

Chemical Composition and Degradability of Xylem and Nonxylem Walls Isolated From Alfalfa

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Introduction

The digestibility of forage legumes declines significantly with plant maturation due to decreasing digestibility of stems and their increasing contribution to plant dry matter. Microscopic studies indicate that, as stems mature, xylem and interfascicular parenchyma form a ring of tissue that is highly resistant to digestion. Other tissues, including pith parenchyma, mesophyll, phloem, phloem fibers, collenchyma, and epidermis appear to have high degradability even in mature stems. These two types of tissues were isolated from alfalfa internodes and analyzed for cell wall composition and carbohydrate degradability as an initial step to elucidate factors limiting fiber degradability in legumes.

Methods

Xylem (xylem and interfascicular parenchyma) and nonxylem tissues (pith parenchyma, mesophyll, phloem, phloem fibers, collenchyma, and epidermis) were isolated by dissection from internode 1 (basal) to internode 6 of Vernal alfalfa plants harvested at the late bud to early flower stage of development. Crude cell walls were prepared by sequentially treating milled tissues with 50 mM NaCl, 80% ethanol, amylase, water, and acetone. Cell walls were analyzed for neutral sugars, uronic acids, hydroxycinnamic acids, and acetyl bromide lignin. Cell walls were degraded with hydrolases from *Trichoderma reesei* (Celluclast, NOVO) and *Aspergillus niger* (Viscozyme L, NOVO). After 3 and 48 h of enzymatic hydrolysis, wall residues were pelleted by centrifugation and an aliquot of the supernatant was analyzed for uronic acids and for neutral sugars after hydrolysis with 2 N trifluoroacetic acid.

Results and Discussion

The cell wall content of alfalfa tissues (estimated by summing neutral sugars, uronates, and lignin) was 604 mg g⁻¹ for whole internodes, 727 mg g⁻¹ for xylem and 497 mg g⁻¹ for nonxylem.

These values slightly underestimate the cell wall content of tissues because they do not include structural proteins and small quantities of pectic sugars removed during preparation of crude cell walls. The carbohydrate fraction of all tissues was composed primarily of glucose (ca 60%). The proportions of other sugars differed considerably between tissue types (Table 1). On a cell wall basis, the lignin content of xylem (288 mg g⁻¹) was about two-fold greater than that of nonxylem (168 mg g⁻¹). Both tissue types contained only trace amounts of ester- and ester/ether-linked hydroxycinnamic acids.

The loss of sugars from alfalfa internodes incubated with fungal enzymes was similar to that reported for alfalfa stems degraded with rumen microorganisms (Table 1). The release of total sugars from xylem walls was extremely low, due in part to their high lignin content. Nonxylem walls, with 42% less lignin, had a 340% greater release of total sugars than xylem, indicating that the effects of lignification on wall degradability differed between tissue groups. The release of all sugars from xylem walls was low, especially for xylose and uronates (probably glucuronic acid). In nonxylem tissues, the release of most sugars was relatively high except for xylose. These results suggest that the degradability of glucuronoxylans in both xylem and nonxylem tissues is limited, possibly due to interactions with lignin. Unlike grasses, interactions between xylans and lignin in alfalfa do not appear to involve ferulic acid. Further work is needed to identify how lignins restrict the degradation of xylans and other associated polysaccharides in legumes.

Conclusions

The degradability of xylem was much lower than nonxylem walls, but in both types of walls the degradation glucuronoxylans was extremely low. Elucidation of lignin-glucuronoxylan interactions is required for developing alfalfa germplasm with improved fiber degradability.

Impact Statement

Studies of cell wall composition, structure, and degradability provide basic information needed for elucidating and rectifying factors which

limit fiber digestion of alfalfa. Improved fiber degradability of alfalfa would reduce both feed costs and manure output for livestock operations.

Table 1. Chemical composition and fungal hydrolase degradability of alfalfa tissues averaged across internodes.

	Rhamnose	Arabinose	Galactose	Uronates	Mannose	Glucose	Xylose	Total Sugars	Lignin
	----- mg g ⁻¹ total sugars -----							mg g ⁻¹ dry matter	
<u>Chemical composition</u>									
Internode	12	32	25	106	29	611	184	460	144
Xylem	8	9	14	77	23	616	253	517	209
Nonxylem	18	59	36	150	32	593	113	413	83
<u>Proportion of sugars released after 3 h</u>									
Internode	0.625	0.668	0.548	0.377	0.348	0.260	0.106	0.274	
Xylem	0.272	0.421	0.404	0.043	0.208	0.154	0.048	0.127	
Nonxylem	0.840	0.708	0.677	0.770	0.478	0.430	0.280	0.499	
<u>Proportion of sugars released after 48 h</u>									
Internode	0.701	0.844	0.649	0.497	0.591	0.471	0.126	0.435	
Xylem	0.371	0.638	0.413	0.108	0.324	0.249	0.066	0.200	
Nonxylem	0.847	0.855	0.758	0.773	0.781	0.709	0.279	0.687	