

Commentary

Lignification of plant cell walls: Impact of genetic manipulation

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Lignin is the second most abundant polymer found in nature after cellulose. Among the many roles lignin plays in plant growth and development are those providing structural support for land plants and as a mechanical barrier in pest resistance. Although vital to plant fitness, lignin negatively impacts paper pulp processing and feed for livestock. Much of the cost, complexity, and pollution associated with the pulping process derives from the removal of lignin from wood to make high-quality paper. Similarly, the amount of digestible energy available to ruminant livestock from fermentation of cell-wall polysaccharides is restricted by lignin, rendering much of the cellulose and hemicellulose inaccessible to rumen microorganisms. Because of the sizable economic benefits that might be achieved, considerable research effort has been targeted toward reducing the amount of lignin or modifying lignin structure to facilitate pulping of trees and improving digestibility of forage crops. As a result, transgenic experiments assessing genes for many of the steps in the lignin biosynthesis pathway have provided important insights (1–3).

In this issue of the *Proceedings*, Ralph *et al.* (4) report on a number of novel structures incorporated into lignins of transgenic tobacco plants down-regulated by homologous antisense genetic constructs of cinnamoyl-CoA reductase (CCR) and cinnamyl alcohol dehydrogenase (CAD). The enzyme products of these genes are responsible for catalyzing the last two steps in the biosynthesis of lignin precursors (CCR converts hydroxycinnamoyl-CoA derivatives to hydroxycinnamaldehydes and CAD reduces these aldehydes to the hydroxycinnamyl alcohols) before export of monolignols into the cell wall where polymerization occurs. Using an antisense CCR transgenic tobacco line, Ralph *et al.* (4) demonstrated that the tyramine ferulates previously shown to be present in cell walls of normal tobacco (5) actually are incorporated into the lignin polymer. Nuclear magnetic resonance analysis was used to authenticate the presence of feruloyl 4-O and 5-position linkages of the tyramine ferulates to lignin. An increase in the syringyl-to-guaiacyl (S/G) ratio of lignin also was found, and this change resulted from decreased guaiacyl units in the lignin of CCR transgenic tobacco (4). In contrast to the antisense CCR transgenic tobacco, the antisense CAD transgenic line accumulated aldehyde structures in lignin rather than tyramine ferulates. In addition to increases in cinnamaldehyde and benzaldehyde, a new group of aldehyde structures not previously reported in plant lignins were found to be predominant components of the lignin. Ralph *et al.* (4) used NMR analysis to demonstrate that these new aldehydes are derived from coniferaldehyde and sinapaldehyde. Associated with this increase in aldehyde components was a decrease in the coniferyl and sinapyl alcohol-derived structures found in normal tobacco lignin. The alteration in synthesis of syringyl lignin caused by the antisense CAD transgene was apparently greater than for guaiacyl lignin because a reduction in the S/G lignin ratio was observed in the transgenic tobacco.

As stated by Ralph *et al.* (4), these unusual lignin structures are further evidence of the apparent plasticity of lignin formation in plants where numerous phenolic precursors can be incorporated into the polymer. Similar aldehyde structures to those observed for the down-regulated CAD transgenic tobacco would be expected in the brown midrib-1 maize mutant, which recently was shown to be a CAD mutation (6). Ralph *et al.* (4) noted that a natural CAD-deficient mutant in loblolly pine (7) also accumulated aldehyde structures, although not to the same degree as the tobacco CAD transgenic. But the pine CAD mutant also had an additional unusual lignin component derived from dihydroconiferyl alcohol that was not observed in the CAD transgenic tobacco. These observations demonstrate how flexible plants are in adapting to metabolic change related to lignin biosynthesis. Plants appear to have adapted several strategies to circumvent genetic and metabolic obstacles to making lignin. In addition to using various monolignol precursors, plants also appear to possess a metabolic grid for monolignol biosynthesis (8). In the metabolic grid, similar reactions are carried out by the same enzyme or isozymes with differential substrate specificity (9, 10). These enzymes are encoded by multigene families and are differentially expressed during development and in response to environmental stress. The flexibility of the metabolic grid yields lignins that differ in composition and structure according to cell types and tissues, stages of development, and in response to environmental stimuli (9). It should be noted that although there is some controversy as to whether such unusual lignin structures as reported by Ralph *et al.* (4, 7) are actually incorporated into the lignin polymer of the cell wall (11), current evidence strongly supports such a conclusion.

Another important observation made by Ralph *et al.* (4) is that normal tobacco appears to produce a “bulk” type lignin where monolignols react to form dimers before incorporation into a polymer. Theirs is the first report of a plant lignin that contains the high proportions of the α -ethers and cinnamoyl alcohol endgroups found in synthetic lignins caused by bulk polymerization rather than the low concentrations of these structures in lignins derived from the typical endwise addition process generally believed to occur in plants. Given this unusual pattern of lignin biosynthesis in tobacco it will be of interest to learn whether other plant species exhibiting the typical endwise lignin polymerization pattern deposit unusual lignin structures similar to those observed in tobacco in response to genetic modifications.

Although it might have been expected that down-regulation of enzymes in the lignin biosynthetic pathway should reduce lignin concentration caused by reduced precursor supply, research with natural mutants and transgenic plants has shown that the results of such down-regulation are very dependent on the enzyme involved. Reduction in CCR activity has been reported to decrease lignin concentration in tobacco and

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Arabidopsis (4, 12, 13). In a conflicting report, Kajita *et al.* (14) found that lignin concentration was not reduced in sense-suppressed and antisense CCR transgenic tobacco. This study (14) was also unusual in that it reported a reduced S/G lignin ratio whereas the other reports on down-regulated CCR transgenics found increased S/G ratios (4, 12, 13). In contrast to the situation with CCR transgenics, all reports on down-regulated CAD transgenics have found no reduction of lignin deposition and a consistent decline in the S/G lignin ratio (15–18). Obviously we still have much to learn about the control points in the lignin biosynthesis pathway.

It was noted by Ralph *et al.* (4) that although the antisense CAD transformant was normal in appearance and growth, other than a red-brown xylem color reminiscent of brown midrib mutants in maize and sorghum, the antisense CCR transformant had both altered xylem color (orange-brown) and reduced growth with abnormal leaf morphology and collapsed vessels. This phenomenon of abnormal plant growth and development in some transgenic plants with altered lignin biosynthesis had been reported previously. The abnormal growth appears to be limited to cases where lignin concentration has been markedly reduced by the transgene (4, 12, 13, 19, 20). The abnormality probably is associated with a weakened vascular system caused by collapsed vessels (13). In contrast, even very extreme changes in lignin composition, such as the complete absence of syringyl-type lignin caused by down-regulation of the ferulate 5-hydroxylase (F5H) gene in the *Arabidopsis fah1* mutant (21), have not altered growth and development when lignin concentration has remained normal. This finding suggests that lignin quantity may play a more important role than lignin composition and structure in overall growth. Improved understanding of how much lignin plants need for normal growth and development will be of great importance to successful manipulation of lignin biosynthesis.

Substantial progress is being made in modification of lignin biosynthesis through biotechnology; however, the critical issue is whether the modifications will achieve the desired practical benefit. In the area of improving cell-wall digestibility of forage crops the results have been mixed. Bernard Vailhe *et al.* (22, 23) reported improved cell-wall digestibility of down-regulated *O*-methyltransferase (OMT) and CAD transgenic tobacco plants. The increased digestibility was attributed to an altered lignin composition (reduced S/G ratio) because lignin concentration was not changed in these transgenics. In contrast, Sewalt *et al.* (24) attributed the improved cell-wall digestibility of down-regulated phenylalanine ammonia-lyase and OMT tobacco transgenics to the observed reduction in lignin concentration rather than the altered lignin composition of these transformants. Finally, recent work in our laboratory with the F5H *Arabidopsis* mutant detected no change in cell-wall digestibility even though lignin composition was shifted dramatically from a mixed syringyl/guaiacyl lignin to a syringyl-free lignin by this mutation (25). Further work remains to be done to define what types of lignin modifications will improve cell-wall digestibility and how to achieve such changes through biotechnology. However, data from the brown midrib mutants clearly indicate that altered lignification can result in forage crops with improved cell-wall digestibility.

Limited information is also available on the impact genetic manipulation of lignification has on the pulping process. Halpin *et al.* (26) reported that down-regulated CAD transgenic tobacco required less bleaching for pulp production. Soon afterward Baucher *et al.* (16) reported that down-regulated CAD transgenic poplar exhibited a reduced kappa number during pulping, indicating more efficient lignin removal, both in 3-month-old seedlings and after 2 years of growth. Recently it was found that down-regulated *O*-methyltransferase transformants of poplar had higher kappa numbers for pulping (W. Boerjan, personal communication), indicating that the reduced syringyl-rich lignin produced a

lignin structure less amenable to extraction than the aldehyde-rich lignin of the CAD transformants.

It is clear that lignification can be extensively modified through biotechnology, although the phenotype of the resultant plants is not always predictable because of the complex nature of the biosynthetic pathway for lignification. However, such genetic manipulations have increased our awareness of the intricacies and complexity of lignin synthesis. Ralph *et al.* (4) now have increased our knowledge about the types of precursors plants can incorporate into lignin in response to genetic manipulation. With increased understanding of lignification it appears reasonable to expect that useful technologies will emerge for improving paper pulp production and feed utilization of fibrous crops in the not too distant future.

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