Genetic variation in maize cell wall for lignin content, lignin structure, p-hydroxycinnamic acid content, and digestibility in set of 19 lines at silage harvest maturity

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GENETIC VARIATION IN MAIZE CELL WALL FOR LIGNIN CONTENT, LIGNIN STRUCTURE, *p*-HYDROXYCINNAMIC ACID CONTENT, AND DIGESTIBILITY IN SET OF 19 LINES AT SILAGE HARVEST MATURITY

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ABSTRACT - Genetic variation for carbohydrate content, lignin content, lignin composition, *p*-hydroxycinnamic acid content and digestibility were investigated in a set of 19 maize lines over a two year period (2002 and 2003) and in two locations (Lusignan and Druelle, France). The studied set was comprised of old and recent elite lines representative of cell wall digestibility variations and several model lines involved in digestibility and intake breeding schemes. A large degree of genetic variation for cell wall composition and digestibility was observed. Lignin content appeared to be the primary determinant in cell wall digestibility variation (r^2 = 0.77). H and S monomeric unit contents also appeared to be negatively correlated with digestibility. The cell wall cross-linkages with ferulic and diferulic acids were significantly correlated with digestibility, but with two apparently contrary effects. A negative correlation was found between etherified ferulic acid content and cell wall digestibility while a positive one was found between diferulic acid content and digestibility. These results strengthened the ambiguity concerning the real proportion of ferulic and diferulic acids measured after an alkaline hydrolysis. A negative correlation was found between *p*-coumarate content and digestibility. This result confirmed the efficiency of these acids as an estimator of feeding value. Finally, a positive correlation between hemicellulose content and digestibility showed that cell wall carbohydrate composition could impact cell wall digestibility. A new ideotype of forage maize can be designed based on the cell wall correlations highlighted in this study, but an elite line improved for feeding value traits (and biofuel production) have to take simultaneously into agronomic traits. Such an ideotype will therefore be used by maize breeders as an allele donor in marker assisted breeding programs.

KEY WORDS: *Zea mays* L.; Silage maize; Digestibility; Intake; Lignin; *p*-Coumaric acid; Ferulic acid.

Abbreviations:
QTL: Quantitative Trait Loci; DM: Dry Matter; NDF: Neutral Detergent Fiber; IVDMD: *In Vitro* Dry-Matter Digestibility; IVNDFD: *In Vitro* NDF Digestibility; ADL/NDF: Acid Detergent Lignin in NDF; Cell: cellulose; Hcell: hemicelluloses; H: *p*-hydroxyphenyl, Hb: 4-*p*-hydroxybenzaldehyde; G: guaiacyl, Va: vanillin; S: syringyl, Sg: syringaldehyde.

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INTRODUCTION

Extensive research has been devoted to silage maize digestibility (review in BARRIÈRE *et al.,* 2007), which has a key role in dairy cow and meat cattle nutrition. Several studies have shown that the first factor influencing whole plant digestibility and energy value was cell wall digestibility (DEINUM and STRUIK, 1985; DOLSTRA and MEDEMA, 1990; BARRIÈRE *et al.,* 1991; WOLF *et al.,* 1993; COORS *et al.,* 1994; ARGILLIER *et al.,* 1995). A maize cell wall is primarily composed of cellulose and hemicelluloses (mainly glucurono-arabinoxylans) and, in lower amounts, of lignins and *p*-hydroxycinnamic acids. The lignin polymer is mostly comprised of guaiacyl (G) and syringyl (S) units, whereas *p*-hydroxyphenyl (H) units occur as a quantitatively minor component. *p*-Coumaric acid is mainly esterified to S units of lignin, while ferulic acid is primarily esterified to arabinosyl residues of arabinoxylans chains. Hemicellulose chains are cross-linked afterwards by diferulic acid bridges. Hemicellulose chains and the lignin polymer are cross-linked by ester-ether ferulic and diferulic acid cross-linking. No covalent linkage has been observed between cellulose and another cell wall component, but hemicelluloses and cellulose are linked with hydrogen bonds.

Lignin is the cell wall component with the most detrimental effect on cell wall digestibility (JUNG and DEETZ, 1993; WOLF *et al.,* 1993; LUNDVALL *et al.,* 1994; MÉCHIN *et al.,* 1998; ARGILLIER *et al.,* 2000; MÉCHIN *et al.,* 2000). Lignin prevents physical access of the ru-

men microbial enzymes to cell wall carbohydrates and thus strongly limits their valorization by cattle (JUNG *et al.,* 1998). Based on investigations in different grass and dicotyledons forage, complementary factors appeared as involved in digestibility limitation. The role of ratios between lignin H, G and S monomeric units in maize feeding value is still being questioned. In maize bm3 mutant plants, which have a higher digestibility and intake primarily due to their lower lignin content, the large reduction of S units in lignins induced a more condensed polymer with a proportionally more inhibiting effect on cell wall degradability (THORSTENSSON *et al.,* 1992; BARRIÈRE *et al.,* 2004). Sinapyl alcohol has indeed a strong tendency to be involved in labile β-O-4 end wise type coupling upon peroxidasic polymerization in plant cell walls. However, the *p*-coumaroylation of sinapyl at C might change the course of the reaction towards the formation of condensed bonds, such as β-β bonds. Nevertheless, MÉCHIN *et al.* (2000) showed a positive correlation between S/G ratio and cell wall digestibility. The contents in ferulic and diferulic acids, and the correlative covalent cross-linkages between lignin and arabinoxylans and between arabinoxylan chains by these acids, also have a negative effect on carbohydrate degradability (JUNG *et al.,* 1998; GRABBER *et al.,* 1998a,b, 2004, 2005). Results of FONTAINE *et al.* (2003) strengthened the interest to use the etherified ferulate content as a criterion in maize breeding for increased cell degradability. Moreover, cross-linkages are considered to play a role in cell wall stiffening (MACADAM and GRABBER, 2002). Because relationships between mechanical properties of maize silage and times of intake and chewing have been reported (FERNANDEZ, 2003; FERNANDEZ *et al.,* 2004), ferulic and diferulic acids cross-linkages were also supposed to influence intake values (BARRIÈRE *et al.,* 2007).

In order to have a better understanding of the effects of each cell wall biochemical trait on feeding value, the cell wall content in lignins, H, G and S lignin units, and *p*-hydroxycinnamic acids have been investigated in a set of 19 maize lines at silage maturity stage. This set was comprised of old and recent elite lines representative of cell wall digestibility variations, including model lines involved in digestibility and intake breeding schemes. Relationships between cell wall components and digestibility were thus investigated towards a definition of an ideotype of maize lines with high feeding values.

MATERIALS AND METHODS

Plant material

The set of 19 maize lines was composed of four INRA lines, F271, F2, F564, and F286, which are of increasing cell wall digestibility. F2 is the French reference line of medium cell wall digestibility. Fifteen lines, L01, L08, L15, L16, L19, L21, L24, L25, L49, L66, L77, L78, L83, L116, and L883, which are of interest for their variable cell wall digestibility and their potential effect on hybrid intake, were simultaneously investigated. Ten lines out of 19 were flint, five were dent and four were flint-dent.

Histological analysis

Tissue histology was investigated in the plant internode located just below the ear for lines F2, L15, L16, L19, L78, L116, F271 and F286, which were chosen for their variable cell wall contents and digestibility. Internodes were sampled at silage maturity, and fixed and conserved in an ethanol / acetic acid solution (75 / 25). Tissues were sectioned as 70 µm slices and colored with the Fasga solution according to the protocol of TOLIVIA and TOLIVIA (1987).

Field experiments, and forage quality evaluation

The 19-line field experiments were carried out over two years (2003 and 2004) in two locations (Lusignan and Druelle, France). In each location, lines were evaluated in bloc designs with 2 replicates. Each experimental plot was a 5.2 m long single row of 37 plants. Row spacing was 0.75 m, and the resulting density was 95,000 plants/ha. Irrigation was applied in Lusignan during summer to prevent water stress. The plots were machine-harvested with a forage chopper at an early silage harvest stage at about 30% of dry matter (DM) without ears that were eliminated by hand the day of harvest. A representative sample of 1 kg chopped material per plot was collected for DM content estimates and biochemical analysis.

Plant samples were dried in a ventilated oven (65°C). Dry samples were then ground with a hammer mill to pass through a 1 mm screen. For all plants, soluble carbohydrates (LILA, 1977), Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF) and Acid Detergent Lignin (ADL) contents were estimated according to GOERING and VAN SOEST (1970). The *in vitro* dry matter digestibility (IVDMD) was estimated according to AUFRÈRE and MICHALET-DOREAU (1983). Contents in esterified *p*-coumaric, ferulic and diferulic acids were estimated after alkaline hydrolysis of the cell wall at 25°C, and contents in total ferulic acid were estimated after alkaline hydrolysis at 170°C (MORRISON *et al.,* 1993). Ether ferulic acid content was estimated as the difference between total ferulic acid and ester ferulic acid. 4-*p*-Hydroxybenzaldehyde (Hb), vanillin (Va) and syringaldehyde (Sg) contents were investigated after alkaline nitrobenzene oxidation (HIGUCHI *et al.,* 1967). Hb, Va and Sg contents are considered as estimates of H, G, S lignin unit contents, despite some *p*-coumarate and ferulate can also be oxidized into *p*-hydroxybenzaldehyde and vanillin, respectively. All these traits were estimated using specific near infrared reflectance spectroscopy (NIRS) calibrations developed at INRA Lusignan for samples of plants without ear (NIRS system 6500 spectrophotometer, with wavelengths spaced every 4 nm from 400 to 2500 nm). Accurate r^2 values (coefficients of determination) have been obtained between laboratory analysis and NIRS predictions (Table 1), based on nearly 1300 laboratory analyses for global traits (IVDMD, NDF, ADF, ADL). These r^2 values were similar to those currently observed for NIRS prediction of these traits on whole

TABLE 1 - *Characteristics of NIRS calibrations developed for cell wall traits in maize plants without ears, at silage stage harvest (for all traits, number is the numbers of analyzed samples, mean is the average values of analyzed samples, RSQ is the coefficient of determination (r^2 value) between laboratory analysis and NIRS prediction, and SECV is the standard errors of cross validation prediction; DM = dry-matter).*

	number	Mean	RSQ	SECV
In vitro DM digestibility (IVDMD)	1281	62.9	0.93	1.89
Neutral detergent fiber (NDF)	1282	55.3	0.97	1.16
Acid detergent fiber (ADF)	1281	27.2	0.98	0.75
Acid detergent lignin (ADL)	1290	2.89	0.80	0.35
Esterified *p*-coumaric acid	1563	15.6	0.87	1.26
Esterified ferulic acid	1561	5.55	0.64	0.52
Total ferulic acid	1499	6.90	0.66	0.54
*p*Hydroxybenzaldehyde	1048	1.43	0.74	0.30
Vanillin	1051	6.38	0.61	1.11
Syringaldehyde	1054	7.22	0.79	1.09
5-5 diferulic acid	516	0.15	0.66	0.03
8-O-4 diferulic acid	514	0.33	0.61	0.05

plant samples. Accurate r^2 values between laboratory analysis and NIRS predictions were also obtained for *p*-hydroxycinnamates and released aldehydes, even if still moderate, but reliable, r^2 values were obtained for ferulate derivatives, Va and Sg lignin monomers (Table 1). In addition, similar r^2 accurate values have been obtained for the two 5-5 and 8-O-4 diferulic acids, despite calibration equations were based on only nearly 500 samples. Calibration equations were validated for each experimental location by a laboratory analysis of 40 samples.

Hemicellulose and cellulose contents were then estimated respectively as NDF – ADF and ADF – ADL. Because these compounds are constituents of the cell wall, hemicellulose, cellulose, and ADL contents were expressed as percentage of NDF. According to STRUIK, (1983) and DOLSTRA and MEDEMA (1990), *in vitro* NDF digestibility (IVNDFD) was computed assuming that the non-NDF part of plant was completely digestible [IVNDFD = 100 x (IVDMD – (100 – NDF))/NDF].

Data analysis

Analyses of variance were carried out following the standard procedure of a fixed model with genotype, environment, block, and genotype x environment interaction effects, as

$$Y_{ijkl} = \mu + E_j + Bk.E_j + G_i + G_i.Ej + \varepsilon_{ijkl}$$

with Y_{ijkl} = observed value for a given trait, μ = grand mean, Ej = environment effect, $Bk.E_j$ = block nested in environment effect, G_i = genotype effect, $G_i.E_j$ = genotype x environment interaction, and ε_{ijkl} = residual error, using Modli and Splus Software (KOBILINSKY, 1983; VENABLES and RIPLEY, 1994).

RESULTS AND DISCUSSION

Histological analysis

Maize stem internodes (Fig. 1) were typified by lignified (red) cortex, vascular bundles and central parenchyma, with a sub-cortical area and a parenchyma area around vascular bundles made of unlignified and cellulosic tissues (in blue). A large variation was observed for the red color intensity of parenchyma tissues, with likely a lower lignification in L78, F286, and L116, and conversely a higher lignification in F271, F2, L15, L16 and L19. The parenchyma around the vascular bundles was also greatly lignified in F271, with only very few cellulosic cells around the bundles, while other lines had at least two unlignified cell layers around these vascular tissues. The structure of vessels seemed relatively conserved between the different lines. Nevertheless, bundle lignification seemed more intense in L15, L19 and L78, while it was significantly weaker in F286. At the same time, the density of peripheric vascular bundles appeared higher in the three lines L15, L19 and L78. These histological analyses probably showed that tissue patterning and lignified tissue organization in maize stems should be considered in maize breeding for its feeding value.

Biochemical analysis

Variance analysis (Table 2) showed that genotype effects were highly significant ($P < 0.001$) for all investigated traits. Genotype effect was particularly high for IVNDFD. Genotype x environment interactions were also significant for all investigated traits. However, genotype effects were higher than genotype x environment interactions for all studied traits except for 5-5 diferulic acid which had comparable genotype and genotype x environment interaction effects.

A large genetic variation for cell wall composition and digestibility was observed among the 19

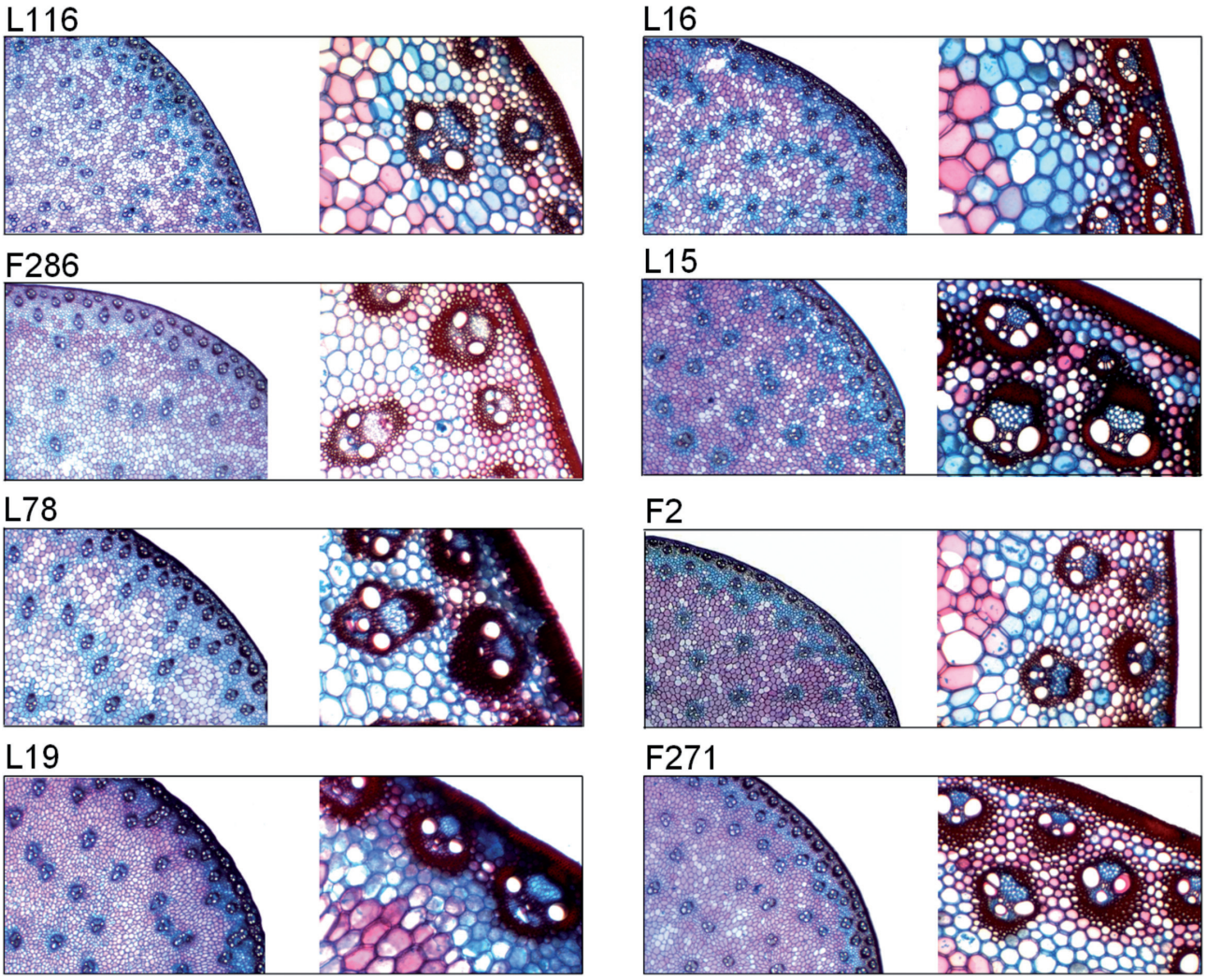

FIGURE 1 - Genetic variation for lignification of parenchyma and vascular tissue in maize below-ear internodes at silage maturity illustrated with Fasga-colored histological slides (lignified areas are colored in red while cellulosic tissue are colored in blue).

maize lines (Table 3). A difference of nearly 11 percentage points was thus observed for cell wall digestibility between lines L08 and F271, which had the highest and lowest IVNDFD values, respectively. Six lines (L08, L19, L21, L24, L66 and L116) had a significantly higher IVNDFD than the control line F2. Two lines (L883 and F271) had significantly lower values. The four lines with higher IVNDFD (L08, L24, L66 and L116) were flint lines.

The genetic variation for ADL/NDF content ranged from 3.14% in L24 to 4.82% in F271. Eleven lines out of 19 had lower ADL/NDF contents than F2, and no line had significant higher lignin content than the F2 control. The lines with the highest IVNDFD also had the lowest ADL/NDF contents.

For lignin structure, based on alkaline nitrobenzene oxidation, most of the investigated lines had significantly lower values than F2 for Va and Sg contents. Nine lines (L01, L08, L49, L66, L116, L883, F271, F286, and F564) had significantly lower contents for Va than F2, together with very different digestibility values. Sg contents lower than in the F2 line were observed in 14 lines out of 19 (L08, L16, L19, L21, L25, L49, L66, L78, L77, L83, L116, L883, F286, and F564). Four lines had higher Hb content than F2 (L15, L49, L883, and F271) and three lines had significantly lower values (L08, L66 and L116). The latter three lines also had significantly lower values for ester *p*-coumaric acid content. A low content for H and *p*-coumaric acid in cell wall is representa-

TABLE 2 - *Estimated genetic and environmental components, and means of cell wall digestibility, lignin contents and phenolic contents in the 19 lines set (effects were significant at P<0.001***, P<0.01 ** and P<0.05 *; IVNDFD* = in vitro *NDF digestibility, NDF = neutral detergent fiber, ADL = acid detergent fiber, Cell = cellulose, Hcell = hemicelluloses).*

Traits	Genotype mean-square	Gen x Environment mean-square	Residual mean-square	Means
IVNDFD (%)	84.64 ***	18.01 **	6.32	38.87
ADL/NDF (%)	1.67 ***	0.44 **	0.14	4.07
Cell/NDF (%)	5.53 ***	1.98 *	1.26	44.15
Hcell/NDF (%)	8.39 ***	2.69 *	1.66	51.78
Esterified *p*-coumaric acid (mg/g NDF)	7.67 ***	1.76 **	0.50	10.31
Esterified ferulic acid (mg/g NDF)	0.40 ***	0.11 *	0.07	5.79
Etherified ferulic acid (mg/g NDF)	0.061 ***	0.016 **	0.008	1.12
5-5 diferulic acid (mg/g NDF)	0.001 ***	0.001 **	0.000	0.16
8-O-4 diferulic acid (mg/g NDF)	0.003 ***	0.002 **	0.001	0.36
p-Hydroxybenzaldehyde (mg/g NDF)	0.143 ***	0.025 **	0.013	1.38
Vanillin (mg/g NDF)	2.020 ***	0.510 **	0.285	6.64
Syringaldehyde (mg/g NDF)	3.592 ***	0.737 **	0.269	6.97

TABLE 3 - *Means of cell wall digestibility, carbohydrate and phenolic contents in the 19 maize lines set (* = significantly higher value than F2, ¯ = significantly lower value than F2).*

Traits	Genotypes	L08	L24	L66	L116	L19	L21	F564	F286	L15	L49	L25	L78	L77	F2	L01	L83	L16	L883	F271
IVNDFD (%)		44.18*	43.70*	42.06*	42.04*	40.61*	40.42*	39.86	39.30	39.18	38.65	38.03	37.92	37.73	37.66	37.56	36.47	36.08	33.74¯	33.27¯
ADL/NDF (%)		3.48¯	3.14¯	3.83¯	3.85¯	3.87¯	3.73¯	4.11¯	3.93¯	4.18	3.86¯	3.99¯	4.22	4.22	4.49	4.63	4.09-	4.19	4.71	4.82
Cell/NDF (%)		42.63¯	44.85	43.44-	43.01¯	44.00	43.83	44.11	44.67	44.53	45.79*	43.22¯	43.83	44.67	44.60	43.79	44.25	44.70	44.22	44.71
Hcell/NDF (%)		53.88*	52.01	52.74*	53.14*	52.14	52.45*	51.78	51.40	51.29	50.36	52.79*	51.95	51.11	50.91	51.58	51.66	51.12	51.07	50.48
Ester *p*-coumaric acid (mg/g NDF)		8.93¯	10.17	8.12¯	9.11¯	10.52	10.65	10.48	10.49	11.28*	11.17*	9.83	10.00	10.01	10.29	11.20*	10.19	10.61	11.47*	11.46*
Ester ferulic acid (mg/g NDF)		5.85	6.00*	5.33¯	5.69	6.01*	6.06*	5.99*	5.72	5.85	5.92*	5.89*	5.72	5.57	5.60	5.40	5.81	5.93*	5.74	5.85
Ether ferulic acid (mg/g NDF)		1.08¯	1.11¯	1.04¯	1.08¯	1.07¯	1.07¯	0.99¯	0.99¯	1.16¯	1.05¯	1.19	1.12¯	1.14¯	1.26	1.23	1.25	1.10¯	1.17	1.17
5-5 diferulic acid (mg/g NDF)		0.17*	0.16	0.18*	0.17*	0.17*	0.18*	0.16	0.16	0.16	0.16	0.16	0.16	0.15	0.15	0.15	0.16	0.15	0.16	0.16
8-O-4 diferulic acid (mg/g NDF)		0.39*	0.37*	0.39*	0.37*	0.38*	0.38*	0.35	0.36	0.35	0.36	0.38*	0.36	0.34	0.34	0.34	0.35	0.33	0.34	0.35
p-Hydroxybenzaldehyde (mg/g NDF)		1.16¯	1.40	1.12¯	1.21¯	1.44	1.37	1.35	1.37	1.51*	1.54*	1.28	1.34	1.31	1.38	1.46	1.43	1.48	1.51*	1.50*
Vanillin (mg/g NDF)		6.50¯	7.29	5.54¯	6.61¯	6.90	6.66	5.96¯	6.34¯	7.18	6.41¯	6.71	6.98	7.05	7.19	6.37¯	6.96	6.71	6.14¯	6.56¯
Syringaldehyde (mg/g NDF)		6.33¯	7.36	5.95¯	6.51¯	6.68¯	7.04¯	5.91¯	6.59¯	7.84	6.77¯	6.94¯	6.82¯	6.83¯	7.77	7.63	7.24¯	7.21¯	6.80¯	8.16

tive of a lignin polymer comprising few condensed bonds (Cabane *et al.,* 2004; Barrière *et al.,* 2007). This lignin structure could partly explain why L08, L66 and L116 belonged to the most digestible lines of the studied set. Conversely, L15, L49, L883 and F271 had probably a more condensed lignin structure with higher H contents and more esterified *p*-coumaric acid. The apparent correlation observed between esterified *p*-coumaric acid and Hg contents has to be considered cautiously, as a part of *p*-coumaric acid is

converted into 4-*p*-hydroxybenzaldehyde similarly as *p*-hydroxyphenyl units during nitrobenzene cell wall oxidation. Little variation for esterified ferulic acid was shown with a tendency to higher releases in lines with higher IVNDFD, probably because of higher intensity of cross-linkages in lines with lower IVNDFD. Significantly lower contents of etherified ferulic acid were found in the six lines with higher IVNDFD (L08, L19, L21, L24, L66 and L116) and in F564 and F286 which are both progeny of INRA F7 (Lacaune landrace) and Argentina germplasm (Cimmyt PI186-223). This weaker etherified ferulic content was also found in lines of lower IVNDFD (L15, L49, L78, L77 and L16). Little variation was shown for diferulic acid contents (5-5 and 8-O-4 diferulic acid contents). Nevertheless, six lines (L08, L19, L21, L24, L66 and L116), which were shown to have significantly higher cell wall digestibility, had significantly higher releases in diferulic acid than F2. Finally, only line L66 was significantly different from F2 for all cell wall investigated traits, while L08 and L116 were significantly different for all traits except for esterified ferulic acid content.

TABLE 4 - *Estimated correlations between cell wall based on genotype means in the 19 lines set (correlations with absolute value lower than 0.46 were not significantly different from the null value, at P = 0.05; IVNDFD* = in vitro *NDF digestibility, NDF = neutral detergent fiber, ADL = acid detergent fiber, Cell = cellulose, Hcell = hemicelluloses).*

Traits	IVNDFD	ADL/NDF
ADL/NDF	- 0.88	–
Cell/NDF	- 0.41	0.19
Hcell/NDF	0.73	- 0.60
Esterified *p*-coumaric acid	- 0.65	0.54
Esterified ferulic acid	0.09	- 0.37
Etherified ferulic acid	- 0.51	0.52
5-5 diferulic acid	0.62	- 0.56
8-O-4 diferulic acid	0.73	- 0.68
p-Hydroxybenzaldehyde	- 0.64	0.46
Vanillin	- 0.02	- 0.11
Syringaldehyde	- 0.50	0.45

Correlations between IVNDFD and cell wall biochemical traits

The highest correlation was found between IVNDFD and ADL/NDF content, and ADL/NDF content accounted for 77% of the IVNDFD digestibility value (Table 4). In agreement with several previous studies (JUNG and DEETZ, 1993; WOLF *et al.,* 1993; LUNDVALL *et al.,* 1994; MÉCHIN *et al.,* 1998; ARGILLIER *et al.,* 2000; MÉCHIN *et al.,* 2000) lignin content thus remains an effective predictor of *in vitro* digestibility. However, the use of lignin content in silage maize breeding program is not fully relevant as the correlation with lignin content has been proven to be lower with *in vivo* digestibility (BARRIÈRE *et al.,* 2003). As previously observed (BUXTON and RUSSELL, 1988; JUNG, 1989; FONTAINE *et al.,* 2003), *p*-coumaric acid content was significantly and negatively correlated with cell wall digestibility. This correlation is probably related to the fact that *p*-coumarate content is a relevant indicator of late lignin deposition (GRABBER *et al.,* 2004; BARRIÈRE *et al.,* 2007). *p*-Coumarate content thus illustrated a specific aspect of lignification as *p*-coumarate content only explained 25% of lignin content (Table 4). Hb content was also correlated negatively with IVNDFD in a similar range as ADL/NDF content. This result showed that the impact of the H minor unit on cell wall properties should not be underestimated. Its detrimental effect on IVNDFD is probably related to the fact that this unit increases the frequency of resistant inter-unit bonds (CABANE *et al.,* 2004). The best linear regression between IVNDFD and investigated traits, with two explanatory variables, was based on ADL/NDF and Hg contents and explained 84% of IVNDFD variation. While no correlation was observed between IVNDFD and Va content, a significant negative correlation was found between cell wall digestibility and Sg content, correlatively with the increase in S unit deposition in physiologically more maturer tissues (BUXTON and RUSSELL, 1988; CHEN *et al.,* 2002). Nevertheless, G or S unit contents could not be reliably used as a breeding trait in maize cell wall digestibility improvement. MÉCHIN *et al.* (2000) has found a positive correlation between IVNDFD and the S/G ratio, but GRABBER *et al.* (1997) showed that the proportions of H, G and S units in lignin polymer are probably not direct factors controlling degradability.

Similarly, significant correlations were also shown between IVNDFD and ferulate or diferulate contents (Table 4). Etherified ferulic acid content was negatively correlated with IVNDFD, strengthening the probable unfavorable effect of ferulate cross-linkages on cell wall digestibility (JUNG, 1996; JUNG *et al.,* 1998; GRABBER *et al.,* 1998b; FONTAINE *et al.,* 2003; BARRIÈRE *et al.*, 2007). In addition, positive correlations were shown between IVNDFD and 5-5 and 8-O-4 diferulic acid contents, results seemingly

in contradiction with the supposed unfavorable role of arabinoxylan cross-linkages in the cell wall. Moreover, GRABBER *et al.* (1998a) have shown a negative linkage between diferulic acid cross-linkages in cell walls and the enzymatic degradation of non-lignified cell suspension of maize. The release of diferulic acid measured after an alkaline hydrolysis of lignified cell walls have therefore to be considered as illustrating a different biological phenomenon. It is indeed not understood how diferulic acid contents measured after an alkaline hydrolysis reflect total diferulic acids present in the cell wall, all the more as the releasable quantity of diferulic acids has been considered to range between 25 to 65% (GRABBER *et al.,* 2004). It is thus be hypothesized that the amount of diferulates released after alkaline hydrolysis reflects more a specific physical organization of the cell wall than the amount of diferulates involved in cross-linkages. A higher release of diferulates should thus correspond to more accessible and degradable cell walls. No correlation was observed between esterified ferulic acid content and IVNDFD, strengthening the fact that only ferulates involved in cross-coupling of components have a detrimental effect on cell wall degradability. Corroborating the importance of *p*-hydroxycinnamates in cell wall digestibility variation, the best linear regression between IVNDFD and investigated traits except lignin content, with two explanatory variables, was based on *p*-coumaric and 8-8 diferulic acid contents and explained 59% of IVNDFD variation.

The positive correlation between Hcell/NDF and digestibility could highlight the influence of cell wall carbohydrate type and organization on feeding value (Table 4). The non-crystalline structure of hemicellulose polymer could correspond to more easily degradable cell walls, even if the correlation between IVNDFD and cellulose was significantly lower. A higher Hcell/Cell ratio could similarly correspond to differently organized cell walls with a correlative higher degradability. Such a different Hcell/Cell ratio could also correspond to different tissue organizations and respective importance, giving a higher whole plant digestibility.

CONCLUSION

Large range of genetic variation in several biochemical components of maize cell walls have been shown, including traits probably related to both tissue degradability and friability, and correlatively with digestibility and intake in cattle. A new ideotype of forage maize can be putatively designed based on the cell wall favorable traits shown from this set of lines. Thus, a maize hybrid of high feeding value should have a low content in ADL/NDF, the first factor negatively influencing whole plant digestibility. The lignin polymer of this ideotype should have few H unit contents, with low proportion of *p*-coumaroylated S units. The ideotype should simultaneously have low etherified ferulic acid content as an indicator of limited cross-linkages between cell wall components. In addition, higher releasable diferulic acids should be favored as a probable indicator of favorably organized cross-linkages in the wall. Finally, higher hemicellulose content with correlative lower cellulose content in the cell wall have to be considered as related to a better feeding value. Only three studied lines out of 19 (L08, L66 and L116) had such a cell wall pattern, which were among the four most digestible lines of the study. Out of these three lines, L08, which had the highest cell wall digestibility of the studied set, could be an efficient parent or model line in future forage maize breeding programs.

However, the possibly of gathering in one line most of all highly favorable traits for both feeding value and agronomic value (DM yield, standability, pest resistance, water conduction and water stress tolerance) is still an unanswered question. Several of the QTL involved in corn borer resistance have been shown in colocalizations with QTL also involved in lignin content and/or cell wall digestibility (RALPH *et al.*, 2004a; BARRIÈRE *et al.*, 2007). Elite maize hybrids currently available with higher cell wall digestibility were always lower yielding, and mostly often more susceptible to stalk lodging or breakage. Highly lignified fibers and vessels are very likely inescapable traits for resistance to dry conditions, and also possibly to an optimal transport of nutriments and photosynthesis products. The best breeding strategy will probably be based on compromise solutions. An elite forage maize line has to be firstly bred for agronomic traits, with the introgression of genome location, QTL, or alleles having the most relevant effect on cell digestibility. Such targeted improvements will be based on a larger knowledge of genetic and genomic basis of phenylpropanoid compounds deposition in each lignified tissue, and of the regulation of lignified tissue patterning. Breeding will be based on the marker assisted introgression of QTL or alleles involved

in maize feeding value in lines of high agronomic values. Moreover, the germplasm bred for higher cell wall degradability will be fully relevant and available for the new use of maize stover in biofuel production.

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REFERENCES

ARGILLIER O., Y. BARRIÈRE, Y. HÉBERT, 1995 Genetic-variation and selection criterion for digestibility traits of forage maize. Euphytica **82:** 175-184.

ARGILLIER O., V. MÉCHIN, Y. BARRIÉRE, 2000 Inbred line evaluation and breeding for digestibility-related traits in forage maize. Crop Sci. **40:** 1596-1600.

AUFRÈRE J., B. MICHALET DOREAU, 1983 In vivo digestibility and prediction of digestibility of some by-products. EEC Seminar, 26-29 September 1983. Mlle Gontrode, Belgique.

BARRIÈRE Y., R. TRAINEAU, J.C. EMILE, Y. HÉBERT, 1991 Variation and Covariation of Silage Maize Digestibility Estimated from Digestion Trials with Sheep. Euphytica **59:** 61-72.

BARRIÈRE Y., C. GUILLET, D. GOFFNER, M. PICHON, 2003 Genetic variation and breeding strategies for improved cell wall digestibility in annual forage crops. A review. Animal Res. **52:** 193-228.

BARRIÈRE Y., J. RALPH, V. MÉCHIN, S. GUILLAUMIE, J.H. GRABBER, O. ARGILLIER, B. CHABBERT, C. LAPIERRE, 2004 Genetic and molecular basis of grass cell wall biosynthesis and degradability. II. Lessons from brown-midrib mutants. C. R. Biol. **327:** 847-860.

BARRIÈRE Y., C. RIBOULET, V. MÉCHIN, M. PICHON, A.J. CARDINAL, T. LUBBERSTEDT, C. LAPIERRE, 2007 Genetics and genomics of lignification in grass cell walls based on maize as a model system. Genes Genomes Genomics **1:** 133-156.

BUXTON D., J. RUSSELL, 1988 Lignin constituents and cell-wall digestibility of grass and legume stems. Crop Sci. **28:** 553-558.

CABANE M., J.C. PIREAUX, E. LEGER, E. WEBER, P. DIZENGREMEL, B. POLLET, C. LAPIERRE, 2004 Condensed lignins are synthesized in poplar leaves exposed to ozone. Plant Physiol. **134:** 586-594.

CHEN L., C. AUH, F. CHEN, X. CHENG, H. ALJOE, R. DIXON, Z. WANG, 2002 Lignin deposition and associated changes in anatomy, enzyme activity, gene expression, and ruminal degradability in stems of tall fescue at different developmental stages. J. Agric. Food Chem. **50:** 5558-5565.

COORS J.G., P.R. CARTER, R.B. HUNTER, 1994 Silage Corn. pp. 305-340. *In:* A.R. Hallauer (Ed.), Specialty corns. CRC Press, Boca Raton, FL.

DEINUM B., P.C. STRUIK, 1985 Improving the nutritive value of forage maize. pp. 77-90. *In:* Proc. 13th Congress of Maize and Sorghum Section of Eucarpia 9-12 Sept. 1985, Wageningen, The Netherlands.

DOLSTRA O., J.H. MEDEMA, 1990 An effective screening method for improvement of cell wall digestibility in forage maize. pp. 258-270. *In:* Proc. 15th Eucarpia Congress Maize and Sorghum, June 4-8, 1990, Baden, Austria.

FERNANDEZ I., 2003 Influence de la finesse de hachage des ensilages de maïs sur leur digestion (amidon et parois) et leur ingestibilité par les vaches laitieres. PHD Université Blaise Pascal Clermont-Ferrand, France.

FERNANDEZ I., C. MARTIN, M. CHAMPION, B. MICHALET-DOREAU, 2004 Effect of corn hybrid and chop length of whole-plant corn silage on digestion and intake by dairy cows. J. Dairy Sci. **87:** 1298-1309.

FONTAINE A.S., M. BRIAND, Y. BARRIÈRE, 2003 Genetic variation and QTL mapping of para-coumaric and ferulic acid contents in maize stover at silage harvest. Maydica **48:** 75-82.

GOERING H.K., P.J. VAN SOEST, 1970 Forage fiber analysis (Apparatus, Reagents, Procedures, and some applications). p. 379. USDA ARS Agricultural handbook US government Printing Office, Washington, DC.

GRABBER J.H., 2005 How do lignin composition, structure, and cross-linking affect degradability? A review of cell wall model studies. Crop Sci. **45:** 820-831.

GRABBER J.H., J. RALPH, R.D. HATFIELD, S. QUIDEAU, 1997 *p*-Hydroxyphenyl, guaiacyl, and syringyl lignins have similar inhibitory effects on wall degradability. J. Agric. Food Chem. **45:** 2530-2532.

GRABBER J.H., R.D. HATFIELD, J. RALPH, 1998a Diferulate cross-links impede the enzymatic degradation of non-lignified maize walls. J. Sci. Food Agric. **77:** 193-200.

GRABBER J.H., J. RALPH, R.D. HATFIELD, 1998b Ferulate cross-links limit the enzymatic degradation of synthetically lignified primary walls of maize. J. Agric. Food Chem. **46:** 2609-2614.

GRABBER J.H., J. RALPH, C. LAPIERRE, Y. BARRIÈRE, 2004 Genetic and molecular basis of grass cell-wall degradability. I. Lignin-cell wall matrix interactions. C. R. Biol. **327:** 455-465.

HIGUCHI T., Y. ITO, I. KAWAMURA, 1967 *p*-Hydroxyphenyl-propane component of grass lignin and role of tyrosine-ammonia lyase in its formation. Phytochemistry **6:** 875-881.

JUNG H.G., 1989 Forage lignins and their effects on fiber digestibility. Agron. J. **81:** 33-38.

JUNG H.G., 1996 Identification of cell wall traits that can be manipulated to improve forage digestibility. US Dairy Forge Research Center, Informational Conference with Dairy and Forage Industries.

JUNG H.J.G., D.A. DEETZ, 1993 Cell-wall lignification and degradability. pp. 315-346. *In:* H.G. Jung, D.R. Buxton, R.D. Hatfield, J. Ralph (Eds.), Forage cell-wall structure and digestibility. American Society of Agronomy, MU.

JUNG H.G., T.A. MORRISON, D.R. BUXTON, 1998 Degradability of cell-wall polysaccharides in maize internodes during stalk development. Crop Sci. **38:** 1047-1051.

KOBILINSKY A., 1983 MODLI, logiciel d'analyses de modéle linéaires. INRA, Laboratoire de Biométrie, 78026 Versailles Cedex, France.

LILA M., 1977 Influence des modalités de séchage sur la mesure de la teneur des fourrages en éléments azotés et glucidiques. Conséquences lors des récoltes d'essais au champ. Ann. Amélior. Plantes **27:** 465-475.

LUNDVALL J., D. BUXTON, A. HALLAUER, J. GEORGE, 1994 Forage quality variation among maize inbreds: in vitro digestibility and cell-wall components. Crop Sci. **34:** 1672-1678.

MACADAM J.W., J.H. GRABBER, 2002 Relationship of growth cessation with the formation of diferulate cross-links and p-coumaroylated lignins in tall fescue leaf blades. Planta **215:** 785-793.

MÉCHIN V., O. ARGILLIER, Y. BARRIÈRE, V. MENANTEAU, 1998 Genetic variation in stems of normal and brown-midrib 3 maize inbred lines. Towards similarity for in vitro digestibility and cell wall composition. Maydica **43:** 205-210.

MÉCHIN V., O. ARGILLIER, V. MENANTEAU, Y. BARRIÈRE, I. MILA, B. POLLET, C. LAPIERRE, 2000 Relationship of cell wall composition to in vitro cell wall digestibility of maize inbred line stems. J. Sci. Food Agric. **80:** 574-580.

MORRISON W.H., D.E. AKIN, D.S. HIMMELSBACH, G.R. GAMBLE, 1993 Investigation of the ester-linked and ether-linked phenolic constituents of cell-wall types of normal and brown midrib pearl-millet using Chemical Isolation, Microspectrophotometry and C-13 Nmr-Spectroscopy. J. Sci. Food Agric. **63:** 329-337.

RALPH J., S. GUILLAUMIE, J.H. GRABBER, C. LAPIERRE, Y. BARRIÈRE, 2004 Genetic and molecular basis of grass cell-wall biosynthesis and degradability. III. Towards a forage grass ideotype. Comptes Rendus Biol. **327:** 467-479.

STRUIK P.C., 1983 Physiology of forage maize (*Zea mays* L.) in relation to its production and quality. Ph D diss Agricultural University Wageningen, The Netherlands.

THORSTENSSON E.M.G., D.R. BUXTON, J.H. CHERNEY, 1992 Apparent inhibition to digestion by lignin in normal and brown midrib stems. J. Sci. Food Agric. **59:** 183-188.

TOLIVIA D., J. TOLIVIA, 1987 Fasga: a new polychromatic method for simultaneous and differential staining of plant tissues. J. Microscopy **148:** 113-117.

VENABLES W., B.B. RIPLEY, 1994 Modern applied statistics with Splus. Springer-Verlag, New York.

WOLF D.R., J.G. COORS, K.A. ALBRECHT, D.J. UNDERSANDER, P.R. CARTER, 1993 Forage quality of maize genotypes selected for extreme fiber concentrations. Crop Sci. **33:** 1353-1359.