UDP-glucose dehydrogenases of maize: a role in cell wall pentose biosynthesis

Anna KÄRKÖNEN*, Alain MURIGNEUX†, Jean-Pierre MARTINANT†, Elodie PEPEY†, Christophe TATOUT†, Bernard J. DUDLEY* and Stephen C. FRY††

*The Edinburgh Cell Wall Group, Institute of Molecular Plant Sciences, School of Biological Sciences, The University of Edinburgh, Daniel Rutherford Building, The King's Buildings, Edinburgh EH9 3JH, U.K., and †BIOGEMMA, Campus Universitaire des Cézeaux, 24, Avenue des Landais, 63170 Aubière, France

INTRODUCTION

UDP-glucuronate (UDP-GlcA) is the glucuronosyl donor for the synthesis of several structural polysaccharides, and is the precursor of other sugar nucleotides including UDP-galacturonate, UDP-Xyl (UDP-D-xylose), UDP-Ara (UDP-arabinose) and UDP-apiose, which are glycosyl donor substrates for hemicellulose and pectin biosynthesis [1–3]. Glucuronoarabinoxylans (synthesized from UDP-GlcA, UDP-Ara and UDP-Xyl) constitute a substantial proportion of the maize cell wall [4], and thus the biosynthesis of UDP-GlcA is a major metabolic activity during maize cell growth.

UDP-GlcA can be formed by two routes: (i) via a sugar nucleotide oxidation pathway involving the UDPGDH (UDP-D-glucose dehydrogenase; EC 1.1.1.22)-catalysed oxidation of UDP-Glc (UDP-D-glucose) to UDP-GlcA (UDP-D-glucuronate), the precursor of UDP-D-xylose and UDP-L-arabinose, major cell wall polysaccharide precursors. Maize (Zea mays L.) has at least two putative UDPGDH genes (A and B), according to sequence similarity to a soya bean UDPGDH gene. The predicted maize amino acid sequences have 95% similarity to that of soya bean. Maize mutants with a Mu-element insertion in UDPGDH-A or UDPGDH-B were isolated (udpgdh-A1 and udpgdh-B1 respectively) and studied for changes in wall polysaccharide biosynthesis. The udpgdh-A1 and udpgdh-B1 homozygotes showed no visible phenotype but exhibited 90 and 60–70% less UDPGDH activity respectively than wild-types in a radiochemical assay with 30 µM UDP-glucose. Ethanol dehydrogenase (ADH) activity varied independently of UDPGDH activity, supporting the hypothesis that ADH and UDPGDH activities are due to different enzymes in maize. When extracts from wild-types and udpgdh-

Al homozygotes were assayed with increasing concentrations of UDP-Glc, at least two isoforms of UDPGDH were detected, having $K_m$ values of approx. 380 and 950 µM for UDP-Glc. Leaf and stem non-cellulosic polysaccharides had lower Ara/Gal and Xyl/Gal ratios in udpgdh-A1 homozygotes than in wild-types, whereas udpgdh-B1 homozygotes exhibited more variability among individual plants, suggesting that UDPGDH-A activity has a more important role than UDPGDH-B in UDP-GlcA synthesis. The fact that mutation of a UDPGDH gene interferes with polysaccharide synthesis suggests a greater importance for the sugar nucleotide oxidation pathway than for the myo-inositol pathway in UDP-GlcA biosynthesis during post-germinative growth of maize.

Key words: arabinose, insertional mutagenesis, pentose biosynthesis, UDP-glucose dehydrogenase, UDP-glucuronate, xylose.

MATERIALS AND METHODS

Maize UDPGDH genes and mutant plants

Two maize contigs of EST (expressed sequence tag) were identified from the GnpSeq GENOPLANTE database (http://genoplante-info.infobiogen.fr/) for their high amino acid sequence similarity to the soya bean UDPGDH gene previously isolated and characterised by [11]. Accession numbers of the contigs on GnpSeq database are 658123 and 658125, here designated as genes UDPGDH-A and UDPGDH-B respectively.

Abbreviations used: ADH, ethanol dehydrogenase; AIR, alcohol-insoluble residue; EST, expressed sequence tag; TFA, trifluoroacetic acid; UDPGDH, UDP-D-glucose dehydrogenase; UDP-GlcA, UDP-D-glucuronate; UDP-Xyl, UDP-D-xylose.

† To whom correspondence should be addressed (email S.Fry@ed.ac.uk).

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A reverse genetics approach was performed at Biogemma on this gene family by use of a resource of 27 500 maize lines in which an endogenous transposon called ‘Mutator’ (Mu) had been allowed to transpose at high frequency. This mutagenesis collection was built up by use of Mu transposon stock kindly provided by B. Taylor (Commonwealth Scientific and Industrial Research Organization, Australia). The Mu transposon stock was crossed with maize hybrids developed by Limagrain (Chappes, France) in order to increase the vigour of the plants and their adaptation to European environmental conditions. The screening for Mu insertions was carried out on F1 plant material and confirmed for their germinal status (heritability) on F2 plant material. Mutant screens were accomplished through a PCR-based approach using a Mu-specific primer (OmuA: 5'-CTTGGTCCAT-AATGGCAATTATCTC-3') that binds to the edges of the element, the so-called TIR (terminal-inverted repeat). OmuA was used in combination with a PCR primer (UDPGDH-c2: 5'-CC-AATCTCTGTGTCCTTGCCC-3'), which has the ability to amplify both maize UDPGDH-A and UDPGDH-B genes. The PCR conditions were standard with 37 cycles and a Tm = 58°C.

One insertion mutation was detected in each gene: udpgdh-A1 and udpgdh-B1. Because of the process used to make the maize mutant collection, each F2 plant for each family has a heterogeneous genetic background. Therefore, in order to minimize phenotypic variation between plants belonging to the same family, we self-pollinated a single plant heterozygous for the insertion event (=mutant allele) for each family investigated. Thus, in each set of kernels, there is a genetic segregation for the mutant allele and therefore a molecular characterization based on DNA extraction and PCR analyses was made for each plant. Because of this approach, any given mutant plant should only be compared with wild-types belonging to the same family. Homozygous plants were collected from greenhouse-grown plants, dried in an oven (50°C) and weighed. Dried stem tissue was ball-milled for 7 min to a fine powder. Dried leaves were crushed with a spatula. AIR (alcohol-insoluble residue) was prepared by incubation in 70% (v/v) ethanol for several days at 60°C with occasional shaking; the ethanol was changed until no more soluble sugars appeared according to an anthrone test for hexoses [13]. The AIR was washed in acetone, dried at room temperature (20°C) and weighed, and the non-cellulosic polysaccharides were hydrolysed with 2 M TFA (trifluoroacetic acid) for 1 h at 120°C (1 ml of TFA/5–20 mg of AIR). The TFA-resistant (cellulosic) residue was rinsed with water and the washings were combined with the TFA hydrolysate, which was then dried in a SpeedVac. Prior to HPLC, 200 µl of the hydrolysate was made up to 0.5 ml with 10 mM pyridinium formate, pH 5.5 (running buffer), and anions were removed by chromatography on a 0.75-ml column of Dowex 1 × 4–200 (formate form) in running buffer. The neutral fraction (2 ml) was eluted with running buffer, dried and redissolved in 200 µl of water. The samples were filtered through 0.45-µm filters and analysed by HPLC. This pre-purification removed some unidentified impurities that impaired the detection of individual sugars in HPLC.

Monosaccharides in the hydrolysates were also analysed by paper chromatography in ethyl acetate/pyridine/water (8:2:1, by vol.) and stained with aniline hydrogen-phthalate [13].

The cellulosic residue remaining after TFA hydrolysis was washed with water (until no soluble sugars appeared) and acetone, dried and assayed by a modification of the method of Updegraff [14]. For this, the residue was shaken for 1 h at approx. 20°C in 1 ml of 67% (v/v) H2SO4, and a portion of the solution was analysed by the anthrone test [13] with a glucose standard curve. Results were expressed as percentage of AIR after correction of free glucose (M, 180) to glucose residues (M, 162).

**Enzyme activity assays**

UDPGDH activity was assayed by spectrophotometric and radiochemical methods. The spectrophotometric assay was modified from that of Robertson et al. [12]. The final reaction mixture contained 5 mM UDP-Glc, 2 mM NAD+, 0.1 M Tris/HCl (pH 8.0), and desalted extract in a final volume of 1.0 ml. The enzyme was preincubated for 10 min with UDP-Glc at approx. 20°C. The reaction was initiated with NAD+ and monitored for 10 min as the increase in A340 due to reduction of NAD+. Controls contained water instead of UDP-Glc. UDPGDH activity was calculated on the basis that 2 mol NADH were formed per mol UDP-Glc oxidized. Ethanol dehydrogenase (ADH) activity was measured in the same way as UDPGDH, except that 5 mM ethanol was used as substrate, the preincubation was omitted, and it was assumed that 1 mol NADH was formed per mol ethanol oxidized.

The radiochemical reaction mixture (10 µl) contained 30 µM UDP-D-[U-14C]Glc (3.7 kBq), 2.5 mM NAD+, 32 mM Tris/HCl (pH 8.0) and enzyme extract. Control samples contained the boiled enzyme. The reaction was started by addition of the enzyme and stopped after 5 min at 25°C by the addition of formic acid (to 10%). Half the reaction mixture was subjected to high-voltage paper electrophoresis at pH 6.5 (2.5 kV, 80 min) as described in [13]. The electrophoretograms were autoradiographed, and the spots of individual compounds were cut out and scintillation-counted in 1.5 ml of OptiScint ‘HiSafe’ (Wallac–EG&G Instruments, Evry, France). Previously, we have shown that the product of the enzymic reaction is UDP-GlcA [10].

To estimate the Kn values of different isoenzymes for UDP-Glc, we conducted the radiochemical activity assay, with increasing concentrations of UDP-Glc (0–32 mM), on extracts from wild-types and udpgdh-A1 individuals. By subtracting the observed mutant value from that of the wild-type, we estimated the velocities of different isoenzymes and estimated their Kn values from Lineweaver–Burk or Hanes plots.

**Cell wall analysis**

For analysis of cell wall polysaccharides, mature leaves (first and third leaf from the soil) and the second-lowest internode were collected from greenhouse-grown plants, dried in an oven (50°C) and weighed. Dried stem tissue was ball-milled for 7 min to a fine powder. Dried leaves were crushed with a spatula. AIR (alcohol-insoluble residue) was prepared by incubation in 70% (v/v) ethanol for several days at 60°C with occasional shaking; the ethanol was changed until no more soluble sugars appeared according to an anthrone test for hexoses [13]. The AIR was washed in acetone, dried at room temperature (20°C) and weighed, and the non-cellulosic polysaccharides were hydrolysed with 2 M TFA (trifluoroacetic acid) for 1 h at 120°C (1 ml of TFA/5–20 mg of AIR). The TFA-resistant (cellulosic) residue was rinsed with water and the washings were combined with the TFA hydrolysate, which was then dried in a SpeedVac. Prior to HPLC, 200 µl of the hydrolysate was made up to 0.5 ml with 10 mM pyridinium formate, pH 5.5 (running buffer), and anions were removed by chromatography on a 0.75-ml column of Dowex 1 × 4–200 (formate form) in running buffer. The neutral fraction (2 ml) was eluted with running buffer, dried and redissolved in 200 µl of water. The samples were filtered through 0.45-µm filters and analysed by HPLC. This pre-purification removed some unidentified impurities that impaired the detection of individual sugars in HPLC.

Enzymes were extracted from developing leaves (basal ~ 3-cm pale green region of the lamina) of 2-month-old plants. Plant material was homogenized in a mortar with pestle in 2 vol. of 50 mM Tris/HCl buffer (pH 7.5) containing 2 mM EDTA and freshly added dithiothreitol (5 mM). The homogenate was centrifuged in Edinburgh, U.K., and used for enzymic and cell wall analyses.

**Extract preparation**

Enzymes were extracted from developing leaves (basal ~ 3-cm pale green region of the lamina) of 2-month-old plants. Plant material was homogenized in a mortar with pestle in 2 vol. of 50 mM Tris/HCl buffer (pH 7.5) containing 2 mM EDTA and freshly added dithiothreitol (5 mM). The homogenate was centrifuged at 15 800 g for 1 h at 4°C to pellet the cell wall debris and the supernatant was re-centrifuged for 2 min. The extracts were freed of low-Mr contaminants by gel-permeation chromatography on PD10 columns (Amersham Biosciences) pre-equilibrated with the homogenization buffer and material in the void volume was used for enzymic assays.
of NaOH concentrations was: 0–1.8 min, 20 mM; 1.8–30 min, 0–100 mM (linear gradient); 31–35 min, 800 mM; 35–36 min, 800 µM.

Samples were run on the HPLC in batches of up to 35, with a mixture of calibration standards (0.2–1.0 mg·ml⁻¹) at the beginning of each batch. Standards of 0.5 mg·ml⁻¹ were run within batches as a check that there was no drift in retention times or sensitivity.

RESULTS

UDPGDH genes

Two contigs of EST (GnpSeq ids 658 123 and 658 125) were identified for their high amino acid sequence similarity to the first isolated gene of the UDPGDH family in soya bean [11]. Each contig contains the whole coding sequence, and ids 658 123 and 658 125 probably correspond to two different homologous genes named UDPGDH-A and UDPGDH-B respectively. The predicted soya bean protein (id AAB58398) is compared with the proteins predicted for the maize and soya bean proteins, 93.9% identity in nucleic acid sequence between the two contigs and the database). Proteins predicted from 658 123 and 658 125 are referred to here as UDPGDH-A and UDPGDH-B respectively. Underline indicates a deviation from the other two sequences; where the two maize contigs of EST (ids 658 123 and 658 125 from the GENOPLANTE GnpSeq database), were added to the radiochemical UDPGPDH assay, a clear difference was observed between the mutant and the wild-type (data for udpghd-A1 mutants; Figure 5). The activity of the mutant never exceeded approx. 20% of that of the wild-type even when the activities approached Vmax. This result supports our earlier finding that majority of the UDPGDH activity in developing maize leaves is due to enzyme(s) other than those (Ei) having an extremely high Km for UDP-Glc [10]. From the total enzymic activities measured in the wild-type and udpghd-A1 mutant, the activities of individual isoenzymes can now be estimated. We assume that the wild-type has at least two functional isoenzymes, with genes A and B encoding isoenzymes UDPGDH-A and UDPGDH-B respectively; thus the homoygous mutant defective in UDPGDH-A is devoid of isoenzyme A. By subtracting the velocity observed in the mutant (which possesses active isoenzyme B) from that of the wild-type we can estimate the velocity of isoenzyme A (Figure 5), assuming the mutant has the same UDPGDH-B activity as the wild-type. The Kn values of approx. 380 and 950 µM for UDP-Glc and Vmax values of 900 and 240 pkat·g fresh weight⁻¹ for isoenzymes A and B respectively, can now be estimated by use of Lineweaver–Burk or Hanes plots (Figure 6). These Kn values are comparable with the range (0.5–1.0 mM) that we previously estimated for enzymes extracted from wild-type developing maize leaves [10]. Thus both isoenzymes A and B more closely resemble isoform Ehf than Ei (see the Introduction section).

Cell wall analysis

Cell wall matrix polysaccharides were acid-hydrolysed and the monosaccharides analysed by paper chromatography and HPLC. The Xyl/Gal and Ara/Gal ratio was chosen to check the pentose/hexose ratio because Xyl and Ara are synthesized from the product of UDPGDH while the route to Gal, on the contrary, diverges before the enzyme of interest. Gal was taken as the representative hexose because some of the Glc in AIR would have arisen from starch rather than the cell wall matrix. It was shown that Ara/Gal and Xyl/Gal ratios were clearly reduced (by ~50%) in the cell wall polysaccharides of leaves defective in UDPGDH-A (Figures 7A and 7B), indicating an important role for UDPGDH-A in pentosan biosynthesis. In stems, on the contrary, the reduction in Xyl/Gal ratio was only 10%, suggesting that the mutants are capable of producing adequate UDP-GlcA, and hence UDP-Xyl, via a UDPGDH other than isoenzyme A in xylan-rich stems. The other possibility is that the myo-inositol oxidation pathway can produce sufficient UDP-GlcA in stems of maize, at least when UDPGDH activity is deficient.
Homozygotes for udpgdh-B exhibited more variability between individual plants. The difference in pentose/hexose ratio was not consistently obvious (Figures 7C and 7D), suggesting that isoenzyme B has a less important role than isoenzyme A in the synthesis of UDP-GlcA and its derivatives, at least in developing maize leaves.

The cellulose content expressed as percentage of AIR was not affected by these mutations (Table 2).

**DISCUSSION**

Reduced UDPGDH activity in the developing leaves of maize mutants obtained by insertion of a transposon into either of the two putative UDPGDH genes, here named as UDPGDH-A and UDPGDH-B, found in public databases supported the assumption that these genes encode functional UDPGDH isoenzymes. The corresponding deduced amino acid sequences are highly similar to each other and also to the soya bean [11], poplar [15], Arabidopsis [16] and tobacco [17] UDPGDH proteins. These observations are in agreement with a high amino acid conservation rate for this enzymic family. It is also possible that additional UDPGDH genes exist in maize, which could account for the occurrence of UDPGDH activities with widely differing $K_m$ values [10].

There is evidence for the existence of both UDPGDH activity and the myo-inositol oxidation pathway in plants, but their relative contributions to the UDP-GlcA pool (and therefore also pentose residues) are still unknown [7]. There has been controversy about the existence of UDPGDH in maize tissues. Roberts and Cetorelli [18] were not able to detect UDPGDH activity in extracts of 5-day-old maize seedlings. The presence of UDP-D-GlcA pyrophosphorylase activity was concluded to suggest that most UDP-GlcA might be synthesized via the myo-inositol oxidation pathway, which generates free GlcA, which must then be phosphorylated and nucleotidylated by a kinase and a pyrophosphorylase. In addition, in maize roots supplied with a high concentration of non-radioactive myo-inositol, the rate of incorporation of $^{14}$C from D-$[6-^{14}$C]Glc into wall-bound $[^{14}$C]galacturonic acid residues was significantly reduced compared with roots treated with lower inositol concentrations, although its incorporation into wall-bound glucose residues was unaffected [19]. This was interpreted as an indication that uronic acid residues of the wall polysaccharides are synthesized via the myo-inositol pathway. However, the observations do not constitute a proof of this hypothesis: it remains possible that although root cells can utilize myo-inositol when the latter is supplied exogenously (and thus acts as a ‘cold trap’, competing in the $[^{14}$C]Glc $\rightarrow$ UDP-$[^{14}$C]GlcA pathway), they normally do not synthesize enough myo-inositol in the appropriate subcellular location for this to be relevant in untreated roots in vivo. Plant cells possess several ‘scavenger’ pathways that may not normally play any major role in vivo [13].
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Figure 3 UDPGDH activities in developing maize leaves: radiochemical assay

(A) UDPGDH activities in two wild-types (wt) and two homozygous udpgdh-A1 mutant individuals (mut). (B) UDPGDH activities in two wild-types (wt) and two homozygous udpgdh-B1 mutant individuals (mut). Activities in ‘pkat’ are reported for the particular assay conditions used (30 µM UDP-[14C]Glc), no account being taken of the Km value(s) for this substrate. FW, fresh weight.

Table 1 UDPGDH/ADH ratios in leaves of wild-type and mutant maize individuals having an insertion in gene UDGDH-A or UDGDH-B

<table>
<thead>
<tr>
<th>Gene</th>
<th>Individual plant</th>
<th>UDPGDH/ADH ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>UDGDH-A</td>
<td>Wild-type 11</td>
<td>0.98</td>
</tr>
<tr>
<td></td>
<td>Wild-type 14</td>
<td>0.70</td>
</tr>
<tr>
<td></td>
<td>Wild-type 15</td>
<td>0.44</td>
</tr>
<tr>
<td></td>
<td>Mutant 1</td>
<td>0.24</td>
</tr>
<tr>
<td></td>
<td>Mutant 7</td>
<td>0.19</td>
</tr>
<tr>
<td></td>
<td>Mutant 8</td>
<td>0.23</td>
</tr>
<tr>
<td>UDGDH-B</td>
<td>Wild-type 10</td>
<td>0.38</td>
</tr>
<tr>
<td></td>
<td>Wild-type 26</td>
<td>0.67</td>
</tr>
<tr>
<td></td>
<td>Mutant 11</td>
<td>0.18</td>
</tr>
<tr>
<td></td>
<td>Mutant 24</td>
<td>0.29</td>
</tr>
</tbody>
</table>

It has been suggested that different pathways for UDP-GlcA synthesis predominate in different organs of the Arabidopsis seedling [16]. In seedlings up to 5 days old the UDGDH gene was mainly expressed in roots; however, older plants showed more uniform gene expression with preference for the vascular system. UDGDH protein concentration and enzymic activity correlated well with the mRNA levels. Radioactivity from [3H]inositol supplied in the culture medium, on the other hand, was more efficiently incorporated into cell walls of the hypocotyl and cotyledons than into those of the roots, suggesting the potential for an active myo-inositol oxidation pathway in these aerial organs (if sufficient inositol is present in them). Arabidopsis has three additional UDGDH genes that are expressed in a developmentally regulated way and often in a transient pattern that suggested that they are only expressed in cells with a need for precursors for cell wall biosynthesis [20].

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In the present study, two different isoenzymes of UDPGDH were observed with $K_m$ values for UDP-Glc of approx. 380 and 950 µM, coded for by genes UDPGDH-A and UDPGDH-B respectively. Both these $K_m$ values for UDP-Glc are high when compared with some published elsewhere. For example, $K_m \sim 18.7$ µM for UDP-Glc was estimated for UDPGDH purified from another gramineous plant, *Saccharum* spp. [21]. In the present study, $K_m$ values were estimated by use of crude cell extracts. It is possible that maize also contains an additional UDPGDH isoenzyme with a very low $K_m$ for UDP-Glc. In suspension-cultured cells of maize, there was an indication of the existence of a UDPGDH isoenzyme (‘E₁’) with a $K_m$ value of $\sim 28$ µM for UDP-Glc [10]. It will be of interest to identify the gene that encodes E₁. Purified isoenzymes are needed to resolve the definitive kinetic values.

The decrease in cell wall pentose content in *udpgdh-A* homozygotes suggests that isoenzyme A is essential for adequate UDP-GlcA and hence UDP-Xyl and UDP-Ara synthesis in maize leaves. The remaining UDPGDH activity (isoenzyme B) was evidently not enough to produce sufficient UDP-GlcA for cell wall pentose synthesis in these mutant plants. It seems that the myo-inositol oxidation pathway is not active enough to compensate for the reduced UDPGDH activity in UDP-GlcA production in developing leaves of maize. In the pathogenic fungus *Cryptococcus neoformans* a disruption of the UDPGDH gene led to a total lack of both UDP-GlcA and UDP-Xyl [22]. Interestingly, the Xyl/Gal ratio was reduced by only 10% in the stems of maize mutants defective in UDPGDH-A, and this decrease was not statistically significant (Figure 7B), suggesting that other isoenzymes of UDPGDH or the alternative myo-inositol pathway do (or can when necessary) produce enough UDP-GlcA in these tissues.

### Table 2  Cellulose content of wild-type and *UDPGDH* insertion mutants of maize

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Cellulose content (% of total AIR)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>First leaf</td>
<td>Third leaf</td>
<td>Stem</td>
</tr>
<tr>
<td>Wild-type-A</td>
<td>16.0 ± 1.0</td>
<td>19.2 ± 1.3</td>
<td>34.7 ± 4.4</td>
</tr>
<tr>
<td>udpgdh-A1 homozygote</td>
<td>19.4 ± 1.3</td>
<td>16.0 ± 0.8</td>
<td>37.4 ± 1.6</td>
</tr>
<tr>
<td>Wild-type-B</td>
<td>20.9 ± 1.8</td>
<td>n.d.</td>
<td>42.5 ± 1.3</td>
</tr>
<tr>
<td>udpgdh-B1 homozygote</td>
<td>17.6 ± 0.5</td>
<td>n.d.</td>
<td>38.2 ± 2.1</td>
</tr>
</tbody>
</table>

Data are means ± S.E.M. ($n$=5 for wild-type-A and *udpgdh-A1*, $n$=4 for wild-type-B and $n$=3 for *udpgdh-B1*). Means do not differ statistically significantly from each other as analysed by Student’s $t$ test ($P > 0.05$), n.d., not determined.
Developing stems were not assayed for UDPGDH enzymic activities in the present study. Cellulose content was not significantly affected in either of the mutants so any surplus UDP-Glc, accumulated because of the block in UDPGDH activity, must be directed elsewhere than into cellulose synthesis in these plants.

Conclusion

Mutants obtained by insertion of a transposon into UDPGDH genes offer a valuable means to study the importance of this enzyme activity in UDP-GlcA and hence cell wall pentose biosynthesis. According to our results it seems that knocking out UDPGDH-A reduces the cell wall pentose content, suggesting an important role for this enzyme in the biosynthesis of UDP-GlcA and other cell wall polysaccharide precursors in maize. However, loss of UDPGDH-B led to no change in cell wall composition. If there are multiple isoenzymes or alternative routes to the product of interest, as in the case of UDPGDH-B, then the knocking out of this may lead to no change in cell wall composition. Cell wall composition mutants like those discovered in this study give an excellent way of elucidating the role of sugar nucleotide interconverting enzymes in cell wall biosynthesis.

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REFERENCES

19 Roberts, R. M. and Loewus, F. (1973) The conversion of o-glucose-6-14C to cell wall polysaccharide material in Zea mays in presence of high endogenous levels of myo-inositol. Plant Physiol. 52, 646–650

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