Effect of Lignin Composition on Cell-Wall Degradability

H.G. Jung and C.C.S. Chapple

Introduction

Lignification of forage cell walls is regarded as the primary mechanism whereby ruminal cell-wall degradability is inhibited. The USDFRC Cell Wall Group has identified ferulic acid cross-linking of lignin to polysaccharide in grass cell walls as an important modifier of the negative effects of lignin on cell-wall degradability. Another hypothesis which has been proposed to explain observed variation in the effect of lignin on cell-wall degradability regards the composition of lignin. Most lignin in forage crops is derived from coniferyl and sinapyl alcohol monolignols. These monolignols differ only in the presence of an extra methoxyl group on the aromatic ring of sinapyl alcohol compared to coniferyl alcohol. This extra methoxyl will prevent bonding of sinapyl monolignols at the C-5 position which should result in a more linear, less highly branched lignin resulting from predominantly sinapyl monolignols. The brown midrib (bmr) mutants of annual C_4 grasses such as maize have defects in lignin biosynthesis such that their lignin is richer in coniferyl alcohol monolignols. These bmr mutants also have cell walls which are more extensively degraded than the normal type. As a result, it has been proposed that a shift from a typical mixed coniferyl/sinapyl alcohol derived lignin to lignins richer in coniferyl alcohol units should alter cell-wall degradability. The identification of a genetic mutant in *Arabidopsis thaliana* that is incapable of producing any sinapyl alcohol affords the opportunity to test the most extreme manifestation of this hypothesis concerning the effect of lignin composition of cell-wall degradability.

Materials and Methods

Wild-type and mutant *Arabidopsis* plants, back-crossed to the wild-type parent for either 2 (*fah*1-2) or 5 (*fah*1-5) generations, were grown in the greenhouse. After initiation of flower stalk development, plants were harvested at weekly intervals to yield stem samples of four different maturities. Approximately 450 plants of each genotype were harvested at each maturity stage. Two replications of the experiment were conducted. The stems were lyophilized, ground to pass a 1-mm screen in a cyclone mill, and analyzed for cell-wall composition. In vitro degradability of cell-wall polysaccharides was determined after 24- and 96-h incubations with rumen fluid.

Results and Discussion

Previous research using nitrobenzene oxidation and pyrolysis-GC-MS demonstrated that the mutant *Arabidopsis* plants produce no sinapyl alcohol derived lignin. While cell-wall concentration and lignin concentration in the cell wall changed with maturity (Fig. 1), virtually no differences in cell-wall composition among the *Arabidopsis* genotypes were observed (Table 1). It should be noted that the cell-wall composition of *Arabidopsis* is very similar to legumes such as alfalfa. The only significant effect of the mutation preventing sinapyl alcohol biosynthesis was an increased concentration of ferulic acid esters in the mutant lines. Degradability of the cell-wall polysaccharides did not differ among genetic lines. As expected, degradability declined with maturity, but there was no increase in degradation associated with the longer fermentation interval (Fig. 2). This indicates that those cell-wall polysaccharides which are susceptible to degradation are degraded very rapidly. This is similar to the pattern for cell-wall degradability seen in legume forages.

Conclusion

While *Arabidopsis* will never be a forage crop for dairy cattle, its cell-wall composition suggests that this species can serve as a model for legumes. Our results indicate very clearly that when lignin concentrations are the same, as was the case for these *Arabidopsis* lines, lignin composition does not impact cell-wall polysaccharide degradation. These results also imply that the effect of the bmr mutation in maize and other grasses on cell-wall degradability is probably a result of reduced cell-wall development and lignin concentration rather than the alteration in lignin composition.

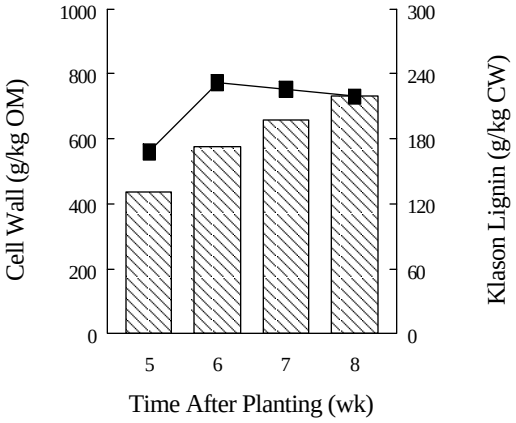

Figure 1. Change in cell-wall concentration (bar) and lignification (line) of Arabidopsis stems harvested at different stages of development, averaged across the three genetic lines.

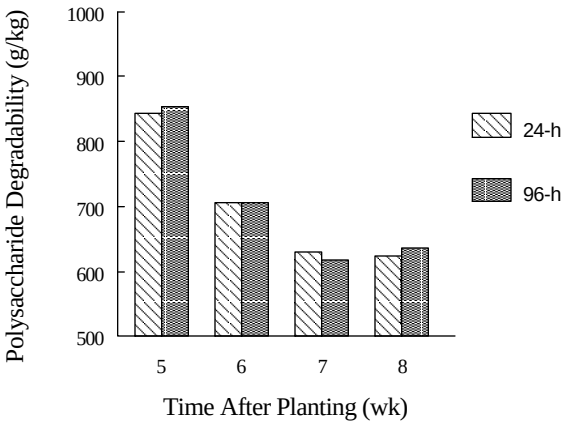

Figure 2. In vitro ruminal cell-wall polysaccharide degradability of Arabidopsis stems harvested at different stages of development after 24- and 96-h fermentations, averaged across the three genetic lines.

Table 1. Stem cell-wall composition of *Arabidopsis* lines averaged across maturity stages.

	Genetic line			
Cell-Wall Trait	Wild-type	*fah*1-2	*fah*1-5	SEM
Cell wall, g kg^{-1} OM	589	615	600	14
Composition, g kg^{-1} CW				
Neutral sugars	577	574	573	7
Uronic acids	207	205	207	3
Klason lignin	208	212	213	7
Ferulic acid				
esters	0.50[a]	0.82[b]	0.94[b]	.10
ethers	4.94	5.54	5.01	.43
p-Coumaric acid				
esters	0.48	0.55	0.37	.08
ethers	1.87	1.13	1.24	.29
Molar proportions of neutral sugars, mol 100 mol^{-1}				
Glucose	57.3	57.5	57.6	.3
Xylose	21.6	21.8	21.1	.3
Arabinose	4.8	4.7	4.9	.1
Galactose	6.9	6.9	7.2	.2
Mannose	5.8	5.9	6.1	.1
Rhamnose	3.1	2.8	2.9	.1
Fucose	0.43	0.42	0.35	.04

[ab]Means in the same row not sharing a superscript are different ($P < 0.05$).