in the different segments (Figs. 2 and 3). The differences in hydroxy-cinnamates and galactose accumulation when comparing the changes over time to changes within a single seedling from the elongating base to the mature tissue at the top shows that the transition from an elongating wall to a mature wall can be varied depending on the specific tissue and its origin.

When calculating the net deposition of arabinoxylan in the seedling a decreasing net synthesis per g fr wt was observed from days 3 to 5 followed by an increase from days 5 to 7 [Fig. 4(a)]. The maximum xylosyl transferase activity is in good agreement with the high de-novo synthesis at the early stage. In contrast, the increased deposition from days 5 to 7 was not accompanied by an increase in the xylosyl-transferase activity. This observation would suggest that the deposition rate is not directly controlled by the amount of the biosynthetic glycosyltransferases, but perhaps by the availability of substrate. The fact that arabinosyltransferase largely follows the xylosyltransferase activity [Figs. 4(a) and (b)] is in agreement with the synthesis of highly substituted arabinoxylan at all times (Gibeaut and Carpita, 1991). The decreasing degree of arabinose substitution during wall maturation is thus likely to reflect increasing activity of arabinofuranosidases.

4. Experimental

4.1. Enzymes and reagents

Scintillation fluid was from Ecoscint (National Diagnostics, Manville, NJ, USA). Homogeneous *endo*-xylanase A from *Aspergillus niger* was a gift from Drs. T. Gravesen and S. Madrid (Danisco Biotechnology, Copenhagen, Denmark). Acetonitrile, super purity solvent grade, was purchased from Romil (Cambridge, England). UDP-[U-^{14}C]-D-xylose with a specific activity of 7.4–11.1 TBq mol^{-1} was from New England Nuclear (Boston, MA, USA). If not otherwise stated all chemicals were purchased from Sigma (St. Louis, MO, USA).

4.2. Plant material

Seedlings of wheat (*Triticum aestivum* L. cv. Cadenza) were grown in trays of vermiculite at 25 °C for 7 days. The seedlings were grown in a greenhouse with supplemental light under a regime of 16 h light and 8 h dark. The entire coleoptile or coleoptile with the first leaf was carefully excised at the point of harvest and used for cell wall preparations. In one experiment 7 day old seedlings were divided into four sections of approximately similar size and weight. The leaf was divided into three zones, the upper 1/3—the most mature tissue, the middle 1/3—an intermediate degree of maturity and the lower 1/3—the youngest tissue including the elongation zone. The fourth section consisted of the remaining coleoptile.

4.3. Cell wall preparation

Cell walls were prepared by a modification of the procedure of Borneman et al. 1990. Harvested material was grounded to a fine powder in liquid N_2 and stirred for 6 h in benzene/ethanol (7/3 v/v). Subsequently, the insoluble material was pelleted by centrifugation, washed twice with acetone and dried. This pellet was stirred for 3 days in chloroform/methanol (3/2 v/v), for removal of all remaining membranes from the sample. Finally the insoluble material was treated for 24 h with α-amylase in 0.1 M Mops (pH 7.0). The degraded starch and water-soluble material were removed by washing the insoluble material three times with water. The final insoluble material, designated "cell walls", was lyophilized to dryness.

4.4. Determination of hydroxy-cinnamates

Hydroxy-cinnamates were extracted from the wall material after hydrolysis overnight in 2 M NaOH according to the procedure by Waldron et al. (1996). *p*-Hydroxybenzoic acid was used as internal standard and individual hydroxy-cinnamates and ferulic acid dimers were identified and quantified by HPLC applying a phosphoric acid-acetonitrile solvent system as described by Obel and Scheller (2000). Standards for individual hydroxy-cinnamates were purchased and ferulic acid dimers were identified and quantified using published spectra and response factors (Waldron et al., 1996).

4.5. *Monosaccharide composition*

Non-cellulose polysaccharides in the cell walls were hydrolyzed in 2 M TFA at 120 °C for 1 h and the monosaccharide composition determined by HPAEC as described by Sørensen et al. (2000) with fucose as internal standard.

4.6. *NMR*

Arabinoxylan was extracted overnight from the 2 g wall material using 2 M NaOH at room temperature in an overnight incubation and the sulubilized arabinoxylan was subsequently desalted using a NAP10 column (PharmaciaBiotech, Uppsala, Sweden). For achieving the best NMR spectra, the samples were digested with xylanase prior to analysis. For this purpose arabinoxylan was suspended in a total volume of 100 μl containing 20 μl of 0.3 M sodium acetate, pH 5.2, and was hydrolyzed by pure *endo*-xylanase A (0.01 U, 1 unit releases 1 μmol of reducing arabinoxylan oligosaccharide per min) for 5 h at 30 °C.

4.7. *Glycosyltransferase activity*

UDP-[U-^{14}C]-D-arabinose was synthesized as described by Pauly et al. (2000). UDP-[U-^{14}C]-D-xylose was fed to microsomes prepared from whole plants harvested from 2–7 days and the incorporation into arabinoxylan was determined as described by Porchia and Scheller (2000). For arabinosyltransferase activity, Golgi vesicles were prepared from wheat seedlings essentially as described by Geshi et al. (2002) and incorporation of ^{14}C-arabinose from UDP-[U-^{14}C]-D-arabinose was determined in the presence of 1 mM UDP-D-xylose and 5 mM $MnCl_2$ in a buffer of 2-(*N*-morpholino)ethanesulfonic acid, pH = 5.5. Characterization of the product was performed in the same manner as for the xylosyltransferase.

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