Adaptation and Growth of Tomato Cells on the Herbicide 2,6-Dichlorobenzonitrile Leads to Production of Unique Cell Walls Virtually Lacking a Cellulose-Xyloglucan Network

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ABSTRACT

Suspension-cultured cells of tomato (Lycopersicon esculentum VF 36) have been adapted to growth on high concentrations of 2,6-dichlorobenzonitrile, an herbicide which inhibits cellulose biosynthesis. The mechanism of adaptation appears to rest largely on the ability of these cells to divide and expand in the virtual absence of a cellulose-xyloglucan network. Walls of adapted cells growing on 2,6-dichlorobenzonitrile also differ from nonadapted cells by having reduced levels of hydroxyproline in protein, both in bound and salt-elutable form, and in having a much higher proportion of homogalacturonan and rhamnogalacturonan-like polymers. Most of these latter polymers are apparently cross-linked in the wall via phenolic-ester and/or phenolic ether linkages, and these polymers appear to represent the major load-bearing network in these unusual cell walls. The surprising finding that plant cells can survive in the virtual absence of a major load-bearing network in their primary cell walls indicates that plants possess remarkable flexibility for tolerating changes in wall composition.

Plants have evolved a variety of complex cell wall polymers which serve various roles in determining cell size, shape, and structural strength; the wall also serves as a barrier against water loss and pathogen invasion, as well a source of oligosaccharides for chemical signaling (23). While it seems clear that differences in cell wall composition have evolved to optimize the functions of various cell and tissue types, it is not at all clear just how much flexibility plants possess for tolerating induced cell wall modifications, some of which might be of commercial importance.

An ideal way to study the flexibility of plants to induced cell wall modifications would be to select for mutants with altered cell wall composition. Unfortunately, due to lack of adequate selection techniques, characterized mutants in plant cell wall structure are virtually non-existent. Recently, Iraki et al. (11, 12) have shown that the cell walls of tobacco cells in suspension culture adapted to water or saline stress possess altered wall composition, indicating a fair degree of flexibility for change. In this paper, we describe the characteristics of a suspension-cultured cell line of tomato (Lycopersicon esculentum VF 36) that possesses a remarkably altered cell wall as a result of adaptation to growth in the presence of the herbicide DCB, an effective and specific inhibitor of cellulose synthesis (4, 5). In particular, the observation that these cells can divide and expand with the virtual absence of a cellulose-xyloglucan network indicates that plant cells do indeed possess surprising flexibility with respect to tolerance for alterations in cell wall composition.

MATERIALS AND METHODS

Adaptation and Growth of Cell Cultures

Suspension-cultured cells of tomato (Lycopersicon esculentum VF36) were obtained from B. Williams (Plant Cell Research Institute, Dublin, CA) and grown on MS medium as described by that group (7). Cells were adapted to growth on DCB by stepwise transfers in gradual increments on increasing levels of DCB beginning at 1 μM DCB. Approximately 15 to 20 generations of growth occurred between each step-up in DCB concentration. Growth curves were obtained by measuring the PCV from 2 mL samples taken at intervals from the cultures.

Microscopy

Cells were fixed in 3% (w/v) glutaraldehyde in 100 mM Na-phosphate buffer (pH 6.8) containing 3% (w/v) sucrose, encapsulated in agar, washed with the same buffer, postfixed with buffered 1% (w/v) OsO₄, then washed again in the same buffer. The samples were then dehydrated in an increasing series of acetone concentrations and embedded in Spurr’s epoxy resin. Blocks were sectioned with an LKB ultratome III ultramicrotome. Sections 3 to 5 μm thick were stained with toluidine blue and viewed and photographed with a Leitz light microscope.

Abbreviations: DCB, 2,6-dichlorobenzonitrile; ECPs, extracellular polysaccharides; NA cells, nonadapted cells; A(0), A(1), A(6), A(12), or A(20) cells, adapted cells growing on 0, 1, 6, 12, or 20 μM DCB, respectively; PCV, packed cell volume, expressed as a percent of total volume of culture; HRGPs, hydroxyproline-rich-glycoproteins; BGR, bound galacturonan-rich cell wall fraction; RG, rhamnogalacturonan.

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Preparation of Extensin Precursors, ECPs, and Cell Walls

All procedures were performed at 4°C, and all resulting samples of precursors, ECPs, and walls were stored frozen at −20°C. Cells were harvested 7 to 8 d after subculturing by filtration on a coarse sintered-glass filter. ECPs were precipitated from the filtered growth medium by addition of four volumes of absolute ethanol; after 18 h at 4°C, the suspension was centrifuged at 13,000g for 30 min. The pellet was resuspended in a small amount of water and reprecipitated with four volumes of ethanol; the last step was repeated twice, and the final pellet resuspended in water.

Filtered cells were washed several times with H2O and weighed. Five small aliquots were taken, weighed, dried at 70°C, and reweighed for fresh weight:dry weight determination. Extensin precursors were salt- eluted from intact cells with 50 mM CaCl2 and partially purified by removal of TCA- precipitable proteins as described elsewhere (20).

Cell walls were prepared from cells not extracted with CaCl2 by suspending a determined weight of water-washed cells in two volumes of 50 mM K-phosphate buffer (pH 6.8). Samples containing large clusters of cells (usually adapted cells growing with DCB) were initially dispersed in a blender. All cells were then broken in a Parr Cell Disruption Bomb; a sample was checked under the microscope to ensure complete cell disruption. The suspension was centrifuged at 20g for 5 min. Using the same time interval and speed of centrifugation, the resulting wall pellets were washed sequentially with 0.5 M K-phosphate buffer (pH 6.8) (2x), 1 M H2O (3−4x), 20% methanol to ensure plastid disruption (1x), and then the supernatant was clear (2−3x). The walls were then digested with α-amylase (porcine pancreas, Sigma; 100 units/mL in 50 mM K-phosphate buffer (pH 6.8) containing 0.05% w/v NaN3). Incubation was for 2 to 3 d at 30°C in a shaking water bath until all starch was hydrolyzed. The walls were then washed as above to 3 to 4 times in dH2O.

Extraction of Cell Walls

All extractions were carried out at 4°C. Clean cell walls were extracted overnight in 0.1 M NaEDTA (pH 7.0) and centrifuged at 13,000g for 5 min, and the pellet was reextracted for 1 h, followed by a brief wash in a small volume of dH2O. All supernatants were pooled as the EDTA-soluble fraction and dialyzed in 10 kD cutoff dialysis bags against several changes of dH2O for 2 to 3 d. Polymers soluble in 4% (w/v) KOH containing 0.1% (w/v) NaBH4 were obtained by two l-h extractions of the pellet; polymers soluble in 24% KOH/0.1% NaBH4 were obtained by extracting the resulting pellet again twice in this mixture. These extracts were neutralized on ice with concentrated acetic acid and dialyzed as above.

The alkali-insoluble pellet was then washed 5 to 6 times in small volumes of dH2O and all washes which contained substantial amounts of carbohydrate were pooled, dialyzed, and referred to as BGR. The remaining pellet is referred to as the Fibers fraction. All fractions were stored frozen at −20°C. Sodium chlorite extraction of EDTA-extracted walls was carried out as previously described (17) at 70°C for 30 min.

Analyses

Prior to assay, fractions which had insoluble material were homogenized, and aliquots were taken for assay while stirring the suspensions. Total carbohydrate was measured by the phenol-sulfuric acid method (6). Individual neutral sugars were quantified by GLC of alditol acetate derivatives (1); uronic acid content was measured according to Blumenkrantz and Asboe-Hansen (3), and protein was assayed using the Pierce Micro BCA Protein Assay. Xyloglucans were quantified according to Kooiman (15), and cellulose was determined by the Updegraff method (22). Methylation analyses were performed using the procedure of Blakeney and Stone (2). All GLC separations were carried out using a 15 m DB-225 capillary column (J & W Scientific, Davis, CA). Amino acid analyses were carried out as described elsewhere (21).

RESULTS

General Properties of DCB-Adapted Cells

Growth measurements show that NA cells, as well as A cells growing in media lacking DCB (Fig. 1A), have approximately the same growth rate, but reach different maxima in PCV. The A(0) cell line represents cells that had been adapted to growth on 25 μM DCB and then transferred back and grown on medium lacking DCB for about 125 generations (one generation = one doubling in PCV). Cells adapted to growth on 12 μM DCB [A(12)] grow with similar rates in the presence or absence of DCB; however, the maximal PCV achieved by A cells growing on DCB is lower (Fig. 1A).

NA cells turn black and die after several days of exposure to 3 μM (Fig. 1B) or 10 μM DCB (Fig. 1C). A(0) cells retain a relatively permanent ability to grow on DCB, since they again grow well on DCB after as many as 125 generations growth without the herbicide (Fig. 1, B and C). When returning to DCB, A cells have a longer lag phase, the length of which depends on the concentration of DCB and the length of time the cells had grown without DCB. Thus, the higher the concentration and the longer the time of growth without DCB, the longer the subsequent lag before growth resumes [compare A(0) cells returned to DCB and A(12) cells returned to DCB after only 30 generations of growth minus DCB].

NA and A(0) cells in culture appear as single cells or in small clusters of round cells (Fig. 2). A cells growing on DCB are irregularly shaped and exist as large clusters. Sometimes groups of cells appear to have developed from a single cell, and cell divisions within the cluster do not appear to be completely haphazard [note especially A(6) cells in Fig. 2]. At this level of magnification, no difference in gross wall structure can be discerned. Although some losses always occur during wall purification, and yields are somewhat variable from preparation to preparation, in general, we observe that the yield of cell walls per gram dry weight of cells is not significantly different for NA and A cells growing with or without DCB, averaging about 150 to 200 mg walls per g dry weight of cells. However, we have noticed that the volume of walls in aqueous solution per unit weight of walls is progressively larger in walls from A cells growing on progressively higher concentrations of DCB [e.g. the volume of A(12) walls per
Figure 1. Growth of NA and A cells in the presence or absence of DCB. (A) All cell lines grown in the absence of DCB, except the curve for A(12) cells growing on 12 μM DCB as indicated. (B) All cell lines grown in the presence of 3 μM DCB. (C) All cell lines grown in the presence of 10 μM DCB. (O-O) NA cells; (●-●) A(0) cells maintained at least 125 generations in the absence of DCB; (△-△) A(12) cells grown 80 generations in 12 μM DCB and then 30 generations in the absence of DCB; (A-A) A(12) cells transferred directly from 12 μM DCB to 12 μM DCB.

unit wt is about twice that of NA walls], suggesting that these walls are more highly hydrated than normal walls.

**Cellulose-Xyloglucan**

The most remarkable difference between A cells growing on DCB and NA or A(0) cells is that the cell walls from A(6), A(12), and A(20) cells contain almost no cellulose [6, 3, and 0.7 μg cellulose per mg cell wall, respectively, compared to 176 and 146 μg per mg cell wall, respectively, in NA and A(0) walls (Fig. 3A)]. Methylation analyses also confirm that the walls of A cells growing on DCB contain greatly reduced levels of cellulose as judged by the substantially reduced quantity of 4-linked glucose in the walls. This finding, as well as the fact that the level of noncellulosic glucose is not substantially elevated in such walls, indicates that the synthesis of cellulose, and not just its crystallization into microfibrils is inhibited. (Noncrystalline cellulose would most likely be hydrolyzed by 2 N trifluoroacetic acid and be detected as noncellulosic.

Figure 2. Photographs of NA and A cells fixed and stained with toluidine blue. Bar = 20 μm.
DCB-ADAPTED CELLS LACK CELLULOSE-XYLOGLUCAN NETWORK

![Graphs and diagrams]

Figure 3. Cellulose and xyloglucan content in NA and A cell walls. (A) Content of cellulose and xyloglucan (XG) as determined colorimetrically by the Updegraff method for cellulose (22) or by formation of iodine complex (15) for xyloglucan present in 24% KOH extract of walls. (B) Content of Xyl and Glc (noncellulosic) and Man in 24% KOH extract of walls as determined by GLC of alditol acetates (1). (C) As for B except determined for the ECPs.

Table I. Analyses of Hydroxyproline Content in NA and A Cells

<table>
<thead>
<tr>
<th>Cells</th>
<th>Hydroxyproline Content</th>
<th>Hydroxyproline Content</th>
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<tbody>
<tr>
<td></td>
<td>Extensin precursors</td>
<td>Wall-bound proteins</td>
</tr>
<tr>
<td></td>
<td>µmol/g dry wt cells</td>
<td>µmol/g dry wt cells</td>
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<tr>
<td></td>
<td>mol%</td>
<td>µmol/g dry wt walls</td>
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<td></td>
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</tr>
<tr>
<td>NA</td>
<td>11.2</td>
<td>11.3</td>
</tr>
<tr>
<td>A(0)</td>
<td>3.6</td>
<td>5.7</td>
</tr>
<tr>
<td>A(1)</td>
<td>3.4</td>
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<td>A(6)</td>
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<td>6.1</td>
</tr>
<tr>
<td>A(12)</td>
<td>1.9</td>
<td>4.8</td>
</tr>
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</table>

glucose during GLC of alditol acetate derivatives.) Since we also did not observe significant levels of 3-linked glucose in any of these walls, there is no evidence that callose [(1→3)-β-glucan] is deposited in place of cellulose during growth on DCB.

Analyses show that the bulk of the wall-bound xyloglucan of NA cells is found in the 24% KOH cell wall extract. Analyses of this extract show that, as for cellulose, A(6), A(12), and A(20) walls also contain greatly reduced levels of xyloglucan (Fig. 3A). The above data for xyloglucan, as determined colorimetrically (15), are further confirmed by the notable decrease in Xyl and Glc in the 24% KOH extract of walls from A cells grown on DCB (Fig. 3B). In contrast, we find greatly enhanced levels of xyloglucan in ECPs of such cells, as determined colorimetrically (Fig. 3B) or by neutral sugar analysis (Fig. 3C). Methylation analyses indicate that the 24% KOH extract from NA walls contains substantial amounts of linkages characteristic of xyloglucan (terminal- and 2-linked Xyl, 4- and 6,4-linked Glc). The levels of these linkages are greatly reduced in this extract from A(12) walls; instead, they are found in increased quantities in the ECPs of these cells (not shown). Thus, we find that synthesis of xyloglucan is not inhibited in A cells growing on DCB; instead, this polymer is secreted in enhanced amounts as an ECP.

The 24% KOH extract from NA walls probably also contains some (1→4)-β-xylan as evidenced by the presence of 4-linked Xyl. Essentially all of the wall-bound Man is found in this fraction in linkages characteristic of (gluco)mannan (4and 4,6-linked Man; 4-linked Glc). As for xyloglucan, we find the level of these linkages, as well as the level of total Xyl and Man, decreases in the cell wall and increases in the ECPs of A cells growing on progressively higher concentrations of DCB (Fig. 3B, B and C).

Protein

No significant difference is found in the percent by weight total protein in cell walls from NA and A cells growing with or without DCB; based upon amino acid analyses of total walls, we recover about 4 to 5% of the weight of all wall types as protein. We considered it possible that these cells might have compensated for loss of the cellulose-xyloglucan network by elevating the level of cross-linked HRGPs in the wall (8, 23). However, amino acid analyses indicate that the absolute amount, as well as the mol% bound Hyp is actually decreased.
Glycin levels of amount absolute similar are in for fraction in the ECPs function of DCB walls from cells growing Hyp which cells however, all from fraction in A(6) polysaccharides, amino acids in many from the ECPs. Neutral sugars were also found in A(12) cells. 

It is clear that the percent of the wall which is EDTA-extractable is substantially higher in adapted cells growing on DCB (Fig. 4). However, Figure 4 also shows that the major fraction found in these walls is a fraction which is insoluble after 24% KOH extraction, but is solubilized by subsequent water washing of the alkali-insoluble residue (labeled H2O in Fig. 4). This fraction is also present in NA and AO walls, but represents a lower percentage of the wall.

A compositional analysis of this fraction is shown in Table II. Both in NA and A(12) cell walls, this fraction is rich in components characteristic of a mixture of homogalacturonans and RGs I and II, thus we refer to it as BGR (for 'bound galacturonan-rich'). Traces of 2-O-methyl-Fuc, 2-O-methyl-Xyl, apiose, 3-deoxy-manno-octulosonic acid, and aceric acid are detectable, indicating the presence of small amounts of RG II (16). Based on the ratio of uronic acid: Rha, about 80% of BGR in NA walls could be long stretches of homogalacturonan and about 20% RG I, assuming a ratio of 1:1 for uronic acid: rha in RG I (13). In A(12) walls these ratios change to 90% and 10%, respectively. Ara and Gal are present in BGR in quantities consistent with their being components of a typical RG I (13). There are increased amounts of xylose and glucose in BGR from A(12) walls compared to NA walls, suggesting the presence of some additional xylan and xyloglucan. However, these amounts in no way compensate for the notable decrease in xyloglucan levels found in the 24% KOH fraction from these walls.

Since the weight percent cellulose and xyloglucan are significantly lower in walls of A cells growing on DCB, the weight percent of remaining components must increase proportionally even if absolute levels remain the same. However, it can be seen in Table II that the amount of BGR synthesized per weight of cells is also increased, indicating that there is an overall stimulation of synthesis of these polymers in A cells growing on DCB.

Analyses of this fraction performed for us by the Complex Carbohydrate Center (University of Georgia) further support the conclusion that this fraction is composed of a mixture of

<table>
<thead>
<tr>
<th>Table II. Composition Analysis of BGR</th>
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<tbody>
<tr>
<td>Component</td>
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<tr>
<td>---------------</td>
</tr>
<tr>
<td>Rha*</td>
</tr>
<tr>
<td>Ara*</td>
</tr>
<tr>
<td>Xyl*</td>
</tr>
<tr>
<td>Gal*</td>
</tr>
<tr>
<td>Glc*</td>
</tr>
<tr>
<td>Other</td>
</tr>
<tr>
<td>Traces of 2-O-me-Fuc, fuc, 2-O-me-Xyl, apiose, 3-deoxy-manno-octulosonic acid, and aceric acid in both NA and A(12)</td>
</tr>
<tr>
<td>Uronic acidb</td>
</tr>
<tr>
<td>Uronic:Rha</td>
</tr>
<tr>
<td>Protein</td>
</tr>
</tbody>
</table>

* Determined by GLC of alditol acetates.  
* Quantified by colorimetric assay; GLC of trimethylsilyl derivatives indicates that >95% is present as galacturonic acid and the remainder as glucuronic acid.

BGR

Since it is clear that walls from adapted cells growing on DCB contain greatly reduced levels of cellulose, xyloglucan, and, to a lesser extent also HRGPs, the question arises as to what are the major load-bearing polymers in these walls.

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Figure 4. Recovery of total noncellulosic neutral sugars (A) and uronic acids (B) during sequential extraction of polymers from NA and A cell walls. Neutral sugars were determined by analyses of alditol acetates (1); uronic acids were determined colorimetrically (3).
Table III. Wall Components Released from EDTA-Extracted Walls by Chlorite

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Uronic Acids*</th>
<th>Neutral Sugars*</th>
<th>Total Extracted</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg/g original cell wall</td>
<td>wt% of wall</td>
<td>mg/g original cell wall</td>
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<tr>
<td>NA</td>
<td>51</td>
<td>61</td>
<td>11.2</td>
</tr>
<tr>
<td>A(0)</td>
<td>54</td>
<td>41</td>
<td>9.5</td>
</tr>
<tr>
<td>A(6)</td>
<td>191</td>
<td>104</td>
<td>29.5</td>
</tr>
<tr>
<td>A(12)</td>
<td>217</td>
<td>141</td>
<td>35.7</td>
</tr>
<tr>
<td>A(20)</td>
<td>235</td>
<td>134</td>
<td>36.9</td>
</tr>
</tbody>
</table>

* Determined colorimetrically.  
* Corrected for background due to uronic acids.

RG I and II and homogalacturonan. Nearly all of the material in BGR from NA walls voids a Bio-Gel A-0.5M column; the same fraction from A(12) walls behaves similarly but is somewhat more heterogeneous, and on the average, slightly smaller in size. Following treatment with endopolygalacturonase (13), 60 to 70% of the uronic acids of NA-BGR are converted to free galacturonic acid, consistent with the presence of a substantial amount of homogalacturonan. About 25% of the total uronic acid and sugar remained in the void volume after treatment, and the rest (excluding that converted to free sugars) was converted to polymers which migrated as two separate peaks in the included volume. The smaller of these contains all of the unique sugars characteristic of RG II, while the larger included peak, as well as that remaining in the void volume, resemble in composition RG I type of polymers. Based on peak areas, the ratio of RG I type to RG II type polymers is about 4:1. A similar degradation pattern was obtained for BGR from A(12) walls, again indicating a great deal of similarity in these fractions.

In titration experiments with KOH or KCl, we find that the represipitation of the BGR polymers in water occurs upon addition of roughly equimolar concentrations of either KOH or KCl, and it appears that insolubility after the 24% KOH extraction is due to a combination of high concentration of polymer and high salt in the extract rather than a specific pH effect. Nevertheless, it is clear that prior alkali treatment is necessary to render the polymers soluble in water. Several preliminary experiments indicate that these polymers may be cross-linked in the wall via conjugated phenolics coupled to the polymers via ester and/or ether linkages. Table III shows that a relatively brief (30 min) treatment of EDTA-extracted walls with chlorite, which oxidizes aromatic rings and thus cleaves linkages between conjugated phenols (19), releases a low proportion (9-11%) of the walls of NA and A(0) cells, and a very much higher proportion (30-37%) of the walls from A cells grown on DCB, and that the polymers released are rich in uronic acids.

We have also analyzed material extracted at pH 2 into ethyl acetate or n-butanol from walls treated with mild alkali to cleave ester linkages (0.1 M NaOH, 18 h, 25°C). UV-absorbing material is released from all types of walls by this procedure; on TLC, it remains immobile under conditions wherein ferulic and diterulic acid are mobile, suggesting the presence of larger phenolic oligomers which may exist in ester-linkage in the wall. Mild alkali treatment releases about twice the amount of carbohydrate from A(12) walls than from NA walls; however, for both types of walls, mild alkali releases <20% the amount of carbohydrate as does treatment with chlorite.

We consider that cross-linking of polysaccharides to proteins is a less likely possibility since treatment of EDTA-extracted walls with protease K did not release significant amounts of material. However, we cannot totally rule out the possibility of a protease-resistant protein being involved in cross-linking.

Returning to Figure 4, we note that a substantial amount of noncellulosic neutral sugars and uronic acids remain in the fiber fraction of NA and A(0) walls. This fraction is virtually nonexistent in A(6), A(12), and A(20) walls. Table IV shows the composition of this fraction in NA cells. With exception of the presence of cellulose, this fraction resembles BGR. Although the protein content is low, it is definitely enriched for HRGPs, since the molepercent Hyp is 22% in this fraction from NA walls and 36% in the fraction from A(0) walls, and the overall amino acid composition indicates that the fraction contains extensin-like protein(s). Here again, we have observed that, for A(1) walls, the molepercent Hyp in this fraction is decreased (to 12 mol%) and that of gly is markedly increased (from 7 to 8 mol% in NA and A(0) to 16 mol% in this fraction from A(1) walls), perhaps indicative of the additional presence of some unique Gly-rich protein in A cells growing on DCB.

**DISCUSSION**

We initiated selection of DCB-resistant cells with the expectation of creating a cell line with a receptor for the herbicide (see refs. 4 and 5) that possessed a greatly lowered affinity for DCB and therefore was able to synthesize cellulose in the presence of much higher concentrations of the herbicide. To our surprise, we obtained instead a cell line, the cells of which have “learned” to divide and expand with a dramatically altered cell wall structure which virtually lacks a cellulose-xylolignan network. Thus, we conclude that the pathway for cellulose synthesis remains inhibited by DCB in these cells as evidenced by their virtual lack of cellulose when grown on high concentrations of DCB. The capacity for cellulose synthesis, however, still exists, since these cells restore cellulose to the wall when returned to medium lacking DCB (Fig. 3A; walls of A(0) cells). Thus, “resistance” to DCB in this cell line results from the ability to survive without cellulose.

Synthesis of xylolignan is clearly not inhibited by growth.
on DCB; however, lacking a cellulose network with which to interact, this polymer is largely secreted to the medium. Thus, this finding further confirms the current model which states that xyloglucan is largely bound in the wall to the cellulose microfibrils.

With respect to protein, it is clear that the level of HRGPs is not elevated as a compensatory mechanism for loss of the cellulose-xyloglucan network, since analyses actually show a significant decrease in the levels of Hyp in these walls, both in the bound and salt-elutable form. This finding is similar to that found by Iraï et al. (11, 12), who found that the mol% hyp in tobacco cells adapted to water or saline stress was only 10% that of nonadapted cells. It is interesting that there are other similarities between the walls of salt-adapted cells and our tomato A cells growing on DCB. Most notable is that Iraï et al. (11, 12) also observed a decrease in cellulose and xyloglucan content, although the reduction observed was only twofold, and therefore not so drastic as that observed by us. Although their rather different extraction and analysis techniques make comparisons tentative, it does not appear that the walls of saline-adapted cells have markedly increased the content of a fraction equivalent to the BGR fraction of tomato. Instead, they observe an increase in the percent of pectic components extracted by chelators as well as changes in branching pattern of these polymers, the latter being a property we have not investigated yet in detail. Other differences are that they do not observe enhanced xyloglucan secretion in these cells, and that the cell size is much smaller in adapted cells. They suggest that some possible inhibition of xyloglucan turnover may limit cell expansion. We do not observe a decrease in xyloglucan secretion nor in cell size indicating our cells are not inhibited in these respects. The tensile strength of saline-adapted cell walls is markedly reduced. We have not yet quantified tensile strength; routine handling and breaking of the cells leads us to suspect that the walls of A cells growing on DCB are less strong, but the cells are clearly not osmotically fragile, as they do not burst in water. A cells should be an ideal system to study the contribution of various polymers to tensile strength, since we can vary the quantity of cellulose and xyloglucan as desired by growing cells on various DCB concentrations.

It appears that the major load-bearing network in A cells growing on DCB is composed of homogalacturonan and rhamnogalacturonan polymers cross-linked via phenolic residues. Further work will be necessary to clarify the types of cross-links involved; at least partial lability to mild alkali treatment and release of some phenolic material suggests that some phenolic-ester cross-links are involved; however, preliminary results indicate that chlorite treatment after mild alkali releases some additional polysaccharides, indicating that some phenolic-ester bridges may also be involved, as indicated also by the work of Selvendran for the pectic polymers of certain plant tissues (18). The existence of substantial amounts of "bound pectins" in primary cell walls of dicots is receiving increased attention (8, 13, 18, 19), and it appears that walls of A cells growing on DCB represent an extreme example of this.

At present, we also cannot rule out cross-linked networks involving protein and/or protein cross-linked to polysaccharides. Although Hyp levels are substantially lower, we do not know if different types of HRGPs may be present. The observation that walls of A cells growing on DCB contain more Gly is intriguing and may indicate the presence of some type of Gly-rich protein as has been found in several types of plant walls (see ref. 14 and refs. therein). We should also note that a summation of all the components analyzed leads to a recovery of only about 75% of our original measured wall weight both for NA and A(12) walls. Some of the unaccounted for weight undoubtedly comes from bound ions, phenolic components, and/or tightly bound water not removed in our drying procedure. Nevertheless, we cannot rule out the possibility of other components of importance to overall wall structure.

Adapted cells clearly possess some relatively stable change which allows them to "remember" how to grow on DCB, although we know nothing of the genetic or epigenetic nature of this change. The parent cell line is not an easy one to regenerate, and we have not attempted to regenerate either NA or A cells. Instead, we have recently selected a DCB-adapted tobacco cell line for testing the heritability of this trait. The only obvious stable change in wall structure which we observe is the reduced level of Hyp, and this may offer some clue as to the mechanism of "memory." Since normal plant protoplasts will not regenerate a wall in the presence of DCB (9), it will be of interest to know if the memory mechanism will allow tomato A cells to do so. Other obvious questions for the future concern the pore size of such walls, and whether or not the putative cellulose synthase complexes observed by freeze-fracture (4) exist in A cells growing on DCB. Since cells of the monocot grasses contain much less uronic-rich polymers of the type found in BGR, it will be of interest to note their mechanism of adaptation to DCB. We know that they can do so, since we have a cell of line of barley endosperm so adapted.

Finally, these results do indicate that plant cells possess remarkable flexibility for tolerating changes in cell wall structure. Although it is unlikely that cells with walls resembling those of A(12) cells could undergo complex morphogenetic changes required for development of an intact plant, the results nevertheless indicate that some potential probably does exist for interesting, and perhaps useful, induced modifications in wall structure in higher plants. Recent advances in genetic engineering in plants offer an additional approach for testing this possibility.

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LITERATURE CITED

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