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Cell Wall Composition in Juvenile and Adult Leaves of Maize (*Zea mays* L.)

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Many leaf characteristics vary with position along the culm in maize (*Zea mays* L.) due to the existence of vegetative phase change and heteroblasty. The objective of this work was to determine if differences in cell wall composition exist among developmental phases and between *Cg1*, a developmental mutant, and wild-type maize. In one experiment, the middle third of fully elongated leaf blades from lower and upper regions of the shoot was harvested (midribs removed) and analyzed for several cell wall components. Averaged over five inbreds (De811, Ia5125, Mo17, P39, and Wh8584), lower leaf blades had higher levels of xylose and lower levels of total uronosyls, glucose, arabinose, and galactose ($P < 0.05$) than did upper leaf blades. With the exception of glucose, upper and lower leaves of *Cg1* plants varied in the same manner as their near-isogenic siblings, except cell walls of *Cg1* plants were more "juvenile" than cell walls of wild-type siblings at the same leaf stage. These data support the hypothesis that *Cg1* delays but does not eliminate the transition from juvenile-vegetative to adult-vegetative phase. In a second experiment, juvenile (leaves 3 and 5), transition (leaf 7), and adult (leaves 9 and 11) leaves from inbreds B73 and De811 were harvested and analyzed as in the first experiment. As leaf number rose, total cell wall content of sample dry matter, total neutral sugars, glucose, xylose, and ester-linked monomers of *p*-coumaric acid and total ferulates including ferulate dimers increased linearly while total uronosyls acids, arabinose, and galactose declined linearly ($P < 0.05$). Glucose and xylose are major cell wall components released from cellulose and xylans after acid hydrolysis. Pectin, a minor component of grass cell walls, is composed of galacturonosyls, arabinose, and galactose. Secondary cell wall deposition increased between leaves 3 and 11 in a heteroblastic series, due to either increased cell wall content concomitant with decreased cell lumen size, changes in proportion of cell types (i.e., sclerenchyma), or a combination of these factors.

KEYWORDS: Maize; cell walls; leaves; *Cg1*; culm; juvenile

INTRODUCTION

Many leaf characteristics vary with position along the culm in maize (*Zea mays* L.). Heteroblastic traits, such as leaf length and width, vary quantitatively (*1*). Heterochronic traits are associated with a particular vegetative phase. Juvenile-vegetative traits, such as epicuticular wax, occur only on leaves one through five or six, whereas adult-vegetative traits, such as pubescence, occur only on leaves higher than those (*2*). One to several transition leaves with juvenile and adult sectors normally occur between vegetative phases.

Previous studies of cell wall composition in sequential leaves along the maize culm, although limited, indicate variation exists. Bergvinson et al. (*3*) reported increased levels of cell wall-bound phenolic acids (*p*-coumaric acid and ferulic acid) between leaves 3 and 10 in the population BS9 (C4). Evans et al. (*4*), using a histological technique to measure relative amounts of lignin, concluded that epidermal cells in adult leaves of several maize inbreds have higher lignin levels than juvenile leaves of the same genotype.

Cell wall composition of leaves and whole plant tissues vary with maturity in a number of forage grasses (*5*, *6*). Generally, tissues of grasses harvested early in development have relatively higher molar fractions of arabinose, galactose, and uronosyls and lower fractions of cellulose, xylans, lignin, and bound phenolic monomers than tissues of plants harvested at later stages of development. Grabber et al. (*7*) have shown that individual sugars that make up polysaccharides in grass cell walls can vary depending upon the grass species and the stage of development. Changes in the cell wall composition can also affect total digestibility (*8*).

Cell wall composition affects resistance to insect herbivory (*9*, *10*) and ruminant digestibility (*8*, *11*) in maize and other

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grass species. Generally, higher levels of lignin, per se, or cross-linking of lignin or other phenolic acids to cell wall carbohydrates results in increased physical resistance to chewing and decreased availability of digestible components (*12*). A more thorough understanding of developmental changes in cell wall composition of sequential maize leaves should increase geneticists' abilities to develop cultivars with improved resistance to herbivory or increased digestibility.

Corngrass1 (*Cg1*) is a heterochronic mutation that alters the timing of vegetative phase change primarily by extending the duration of the juvenile-vegetative phase (*13*, *14*). Relative to wild-type, *Cg1* plants have an elongated transition zone and additional juvenile nodes, resulting in increased ear leaf and total leaf number but not an increased number of leaves above the uppermost ear.

Our objective was to determine if differences in cell wall composition exist among developmental phases and between *Cg1* and wild-type maize.

MATERIALS AND METHODS

All field plantings were conducted at the West Madison Agricultural Experiment Station, Madison, WI, on a Plano silt loam (fine-silty, mixed, mesic Typic Argiudolls). Plots consisted of individual rows spaced 0.75 m apart and 4.3 m long.

Experiment 1. The *Cg1* mutation delays the transition from juvenile-vegetative to adult-vegetative phase to higher nodes than normal (*13*). We compared cell wall composition in upper and lower leaf blades of near-isogenic *Cg1* and wild-type plants in several inbred backgrounds.

Preparation of Leaf Samples. Near-isogenic *Cg1* lines were created by backcrossing *Cg1* into three sweet corn (P39, Ia5125, and Wh8584) and two field corn (De811 and Mo17) inbred lines. The source of *Cg1* was the Maize Genetics Cooperative, Urbana, IL (stock no. 310D). The inbred lines were chosen due to their range in flowering date and endosperm type. The number of backcross generations varied with inbred background (2, 3, 3, 3, and 5 for Wh8584, Ia5125, De811, Mo17, and P39, respectively) because high *Cg1* expression in some backgrounds made seed production difficult.

In 1991, heterozygous *Cg1 cg1* plants were backcrossed to wild-type (*cg1 cg1*) inbreds resulting in families segregating for *Cg1*. In 1992, 20 kernels for each family were hand-planted in individual rows, resulting in *Cg1* and wild-type segregants in each row. Separate plantings were made on May 28 and June 25. Three weeks after emergence, when the mutant phenotype was discernible, rows were thinned to five *Cg1* and five wild-type plants each. The experimental design was a split-plot in randomized complete blocks (three replications) with two whole plot factors, inbred background and planting date, and one subplot factor, *Cg1* genotype (*Cg1* vs wild-type). Whole plot treatments were planted in separate rows.

The two plantings produced plants at the seedling and midwhorl stages of development 1 month after the second planting. When the earliest flowering genotype (Wh8584) from the early planting had nearly reached anthesis (July 31), the middle third of the blade of the topmost, fully elongated leaf (with a visible collar) from three *Cg1* and three wild-type plants in each row was harvested, placed on dry ice, and then frozen at −20 °C in preparation for laboratory analysis. The corresponding node of excised leaves from wild-type plants ranged from node 9 to 12 for the early planting and from node 4 to 5 for the late planting, due to differences in the rate of growth of inbred backgrounds. The corresponding node of excised leaves for *Cg1* plants was three to four nodes higher than for wild-type siblings due to the faster rate of leaf initiation in *Cg1* plants (*15*). All excised wild-type leaves from the early planting were in the adult vegetative phase. All excised wild-type leaves from the late planting were in the juvenile vegetative phase. On the basis of the presence of epicuticular wax, all *Cg1* leaves were juvenile.

Cell-Wall Isolation. Frozen leaf samples were lyophilized before removal of the midrib. Leaf blade material was ground through a 1 mm screen with a cyclone mill (Udy Corp., Fort Collins, CO). Analyses of cell wall components were performed in duplicate, then averaged, for each leaf sample, except for total cell wall sample^{-1}, for which only one analysis per sample was completed. Cell wall (CW) material was isolated using the Uppsala procedure (*16*) with minor changes. Briefly, ground plant material was weighed into 50 mL centrifuge tubes (polypropylene with caps), thoroughly mixed with Tris−acetate buffer (10 mM + 50 mM NaCl, 4 °C, pH 6.0, 40 mL g^{-1} of dry matter), and placed in a sonication bath for 15 min. Insoluble residue was pelleted by centrifugation (1000*g*) and the supernatant carefully decanted so as not to disturb the pellet. Fresh buffer was added to the tubes mixed with the residues and sonicated, and insoluble residues were recovered by centrifugation. Insoluble residues were further washed with 80% ethyl alcohol (EtOH) four times, acetone one time, and chloroform/methanol (2:1) one time, followed by a final acetone wash (two times). For each wash the sample was stirred to thoroughly suspend it in the solvent before sonication for 15 min and recovery of the insoluble material by centrifugation. After the final acetone wash, tubes were left uncovered in the fume hood until dry.

Each crude CW sample was suspended in Tris−acetate buffer (50 mM, pH 6.0, 15 mL g^{-1} of CW) and heated in a boiling water bath for 2.0 h to gelatinize starch. Tubes were cooled to 60−65 °C, 10 IU of α-amylase (Sigma A-3403) and 10 units of amyloglucosidase (Fluka, 10115) were added, and then the tubes were placed in a water bath at 55 °C for 2.0 h. It is important to maintain the incubation temperature above 55 °C to inhibit wall hydrolases present in these enzyme preparations. Cell walls were pelleted at 2500*g* (10 min) following amylase/amyloglucosidase treatment. The supernatant was decanted into a filtration flask fitted with a glass fiber filter (GF-4 Fisher). Thirty milliliters of deionized water (H_2O) was added to each tube and mixed thoroughly, and CW was pelleted as before. After four wash cycles, the CW was transferred to a freeze-drying flask and lyophilized. The amylase extract and washes were combined, dialyzed against H_2O (8−10 L) for 48 h, frozen, and lyophilized. This extract represented a small amount of total cell wall material recovered, but for completeness, the recovered carbohydrate in the water-soluble extract after starch removal was added to the recovered CW material to give a total cell wall fraction.

Chemical Analyses. Klason lignin (KL, determined as acid insoluble lignin) was determined as the residue remaining after two-step sulfuric acid hydrolysis of cell wall polysaccharides (*17*). This method allowed the determination of neutral sugars, total uronosyls, and acid-insoluble residues in isolated CW samples. Neutral sugars were quantified using an HPLC procedure (*18*) (DX-500 Carbohydrate System using a Carbopac PA10 4 × 250 mm column; Dionex Corp., Sunnyvale, CA). Total uronosyls were determined using the phenyl phenol method (*19*). Cell wall fractions are presented on a total cell wall basis.

Experiment 2. *Preparation of Leaf Samples.* Two inbreds (B73 and De811) were planted June 7, 1994, in a split-plot experimental design with randomized complete blocks (two replications). Inbred background and leaf type were the whole plot factor and subplot factor, respectively. Rows were overplanted and thinned at the six to eight leaf stage to 11 plants. Six leaf samples (leaves 3, 5, 7-juvenile, 7-adult, 9, and 11) were harvested from each experimental unit in the following manner. When leaf 7 was fully elongated on the majority of plants, the middle third of blades from leaves 3 and 5 (juvenile phase) and leaf 7 (transition leaf between vegetative phases) was harvested from three plants per row, and midribs were removed. Leaf 7 was further divided into juvenile and adult sectors, based on the presence or absence of epicuticular wax, producing leaf samples 7-juvenile and 7-adult, respectively. When leaf 11 was fully elongated on the majority of the remaining unharvested plants, the middle third of blades from leaves 9 and 11 (adult phase) was harvested from three previously unharvested plants per row, and midribs were removed. Immediately after harvest, all leaf samples were stored separately on dry ice and then frozen at 20 °C in preparation for laboratory analysis.

Cell-Wall Isolation. CW material was isolated as in experiment 1.

Chemical Analyses. Cell wall analysis was performed as in experiment 1, except that samples from experiment 2 were also analyzed for

Table 1. Cell Wall Component Means in *Cg1* and Wild-Type Segregants at Two Leaf Stages, Averaged over Inbred Background and Replications

			mg/g of CW[a]						
leaf stage[b]	genotype	total cell wall (mg/g of leaf sample)	total uronosyls	lignin	total neutral sugars	GLU[c]	XYL[c]	ARA[c]	GAL[c]
lower	*Cg1*	481.7	32.3	141.4	462.7	310.0	111.0	42.5	18.1
	wild-type	550.3	30.1	150.1	491.9	290.2	167.2	41.0	14.9
upper	*Cg1*	537.6	27.4	158.0	501.2	305.7	140.3	39.2	15.9
	wild-type	589.2	23.4	163.8	512.2	276.6	189.5	37.3	11.7
LSD ($P < 0.05$)		55.3	1.5	18.8	20.0	10.6	13.9	3.1	2.7

[a] CW, total cell wall recovered. [b] The lower leaf stage corresponds to leaves 4–5 in wild-type plants and leaves 7–9 in *Cg1* plants. The upper leaf stage corresponds to leaves 9–12 in wild-type plants and leaves 12–16 in *Cg1* plants. [c] GLU, glucose; XYL, xylose; ARA, arabinose; GAL, galactose.

Table 2. Cell Wall Component Means in Sequential Leaves of B73 and De811, Averaged over Inbred Background and Replications

		mg/g of CW[a]						
leaf no.	total cell wall (mg/g of leaf sample)	total uronosyls	lignin	total neutral sugars	GLU[b]	XYL[b]	ARA[b]	GAL[b]
3	428.9	30.6	161.9	414.6	248.9	100.4	43.2	18.7
5	538.3	31.3	162.2	434.8	271.9	104.5	40.1	15.6
7J[c]	549.1	27.5	150.0	492.6	290.8	142.3	41.7	15.7
7A[c]	581.8	23.4	153.2	491.1	279.8	160.0	35.7	12.9
9	605.2	21.9	144.2	522.0	295.3	178.1	34.8	11.4
11	643.1	19.5	154.5	549.5	300.2	202.8	35.3	10.0
LSD ($P < 0.05$)	47.2	1.5	NS[d]	35.4	27.1	12.6	3.9	3.0
b[e]	37.3[f]	–2.5[f]	NS	26.7[f]	9.0[g]	21.4[f]	–1.8[g]	–1.7[f]

[a] CW, total cell wall recovered. [b] GLU, glucose; XYL, xylose; ARA, arabinose; GAL, galactose. [c] J, juvenile wax sectors; A, adult wax sectors. [d] NS, not significant at $P < 0.05$. [e] Linear regression coefficient for cell wall component means regressed on leaf number. [f] $P < 0.01$. [g] $P < 0.05$.

cell wall-bound *p*-coumarate (*p*-CA), ferulate (FA), and ferulate dimers (DFA). Walls were analyzed for ester- and ether-linked phenolics using the procedure of Grabber et al. (*20*). The alcohol-insoluble residues (≈50 mg) were treated with degassed 2 M NaOH in glass culture tubes (25 mm × 150 mm) fitted with Teflon-lined caps. Samples were incubated for 20 h at room temperature before acidifcation with 10 N HCl (pH <2) and extracted three times with 2 mL of ether. The combined ether extracts were dried over anhydrous Na_2SO_4, transferred to 2 mL reaction vials, and evaporated under a stream of nitrogen. Total quantities of *p*-CA, FA, and DFA were determined by FID-GLC using a DB-1 column (30 m × 0.26 mm) (*20*).

Entry means were tested by analysis of variance (ANOVA), using the mixed model procedure written by Statistical Analysis System (SAS) software (*21*). The mixed model procedure written by Statistical Analysis System (SAS) software (*21*) was used to calculate least significant differences (LSDs) with a *P* value of 0.05.

RESULTS

Inbred background effects were nonsignificant in both experiments 1 and 2; therefore, data are presented averaged across inbred background.

In experiment 1, significant differences ($P < 0.05$) occurred for many cell wall components between lower and upper leaf blades on both wild-type and *Cg1* plants (**Table 1**). Compared to lower leaf blades of wild-type plants, upper wild-type leaf blades had higher levels of total neutral sugars and xylose and lower levels of total uronosyls and glucose. For *Cg1* plants, upper leaf blades had higher levels of total neutral sugars and xylose and lower levels of total uronosyls and arabinose and galactose compared to lower leaf blades. The glucose/xylose ratio decreased significantly ($P < 0.05$; data not shown) between lower and upper leaf blades of each plant type, indicating that xylose-containing polysaccharides, primarily xylans, increased as a proportion of the total CW relative to the glucose-containing polysaccharides as the plant developed. Total cell wall as a proportion of the dry matter and lignin tended to increase (although not significantly; $P > 0.05$) between lower and upper leaf blades of each plant type.

Table 3. Means of Phenolic Acid Monomers and Dimers in Sequential Leaves of B73 and De811, Averaged over Inbred Background and Replications

	mg/g of CW[a]					
	ester-linked			ether-linked		
leaf no.	*p*-CA[b]	FA[b]	DFA[b]	*p*-CA	FA	DFA
3	1.27	2.97	2.31	0.13	1.67	3.52
5	1.50	3.52	2.04	0.14	1.34	3.79
7J[c]	1.96	4.41	2.82	0.03	0.98	3.16
7A[c]	2.12	3.95	3.23	0.09	1.59	3.46
9	2.53	4.34	3.75	0.12	1.68	2.66
11	4.44	5.39	3.38	0.04	1.77	3.39
LSD ($P < 0.05$)	0.32	0.71	0.77	NS[d]	NS	NS
b[e]	0.55[f]	0.40[f]	0.31[f]	NS	NS	NS

[a] CW, total cell wall recovered. [b] *p*-CA, *p*-coumaric acid monomers; FA, ferulic acid monomers; DFA, ferulic acid dimers. [c] J, juvenile wax sectors; A, adult wax sectors. [d] NS, = not significant at $P < 0.05$. [e] Linear regression coefficient for cell wall component means regressed on leaf number. [f] $P < 0.05$.

Results for experiment 2 agreed with those for experiment 1 except that the glucose fraction increased significantly ($P < 0.05$) between lower and upper leaves in experiment 2. Between leaves 3 and 11, we observed linear increases in total cell wall per unit of dry matter and, on a cell wall basis, linear increases in total neutral sugars, glucose, and xylose and linear decreases in total uronosyls, arabinose, and galactose ($P < 0.05$; **Table 2**). Ester-linked phenolic acids also rose linearly ($P < 0.05$) as leaf number increased (**Table 3**). Juvenile and adult sectors of leaf 7 (vegetative-phase transition leaf) generally had levels closer to leaves 5 and 9, respectively, indicating that sectors visible in the transition leaf based on epidermal characteristics (e.g., the presence or absence of epicuticular wax) differ in cell

wall composition in a manner consistent with whole-leaf differences in the rest of the plant.

DISCUSSION

Most cell wall components of leaf blades in experiments 1 and 2 showed gradual, quantitative changes between juvenile and adult leaves, indicating the cell wall is influenced by heteroblasty, not heterochrony. In grasses, glucose and xylose are predominately components of cellulose and xylans, respectively. The origin of arabinose, galactose, and uronosyls is a little more complicated in that they are associated with pectin. Arabinose is also found as a major substitution residue on xylans, particularly in primary cell walls. We believe the decrease in the glucose/xylose ratio indicates that there is a shift from highly branched or substituted polysaccharides to increased cellulose and fewer substituted xylans in leaf blades during ontogeny due to greater secondary cell wall deposition in upper compared to lower leaf blades. This hypothesis is also consistent with the increase in total cell wall recovery observed in higher relative to lower leaf blades.

The acid-insoluble residue (Klason lignin) did not vary significantly in either experiment, but changes could be masked by the inclusion of protein with the acid-insoluble residue. Leaf tissues of plants typically have high levels of protein and low levels of lignin. This can artificially elevate Klason lignin values using the acid-insoluble residue (*17*). Ester-linked *p*-CA is correlated with lignin in grasses. Because *p*-CA increased during ontogeny, the level of lignin may actually have risen as well. Because *p*-CA does not enter into cross-coupling reactions, it does not contribute to the total cross-linking found within grass walls. Its functional role within the cell wall remains unclear at this time, although there is some evidence to suggest that *p*-CA may have a role in aiding radical coupling reactions involving sinapyl alcohol (*22*). Ferulates do become involved in cross-coupling reactions, resulting in cross-links between arabinoxylans and lignin. Ferulate dimers (ester-linked only) result from the oxidative mediated cross-coupling of ferulates on arabinoxylans. Ether-linked FA and DFA result from cross-coupling reactions involving ferulated arabinoxylans and lignin (*23*). As the leaf number increased there was a significant increase in ester-linked FA and DFA, reflecting an increased level of maturity.

For all cell wall components, except glucose, *Cg1* plants had more "juvenile" levels than wild-type plants at the same leaf stage. In other words, the differences in cell wall composition between *Cg1* and wild-type leaf blades at the same leaf stage paralleled the differences between lower (juvenile) and upper (adult) wild-type leaf blades (**Tables 1** and **2**). For example, xylose levels in both the lower and upper leaf stages of wild-type plants were greater than in the corresponding *Cg1* leaf stages, the same trend that occurred between lower and upper wild-type leaf blades. The *Cg1* leaves were more juvenile despite being three to four nodes higher on the plant than leaves of wild-type siblings at the same leaf stage. In addition, cell wall component fractions of upper *Cg1* leaf blades tended to be similar to those of lower wild-type leaf blades.

The data from experiment 1 support the hypothesis that *Cg1* delays but does not eliminate the transition from juvenile to adult vegetative phase (**Table 1**). More juvenile levels of cell wall components were observed in *Cg1* plants than in wild-type plants at the same leaf stage, and upper *Cg1* leaf blades tended to have cell wall composition similar to that of lower wild-type leaf blades. The changes in cell composition are in agreement with trends observed in previous studies comparing leaf blade anatomy, disease resistance, and insect resistance of *Cg1* and wild-type isogenic siblings (*13*, *24*).

LITERATURE CITED

(1) Greyson, R. I.; Walden, D. B.; Smith, W. J. Leaf and stem heteroblasty in *Zea*. *Bot. Gaz.* **1982**, *143*, 73−78.

(2) Poethig, R. S. Phase change and the regulation of shoot morphogenesis in plants. *Science* **1990**, *250*, 923−930.

(3) Bergvinson, D. J.; Larsen, J. S.; Arnason, J. T. Effect of light on changes in maize resistance against the European corn borer, *Ostrinia nubilalis* (Hubner). *Can. Entomol.* **1995**, *127*, 111−122.

(4) Evans, M. S.; Passas, H. J.; Poethig, R. S. Heterochronic effects of *glossy15* mutations on epidermal cell identity in maize. *Development* **1994**, *120*, 1971−1981.

(5) Burritt, E. A.; Bittner, A. S.; Street, J. C.; Anderson, M. J. Comparison of laboratory methods for the prediction of in vitro dry matter digestibility in three maturing grasses. *J. Agric. Food Chem.* **1985**, *33*, 725−728.

(6) Morrison, I. M. Changes in the lignin and hemicellulose concentrations of ten varieties of temperate grasses with increasing maturity. *Grass Forage Sci.* **1980**, *35*, 287−293.

(7) Grabber, J. H.; Jung, G. A.; Hill, R. R., Jr. Chemical composition of parenchyma and sclerenchyma cell walls isolated from orchardgrass and switchgrass. *Crop Sci.* **1991**, *31*, 1058−1065.

(8) Grabber, J. H.; Jung, G. A.; Abrams, S. M.; Howard, D. B. Digestion kinetics of parenchyma and sclerenchyma cell walls isolated from orchardgrass and switchgrass. *Crop Sci.* **1992**, *32*, 806−810.

(9) Coors, J. G. Resistance to the European corn borer, *Ostrinia nubilalis* (Hubner), in maize, *Zea mays* L., as affected by soil silica, plant silica, structural carbohydrates, and lignin. In *Genetic Aspects of Plant Mineral Nutrition*; Gabelman, H. W., Loughman, B. C., Eds.; Martinus Nijhoff: Dordrecht, The Netherlands, 1987; pp 445−456.

(10) Bergvinson, D. J.; Arnason, J. T.; Hamilton, R. I.; Tachibana, S.; Towers, G. H. N. Putative role of photodimerized phenolic acids in maize resistance to *Ostrinia nubilalis* (Lepidoptera: Pyralidae). *Environ. Entomol.* **1994**, *23*, 1516−1523.

(11) Hartley, R. D.; Ford, C. W. Phenolic constituents of plant cell walls and wall biodegradability. In *Biogenesis and Biodegradation*; ACS Symposium Series 399; Lewis, N. G., Paice, M. G., Eds.; American Chemical Society: Washington, DC, 1989; pp 137−145

(12) Grabber, J. H.; Ralph, J.; Hatfield, R. D. Ferulate cross-links limit the enzymatic degradation of synthetically lignified primary walls of maize. *J. Agric. Food Chem.* **1998**, *46*, 2609−2614.

(13) Galinat, W. C. The corn grass and teopod loci involve phase change. *Maize Genet. Coop. Newsl.* **1966**, *40*, 102−103.

(14) Poethig, R. S. Heterochronic mutations affecting shoot development in maize. *Genetics* **1988**, *119*, 959−973.

(15) Whaley, W. G.; Leech, J. H. The developmental morphology of the mutant "corn grass". *Bull. Torrey Bot. Club* **1950**, *77*, 274−286.

(16) Theander, O.; Westerlund, E. A. Studies on dietary fiber. 3. Improved procedures for analysis of dietary fiber. *J. Agric. Food Chem.* **1986**, *34*, 330−336.

(17) Hatfield, R. D.; Jung, H. G.; Ralph, J.; Buxton, D. R.; Weimer, P. J. A comparison of the insoluble residues produced by the Klason lignin and acid detergent lignin procedures. *J. Sci. Food Agric.* **1994**, *65*, 51−58.

(18) Hatfield, R. D.; Weimer, P. J. Degradation characteristics of isolated and *in situ* cell wall lucerne pectic polysaccharides by mixed ruminal microbes. *J. Sci. Food Agric.* **1995**, *69*, 185−196.

(19) Blumenkrantz, N.; Asboe-Hansen, G. New method for quantitative determination of uronic acids. *Anal. Biochem.* **1973**, *54*, 484−489.

(20) Grabber, J.; Hatfield, R. D.; Ralph, J.; Zon, J.; Amrhein, N. Ferulate cross-linking in cell walls isolated from maize cell suspensions. *Phytochemistry* **1995**, *40*, 1077−1082.
(21) SAS Institute. *The SAS System for Windows*, release 8.2; SAS Institute: Cary, NC, 1999.
(22) Hatfield, R. D. A potential role of sinapyl *p*-coumarate in grass lignin formation. *Plant Physiol.* **1997** *114*, 346.
(23) Ralph, J.; Grabber, J. H.; Hatfield, R. D. Lignin-ferulate cross-links in grasses: active incorporation of ferulate polysaccharide esters into ryegrass lignins. *Carbohydr. Res.* **1995**, *275*, 167−178.
(24) Abedon, B. G.; Tracy, W. F. *Corngrass1* of maize (*Zea mays* L.) delays development of adult plant resistance to common rust (*Puccinia sorghi* Schw.) and European corn borer (*Ostrinia nubilalis* Hubner). *J. Hered.* **1996**, *87*, 219−223.

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