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ORIGINAL PAPER

QTLs for agronomic and cell wall traits in a maize RIL progeny derived from a cross between an old Minnesota13 line and a modern Iodent line

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Abstract In order to contribute to the inventory of genomic areas involved in maize cell wall lignification and degradability, QTL analyses were investigated in a RIL progeny between an old Minnesota13 dent line (WM13) and a modern Iodent line (RIo). Significant variation for agronomic- and cell wall-related traits was observed for the RIL per se (plants without ears) and topcross (whole plants) experiments after crossing with both old (Ia153) and modern tester (RFl) lines. Three QTLs for stover (plant without ear) yield were observed in per se experiments, with alleles increasing yield originating from RIo in two genomic locations with the highest effects. However, no QTL for whole plant yield was detected in topcross experiments, despite the fact that two QTLs for starch content were shown with increasing alleles originating from the modern RIo line. Fifteen lignin QTLs were shown, including a QTL for Klason lignins in per se experiments, located in bin 2.04, which explained 43 % of the observed genetic variation. Thirteen QTLs for *p*-hydroxycinnamic acid contents and nine QTLs related to the monomeric composition of lignin were shown in per se experiments, with syringaldehyde and diferulate QTLs explaining nearly 25 % of trait variations. Nine and seven QTLs for cell wall digestibility were mapped in per se and topcross experiments, respectively. Five of the per se QTLs explained more than 15 % of the variation, up to nearly 25 %. QTL positions in bins 2.06, 5.04, 5.08 and 8.02 for ADL/NDF, IVNDFD, lignin structure and/or *p*-hydroxycinnamic acid contents have not been previously shown and were thus first identified in the RIo × WM13 progeny. Based on QTL colocalizations, differences in cell wall degradability between RIo and WM13 were less often related to acid detergent lignin (ADL) content than in previous RIL investigations. QTL colocalizations then highlighted the probable importance of ferulate cross linkages in variation for cell wall digestibility. No colocalizations of QTL for cell wall phenolic-related traits were shown with genes involved in monolignol biosynthesis or polymerization. In contrast, colocalizations were most often shown with MYB and NAC transcription factors, including orthologs of master genes involved in *Arabidopsis* secondary wall assembly. QTL colocalizations also strengthened the probable involvement of members of the CoA-dependent acyltransferase PF02458 family in the feruloylation of arabinoxylan chains.

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Introduction

After the Second World War, the period between 1947 and 1960 marked the emergence of European maize pedigree breeding. Then, from 1958 to 1980, maize hybrids were thus bred based on the heterotic pattern between Wisconsin- and Minnesota-related lines adapted to European conditions and European early flint lines related to the Lacaune and northern flint groups. In 1970, the registration in France of LG11, the first three-way hybrid, also marked the beginning of the rapid increase in silage maize areas.

The registration of DEA in France (1980), a single cross between Pioneer Pio165 and INRA F2 lines, with outstanding grain yield performances and standability, illustrated tremendous changes in European maize germplasm and marked the beginning of a second era in modern European early maize breeding. The dent parental line of DEA is related to the Iodent (or Iodent Reid) germplasm that was not used in European dent lines breeding before the registration of this hybrid. Iodent germplasm became the foundation of all early hybrids and is still at the basis of early maize improvements in Europe. The present maize breeding period is also characterized by a broadening of the genetic basis of early maize breeding through introgression of medium late germplasm. Current female dent lines include Iodent, BSSS, and to a lesser extent Lancaster germplasm, while flint resources have been broadened from Lacaune (INRA F2) or northern flint lines with other flint origins, and/or introgressed by dent origins. In addition, these modern hybrids also have a different physiology. They are later flowering than the earlier ones, but have faster grain filling and drying, an important stay green, a more erected habit, and most often a stiff stalk and lower cell wall digestibility.

As a consequence of the introduction of Iodent and BSSS resources, the phasing out of flint lines with high cell wall digestibility but with poor standability and yield, and the focus of breeders on (grain) yield and standability, a significant drift of hybrids towards lower cell wall digestibility values was observed (Barrière et al. 2004a, 2006). The decline in average cell wall digestibility, which has been mainly observed since 1980, has now ceased with the breeding of specialized silage maize and the use of a digestibility criterion in forage maize registration since 1986 in the Netherlands and since 1998 in France. While the cell wall degradability of the best modern hybrids does not yet equal that of the best older types, such as INRA258, several currently registered French hybrids have cell wall digestibility close to that of DEA-type hybrids. However, unlike yield or stress tolerance for which regular improvements have been observed, energy value of currently released silage maize varieties plateaus due to the insufficient improvement in cell degradability. Economically profitable milk production, nevertheless, requires forage with high cell wall digestibility and intake. Similarly, second generation biofuel production, based on grass stover or straw, will be profitable only with plants with high cell wall degradability and saccharification levels. Hence, a breeding scheme more directly focused on stover quality is required for further improvement in maize plant energy value. This strategy will be more efficient if the genetic determinants of cell wall assembly and degradability are discovered and marker-assisted selection is made possible.

To date, QTL analyses for cell wall-related traits have been investigated in publicly available lines used by European maize breeders over the past 20 years, including flint and dent germplasm (Méchin et al. 2001; Roussel et al. 2002; Fontaine et al. 2003; Barrière et al. 2008, 2010; Riboulet et al. 2008a). Hence, the purpose of this research was to highlight some of the still unknown genomic areas involved in cell wall-related trait variation in a study based on two lines expected to be significantly distinct from each other, given their temporal and genetic differentiation. A RIL progeny was thus descended between an old US Minnesota13 dent line used since 1936 (WM13, also named M13 and W13) and a currently used private Iodent French line (RIo). The two WM13 and RIo lines cannot be considered as representative of the whole universe of lines released for dent heterotic patterns at the two specific breeding eras. However, WM13 and RIo are two lines typical of Minnesota13 and Iodent germplasms used in the 1940–1950s and 2000–2010 eras of breeding, respectively. The search for QTLs in the progeny of these two lines, with a diversification of the currently considered genetic resources, is expected to increase the inventory of genomic areas involved in maize cell wall lignification and degradability. These investigations could also contribute to highlight differences in cell wall composition and degradability between old and modern maize lines. In addition to QTL analyses, the search for candidate genes underlying QTLs was simultaneously considered, with a focus on genes involved in phenolic component biosynthesis and lignified-tissue assembly.

Materials and methods

RIL progeny

A progeny consisting of 163 RILs was developed by single seed descent (SSD) up until the F7 generation in a cross between the modern elite line RIo and the old Wisconsin line WM13. The medium-early WM13 line is one of the 25 outstanding inbred lines bred from the Minnesota13 yellow dent open-pollinated variety before the Second World War (Jenkins 1936, quoted in Troyer and Hendrickson 2007; Gerdes et al. 1993; Troyer and Hendrickson 2007). WM13 was used in numerous Wisconsin old hybrids (G Amberson, com. pers.) including the medium-early hybrid W355 [(Ia153 × W25) × (W9 × WM13)] that was registered in France in 1951. The RIo line, bred by the RAGT-R2n company, is a medium-early dent line which only belongs to the Iodent genetic group and has been used in registered hybrids since 2005. In order to estimate hybrid values of RIo × WM13 lines, topcrosses of all 163 RILs were considered with two different male lines. RILs were crossed

with the modern line RFl, which is the male line in the registered RIo × RFl hybrid and belongs to the "flint" heterotic group. Its germplasm is two-thirds related to the flint Lacaune/Galicia groups and one-third to the B14-related Stiff-Stalk group. RILs were also crossed with Ia153, which is a heterotic partner of WM13 in the W355 hybrid. Ia153 was bred in the open-pollinated variety US Selection n°133 which also, in turn, was bred from Minnesota13.

Field experiments and plant harvests

Field experiments were carried out over 2 years (2005 and 2006) for RIL topcross evaluation in two locations (Lusignan, Vienne, and Le Pin, Orne) with an extra location in 2005 (Druelle, Aveyron). Field experiments were similarly carried out over 2 years (2006 and 2007) for RIL per se evaluation in two locations, Lusignan (Vienne) and Le Pin (Orne). Each year, RILs or hybrids were evaluated using a generalized alpha-lattice design with two replicates. Each experimental plot was a 5.2 m long single row of 37 plants sown in Lusignan, and a similar two row plot in Le Pin and Druelle. Row spacing was 0.75 m, and the density was 90,000 plants ha^{-1}. Irrigation was applied in Lusignan during the summer to prevent water stress. Silking dates were recorded as days from July 1st. In order to have an estimate of leaf erectness, ear leaf angle was recorded in Lusignan topcross experiments on five plants per row as the angle (degrees) between the internode bearing the ear and the basal dorsal part of the ear leaf. The average value of the five plants was then considered in data analyses. In RIL per se experiments, ears were removed by hand from all plants the day before and/or on harvesting day in all plots, allowing an estimate of stover ferulate content without confusing effect with grain ferulates. Plots of RIL per se and topcross experiments were machine harvested with a forage chopper at silage maturity stage. A representative sample of 1 kg chopped material per plot was collected for dry-matter (DM) content, DM yield estimates, and biochemical analysis. Samples were dried in a ventilated oven (65 °C), and dry samples were ground with a hammer mill to pass through a 1 mm screen for further analyses.

Cell wall analyses

Neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) were investigated according to Goering and Van Soest (1970). Klason lignin (KL) was estimated according to Dence and Lin (1992). KL values are two to four times greater in grasses than ADL estimates, corresponding to the loss of an acid-soluble part of lignins in the first step of the ADL procedure (Hatfield et al. 1994; Jung et al. 1997; Hatfield and Fukushima 2005). Cellulose (Cell) and hemicelluloses (Hcell) were estimated as ADF–ADL and NDF–ADF, respectively (Goering and Van Soest 1970), Cell, Hcell, ADL, and KL, which are components of the cell wall, were expressed as percentages of NDF (Cell/NDF, Hcell/NDF, ADL/NDF, and KL/NDF). The in vitro dry matter digestibility (IVDMD) was estimated according to Aufrère and Michalet-Doreau (1983). Cell wall digestibility was investigated according to Struik (1983) and Dolstra and Medema (1990). In vitro NDF digestibility (IVNDFD) was thus computed assuming that the non-NDF part of plant material was completely digestible [IVNDFD = 100 × (IVDMD − (100 − NDF))/NDF]. Cell wall digestibility was also estimated as the in vitro digestibility of the "non starch, non soluble carbohydrates, and non crude protein" part (DINAGZ, Argillier et al. 1995; Barrière et al. 2003). The latter is computed assuming these three constituents are completely digestible [DINAGZ = 100 × (IVDMD − ST − SC − CP)/(100 − ST − SC − CP)] where ST, SC and CP are starch, soluble carbohydrate, and crude protein contents, respectively (and considering a null value for starch content in plant without ears).

p-Hydroxycinnamic acid contents were measured after treating NDF fractions with NaOH according to the double procedure previously described by Morrison et al. (1993) and used by Méchin et al. (2000). This procedure involves a mild alkaline treatment allowing the release of esterified ferulic (esterFA) and *p*-coumaric (pCA) acids, and a severe alkaline treatment allowing the release of etherified ferulic acid (etherFA). In the light of the fact that almost all the pCA is esterified (Ralph et al. 1994; Hatfield et al. 1999), only esterified pCA content was investigated. The concentration of etherFA was calculated as the difference between FA amounts released by the severe and mild alkaline treatments, as all etherFA is also involved in esterified linkages. Besides esterFA and etherFA contents, two FA dimers were also reported as the content in the 5-5 and 8-O-4 FA dimers. The latter is the predominant FA dimer out of the six shown in maize cell wall (Lindsay and Fry 2008). In order to investigate the monomeric composition of lignins, oxidation of cell wall residues with alkaline nitrobenzene was performed according to a method adapted from Roadhouse and MacDougall (1956) according to Higuchi and Kawamura (1966). During alkaline nitrobenzene oxidation, *p*-hydroxyphenyl (H), guaiacyl (G), and syringyl (S) monomers, resulting from *p*-coumaryl, coniferyl and syringyl alcohol polymerization, respectively, are oxidized into *p*-hydroxybenzaldehyde (pHb), vanillin (Va) and syringaldehyde (Sg), respectively, with disruption of Cα and Cβ linkages. Parts of pCA and FA are also oxidized into pHb and Va, respectively. Consequently, amounts of H and G units of lignins may be

partly overestimated. Extracted pHb, Va, and Sg were analyzed by HPLC.

Due to the large number of samples, all biochemical traits were estimated using near infrared reflectance spectroscopy (NIRS, NIRS system 6500 spectrophotometer). Calibrations available from the Centre de Recherches Agronomiques in Libramont (Belgium, Dardenne et al. 1993) were used for hybrid experiments. Specific calibrations, which were developed at INRA Lusignan for traits investigated in plants without ears, were used for RIL per se experiments (Barrière et al. 2008; Riboulet et al. 2008b). Calibration equations were validated by a laboratory analysis of 20 samples per location with a sample choice based on spectral data.

Data analyses

Variance analyses were carried out using the standard procedure of a fixed model for both per se and topcross experiments with genotype, environment (year-location), block, sub-block and interaction effects, as $Y_{ijkl} = \mu + E_j + B_kE_j + SB_lB_kE_j + G_i + G_iE_j + \varepsilon_{ijkl}$, with Y_{ijkl} = observed value for a given trait, μ = grand mean, E_j = environment effect, B_kE_j = block within environment effect, $SB_lB_kE_j$ = sub-block within block and environment effect, G_i = genotype effect, G_iE_j = genotype × environment interaction, and ε_{ijkl} = residual error ($=\sigma_r^2$), using Modli (least square means, Kobilinsky 1983) and Splus Softwares (Venables and Ripley 1994). Tester effect (RFl vs. Ia153) in topcross experiments was similarly estimated after adding tester and tester × genotype interaction effects in the linear fixed model. Variances of genetic effects σ_g^2 were estimated with the standard procedure of a mixed model with random genotype and genotype × year-location interaction effects, using the SAS statistical package (SAS 1990) with the "varcomp" procedure and a restricted maximum likelihood method. Broad sense heritabilities were estimated as $\sigma_g^2/(\sigma_g^2 + \sigma_{g\times e}^2/e + \sigma_r^2/er)$ with $\sigma_{g\times e}^2$ = variance of genotype × environment interaction, e = number of environment, and r = number of replicates. Pearson's phenotypic correlations between traits were computed on the mean basis over environments.

Development of the linkage map and QTL identification

In accordance with their bin locations, nearly 300 simple sequence repeat (SSR) markers were chosen in the MaizeGDB database (http://www.maizegdb.org) throughout the genome. Out of these markers, 108 SSRs were polymorphic between the two parental lines RIo and WM13 and were successfully used on 163 RILs (91 markers run by Eurofins Company and 17 by RAGT-R2n and INRA Lusignan). The linkage map was developed using Mapmaker version 3.0b (Lincoln et al. 1992) and Biomercator (Arcade et al. 2004) softwares.

QTL mapping was based on trait means over years and locations for the 163 RILs, using the composite interval mapping (CIM, Zeng 1994) method implemented in the PLABQTL computer package (Utz and Melchinger 1996). PLABQTL uses the regression method (Haley and Knott 1992) in combination with markers which are selected by stepwise regression as cofactors. LOD support intervals are constructed in PLABQTL according to Lander and Botstein (1989) and are likely underestimated in the case of CIM. The percentage of phenotypic variance ascribed to an individual QTL was estimated with the approximate standard error of Kendall and Stuart (1961). The additive effects of QTLs were estimated as half the difference between the phenotypic values of the respective homozygotes. LOD threshold estimates were based on the permutation-test method of Churchill and Doerge (1994) implemented in PLABQTL, with 1,000 permutations. The maize bin was given for each QTL as the position of the left flanking marker, according to MaizeGDB.

In order to highlight colocalizations with candidate genes, QTL physical positions were estimated based on physical positions of the two flanking markers available in MaizeGDB database (B73 release 4a.53), and assuming a constant relationship between recombination and physical distances within this interval. The physical lengths of QTL support intervals were difficult to estimate in several instances due to their overlapping centromer positions. Consequently, the latter were not reported. Their gross values could be considered to be between 20 and 50 Mbp. The search for candidate genes underlying cell wall-related traits focused on genes involved in secondary wall biosynthesis and assembly, and was based on the list previously proposed by Barrière et al. (2009). The list also included maize orthologs of newly described transcription or regulation factors in *Arabidopsis* or woody plants (poplar, eucalyptus, …) and currently comprises 394 genes. Gene physical positions were based on the maize B73 sequence (http://www.maizesequence.org), assuming a similar genomic structure in RIL parental lines and B73 within QTL support intervals.

Results

Mean and variances estimates in RIL per se experiments

Genotype effects in per se experiments (plants without ears) were highly significant for all investigated traits

($P < 0.001$), and much higher than genotype × environment (GE) interaction effects (Table 1). Based on mean-square (MS) values, GE interaction effects were thus at least 6 times lower than genotype effects for most traits. However, MS GE interactions were slightly more important for crude protein, cell wall carbohydrate contents, and also for etherFA contents. Similar trends were also observed for genotype and GE interaction variances. QTL analyses were therefore relevantly investigated based on mean over environments for each trait, even if some specific-environmental information was likely lost. Broad sense heritabilities were high for all investigated traits, apart from a lower heritability estimate for etherFA following a low genetic variance estimate.

Average RIL stover DM content ranged in per se experiments from 24.1 to 27.9 % across years and locations, with an average value equal to 26.3 %. These values corresponded to maturity stages suitable for investigations of agronomic and lignification traits. Average silking dates were nearly 19 days later in the colder and northern Le Pin location than in the more southern and oceanic Lusignan location. Average lignin content and cell wall digestibility were comparable across environments and ranged from 4.97 to 5.47 % (ADL/NDF), and 33.1 to 27.5 % (IVNDFD), in Lusignan (2007) and Le Pin (2006), respectively. Stover DM yield was nearly 0.4 t ha^{-1} lower in WM13 than in RIo and the best transgressive RIL yielded nearly 3 t ha^{-1} more than the best parent (Table 1). Average silking date was 4 days later in WM13 than in RIo, with large transgressions for earliness and lateness. Stover crude protein content was slightly, but not significantly, higher in WM13. Lower lignin content and higher cell wall degradability were observed in WM13 in comparison to RIo, with important transgressive values in the RIL progenies, especially towards higher ADL/NDF contents. While the two parental lines had similar *p*-hydroxycinnamic acid contents or lignin monomeric composition, large transgressions were observed towards both higher and lower values for this set of traits, and especially for lower pCA and Sg contents. The unusually high values observed for pHb, nearly 10 % of aldehyde (pHb + Va + Sg) releases, illustrated a partial oxidation of pCA into pHb during alkaline-nitrobenzene attack of cell wall components. The *p*-hydroxyphenyl monomer indeed represents only 3 % of maize lignin monomers released after thioacidolysis.

Table 1 Variance analysis, mean, maximum, and minimum values of traits for RIo × WM13 RIL progenies in per se experiments and plant without ear at silage harvest

Traits	Genotype MS	Gen × env MS	σ^2_r	σ^2_g	$\sigma^2_{g\times e}$	h^2	Mean	Mini	Maxi	RIo	WM13
Yield	10.9	1.20	0.47	1.33	0.39	0.89	5.78	3.04	8.78	5.95	5.58 ns
Dry-matter	31.1	4.87	1.10	3.54	2.02	0.85	26.3	22.4	32.8	25.9	24.9 ns
Silking date	74.4	6.50	2.92	9.27	1.91	0.91	34.5	26.1	42.5	32.9	36.5*
Sol carbohyd	44.2	9.81	2.47	4.59	3.94	0.80	18.8	12.5	24.9	18.3	21.5*
Crude protein	4.86	0.98	0.36	0.53	0.33	0.78	8.48	6.66	11.0	7.92	8.60 ns
Cell/NDF	6.67	0.99	0.59	0.77	0.22	0.85	45.7	43.4	48.0	45.2	46.0 ns
Hcell/NDF	10.2	1.48	0.96	1.19	0.28	0.86	49.1	45.8	52.4	49.4	49.1 ns
ADL/NDF	1.21	0.16	0.11	0.14	0.03	0.87	5.24	4.12	6.40	5.44	4.86*
KL/NDF	3.82	0.61	0.32	0.44	0.14	0.84	14.5	12.8	16.8	14.4	13.9 ns
IVNDFD	35.4	6.3	3.05	3.93	1.80	0.82	30.2	24.1	36.5	28.8	33.9*
DINAGZ	36.4	7.2	3.15	4.00	1.97	0.81	50.5	45.0	56.1	49.6	53.2*
pCA	6.44	1.37	0.92	0.70	0.22	0.80	16.1	14.2	18.2	16.0	16.0 ns
EsterFA	0.35	0.06	0.03	0.04	0.016	0.82	6.08	5.55	6.62	6.10	6.13 ns
EtherFA	0.07	0.02	0.01	0.009	0.015	0.62	1.36	1.06	1.67	1.31	1.25 ns
5-5diFA	0.001	0.0002	0.0001	0.0002	0.00003	0.86	0.15	0.12	0.19	0.15	0.16 ns
8-O-4diFA	0.004	0.001	0.0004	0.0005	0.00009	0.86	0.36	0.26	0.39	0.32	0.32 ns
pHb	0.19	0.04	0.02	0.02	0.0009	0.79	2.09	1.73	2.50	2.07	2.05 ns
Va	1.71	0.33	0.19	0.19	0.07	0.81	7.55	6.16	8.70	7.58	7.30 ns
Sg	2.63	0.51	0.30	0.29	0.10	0.82	10.6	8.86	12.0	10.5	10.6 ns

All genotype and genotype × environment mean-square (MS) effects were significant at $P < 0.001$; differences between RIo and WM13 parents are given in the WM13 column, significant at $P < 0.05$ (*) or non significant (ns). Yield as t/ha, silking dates as days in July, pCA, FA, pHb, Va, and Sg as mg/g NDF, other traits as percentages

Sol carbohyd soluble carbohydrates, *NDF* neutral detergent fiber, *ADL* acid detergent lignin, *KL* Klason lignin, *Cell and Hcell* cellulose and hemicelluloses, *IVNDFD* in vitro NDF digestibility, *DINAGZ* in vitro cell wall digestibility according to Argillier et al. (1995), *pCa p*-coumaric acid, *EsterFA and EtherFA* esterified and etherified ferulic acids, *5-5 diFA and 8-O-4 diFA* 5-5 and 8-O-4 diferulic acids, *pHb p*-hydroxybenzaldehyde, *Va* vanillin, *Sg* syringaldehyde

Mean and variances estimates in topcross experiments

In topcross experiments (whole plants), variance analysis showed that tester effect (RFl vs. Ia153) was highly significant for all investigated traits, and to a greater extent for agronomic traits than for cell wall-related traits (Table 2). In addition, all genotype effects were highly significant either for crosses with the old Ia153 line or with the modern RFl line (Table 3). Significant GE interactions were shown in the two topcrosses which, for most traits, were of lower importance than in per se experiments based on GE MS or variance values. Crossing with either the modern or old line did not cause differences in trait genetic variances, except the greater value in yield genetic variance in the Ia153 topcross progenies. Similarly, a higher GE interaction variance was observed for yield in the topcross with Ia153 than in the topcross with RFl (Table 3). Broad sense heritabilities were high for yield, DM content, and silking date, but less high for starch, carbohydrate, and crude protein contents (Table 3). Heritabilities were also moderately high for cell wall-related traits, with lower values for KL/NDF than for ADL/NDF. Heritabilities of digestibility traits ranged from 0.55 to 0.61.

Average DM yield was nearly 1 t ha^{-1} higher in crosses with the modern line RFl than with the older one Ia153 (Table 3). Average DM content was also nearly 2.5 % higher in crosses with RFl. Similarly, highest yield values were shown in the two topcrosses, while hybrids with lower yields were shown in the topcross with Ia153. As observed in the per se RIo and WM13 comparison, there was a significantly higher average crude protein content in the topcross with the older line, with simultaneously higher minimum and maximum values. Average lignin contents were not significantly different in the two topcrosses, but there was a tendency to higher cell wall digestibility values in the topcross with the older line, especially when considering maximum values. Leaf angle was 10 angle degrees higher in the topcross with the modern RFl line (Table 3) with a highly significant tester effect (Table 2).

Correlations between traits

Correlations between agronomic traits (DM yield, DM content, silking, and leaf angle) and lignin or degradability traits were low or very low in both per se and topcross experiments (Table 4). As previously observed (Riboulet et al. 2008b), correlations between cell wall degradability traits and cellulose content were negative, while they were positive with hemicellulose content. Correlations between cell wall degradability traits and ADL/NDF lignin content were negative. No correlations were unexpectedly shown between lignin content and leaf erectness, or between cell wall degradability and leaf erectness. Finally, significant negative correlations were shown in per se experiments between cell wall degradability traits and lignin monomeric composition or *p*-hydroxycinnamic acid contents, except with esterified ferulic acid, corroborating previous observations (Méchin et al. 2000; Barrière et al. 2008; Riboulet et al. 2008b).

Map of the RIo × WM13 progeny

The map of the RIo × WM13 RIL progeny with a total length of 1,469 cM agreed with and was even a bit shorter than previously published maize maps and results available in the MaizeGDB database (1,759 cM for the UMC98 standard reference map for maize in MaizeGDB database). Chromosomes of the RIo × WM13 map were nearly 30 cM shorter than those of UMC98 reference map, except for chromosomes 1 and 3 which were of similar length and chromosome 8 that was 70 cM shorter. However, no polymorphic marker was obtained in bins 8.00 and 8.01.

Table 2 Variance analysis for tester effects (modern RFl and old Ia153 lines) in topcross experiments of the RIo × WM13 RIL progenies at silage harvest

Traits	Tester MS	Genotype MS	GT MS	GE MS	σ_r^2
Yield	1,133.5	26.7	7.46	4.23 ns	3.87
Dry-matter	1,447.8	30.6	5.72	4.30 ns	4.28
Silking date	877.7	21.4	4.93	3.46	2.45
Leaf angle[a]	31,065.2	103.8	42.4	31.4	37.9
Sol carbohyd	43.7	6.54	1.42 ns	1.79	1.35
Starch	1,232.7	59.6	13.5 ns	17.4	11.7
Crude protein	134.3	0.52	0.18 ns	0.16 ns	0.27
Cell/NDF	49.1	3.10	0.64 ns	0.73 ns	0.68
Hcell/NDF	90.9	3.91	0.88 ns	0.98 ns	1.00
ADL/NDF	6.39	0.21	0.08 ns	0.07 ns	0.09
KL/NDF	50.5	1.05	0.50 ns	0.46 ns	0.68
IVNDFD	73.4	6.37	2.59	2.21 ns	1.97
DINAGZ	19.3	6.01	2.53	1.75 ns	1.79

Genotype, genotype × tester (GT), and genotype × environment (GE) effects were significant at $P < 0.001$, except non significant (ns) ones; trait legends as in Table 1, starch content as DM %

[a] Leaf angle data from only two locations

Table 3 Variance analysis, mean, maximum, and minimum values of traits for RIo × WM13 RIL progenies in topcross experiments with the modern (RFl) and old (Ia153) lines at silage harvest

Traits	Genotype MS	GE MS	σ^2_r	σ^2_g	σ^2_{gxe}	h^2	Mean	Mini	Maxi	RIo × RFl
RIL × RFl										
Yield	10.0	2.99	2.08	0.79	0.32	0.74	16.6	13.5	19.8	19.4*/ns
Dry matter	16.8	3.78	1.92	1.36	0.43	0.83	38.0	34.1	42.6	39.6*/*
Silking date	13.4	3.28	1.62	1.16	0.55	0.81	25.4	21.8	29.8	24.7*/*
Leaf angle[a]	53.5	21.0 ns	24.9	–	–	–	153.2	144.4	160.5	158.5*/ns
Sol carbohyd	4.64	1.46	0.49	0.38	0.48	0.70	5.56	3.84	8.03	4.88*/*
Starch	36.8	16.9	6.47	1.98	4.97	0.55	31.6	25.4	37.8	34.7*/ns
Crude protein	0.27	0.14	0.09	0.022	0.001	0.72	7.17	6.45	7.82	7.06*/*
Cell/NDF	1.63	0.69	0.50	0.10	0.002	0.67	49.5	48.0	50.6	49.1 ns/ns
Hcell/NDF	2.06	0.91	0.65	0.13	0.000	0.67	44.4	43.1	46.0	44.7*/*
ADL/NDF	0.14	0.06	0.04	0.011	0.003	0.71	6.10	5.74	6.55	6.24*/*
KL/NDF	0.66	0.42	0.22	0.026	0.101	0.38	14.2	13.5	15.2	14.5*/*
IVNDFD	4.08	1.93	1.24	0.19	0.08	0.63	22.7	20.8	24.6	22.0*/*
DINAGZ	3.96	1.58	1.16	0.27	0.25	0.62	45.4	43.7	47.4	44.6*/*
RIL × Ia153										
Yield	25.4	4.59	2.71	1.65	1.45	0.75	15.7	11.4	19.9	19.9*/ns
Dry-matter	18.2	3.69	2.28	1.68	0.21	0.86	35.6	30.8	40.2	39.2*/ns
Silking date	11.4	2.44	1.49	0.83	0.32	0.79	26.2	21.8	35.4	24.3*/*
Leaf angle[a]	82.3	31.4 ns	36.6	–	–	–	143.5	131.9	150.4	156.5*/*
Sol carbohyd	3.10	1.23	0.43	0.23	0.51	0.64	5.29	3.29	8.03	4.94*/*
Starch	32.1	11.9	5.46	1.72	3.87	0.57	30.2	24.3	37.4	34.9*/*
Crude protein	0.41	0.13	0.09	0.024	0.023	0.61	7.70	7.08	8.30	7.21 ns/*
Cell/NDF	1.98	0.62	0.42	0.11	0.11	0.72	50.0	48.8	51.5	49.5 ns/*
Hcell/NDF	2.61	0.86	0.58	0.15	0.13	0.63	43.7	42.4	45.2	44.3*/ns
ADL/NDF	0.14	0.07	0.04	0.010	0.003	0.70	6.21	5.74	6.64	6.25*/*
KL/NDF	0.74	0.36	0.26	0.028	0.08	0.40	14.1	13.13	14.9	14.4*/ns
IVNDFD	4.18	1.94	1.27	0.28	0.10	0.66	23.1	21.1	26.4	23.1*/*
DINAGZ	3.76	1.64	0.97	0.19	0.14	0.61	45.8	43.9	48.5	46.0*/*

Genotype and genotype × environment (GE) effects were significant at $P < 0.001$, except ns ones; trait legends as in Table 1; values of traits in the RIo × RFl hybrid were significantly different ($P < 0.05$) or not (ns) from RIL minimum or maximum values (mini/maxi in the RIo × RFl column)

[a] Leaf angle data from only two locations

The average distance between markers was 15.0 cM, but in 17 areas the distance between markers was higher than 25 cM, including four areas with distance between markers greater than 30.0 cM (30.4, 30.7, 38.3, and 41.7 in bins 3.08, 1.08, 6.06, and 3.05, respectively). The largest area between markers in bin 3.05 was close to centromer position.

LOD threshold and QTLs in the FR × WM13 progeny

Based on the permutation-test method of Churchill and Doerge (1994), LOD threshold estimates equal to 3.1/3.0 and 3.7/4.0 yielded experiment-wise error rates equal to 5 and 1 % in per se (plant without ears)/topcross (whole plant) experiments, respectively. QTLs were therefore considered for LOD higher or equal to 3.0. However, a LOD threshold equal to 2.6 ($P < 0.10$) was considered for QTLs colocalizing with more significant ones. When including both agronomic and quality traits, 66 QTLs were thus observed on nine chromosomes in per se experiments. No QTLs were observed on chromosome 6, while most QTLs (56) were observed on chromosomes 1, 2, 3, 4, and 8. Again when including both agronomic and quality traits, only 35 QTLs were observed on eight chromosomes in topcross experiments, including 21 and 14 QTLs in RFl and Ia153 topcrosses, respectively. No topcross QTLs were shown on chromosomes 7 and 8, only 1 was shown on chromosome 10 and 2 were shown on chromosomes 5 and 6. In addition, four major QTL clusters were found in the vicinity of centromers of chromosomes 2, 3, 4, and 8, respectively.

Table 4 Correlations between observed traits and lignin and cell wall degradability traits, based on average values over environments of the 163 RIL progenies in per se and topcross experiments

Traits	RIL per se experiments			RIL × RFl experiments			RIL × Ia153 experiments		
	ADL/NDF	IVNDFD	DINAGZ	ADL/NDF	IVNDFD	DINAGZ	ADL/NDF	IVNDFD	DINAGZ
Yield	0.05	0.12	0.06	−0.12	0.19	−0.03	0.15	0.04	−0.12
Dry matter	−0.09	−0.12	−0.36	0.02	0.03	−0.38	−0.19	0.10	−0.13
Silking date	−0.03	0.28	0.22	−0.06	0.16	0.19	0.07	0.39	0.07
Leaf angle[a]	–	–	–	−0.08	0.12	0.20	−0.03	0.13	0.21
Sol carbohyd	0.01	0.43	0.54	−0.13	0.19	0.51	−0.12	0.12	0.51
Starch	–	–	–	0.04	−0.23	−0.22	−0.17	−0.04	−0.07
Crude protein	−0.17	0.30	0.42	−0.25	0.05	0.31	0.21	−0.26	−0.02
Cell/NDF	0.42	−0.33	−0.53	0.36	−0.17	−0.34	0.27	−0.16	−0.23
Hcell/NDF	−0.68	0.47	0.61	−0.61	0.35	0.49	−0.54	0.33	0.41
ADL/NDF	1.00	−0.60	−0.51	1.00	−0.70	−0.65	1.00	−0.66	−0.70
KL/NDF	0.69	−0.47	−0.25	0.44	−0.57	−0.20	0.34	−0.60	−0.12
IVNDFD	−0.60	1.00	0.90	−0.70	1.00	0.53	−0.66	1.00	0.49
DINAGZ	−0.51	0.90	1.00	−0.65	0.53	1.00	−0.70	0.49	1.00
pCA	0.50	−0.65	−0.71	–	–	–	–	–	–
EsterFA	−0.62	0.15	−0.01	–	–	–	–	–	–
EtherFA	0.25	−0.60	−0.66	–	–	–	–	–	–
5-5diFA	−0.59	0.45	0.48	–	–	–	–	–	–
8-O-4diFA	−0.61	0.40	0.40	–	–	–	–	–	–
pHb	0.55	−0.61	−0.74	–	–	–	–	–	–
Va	0.42	−0.45	−0.54	–	–	–	–	–	–
Sg	0.53	−0.70	−0.71	–	–	–	–	–	–

Correlations with absolute values higher than 0.21 are significant at $P = 0.01$, trait legends as in Table 1—are traits not investigated in topcross experiments

[a] Leaf angle data from only two locations

QTLs for agronomic traits

Three QTL positions for silking date were observed, out of which one in bin 7.01 was only observed in per se experiments (Table 5). Three QTLs for stover (plant without ear) DM content at harvest were observed in per se experiments with only one colocalization with silking date QTL in bin 7.01 where the WM13 allele induced both a later silking date and a lower DM content at harvest. Interaction was significant between DM content QTLs located in positions 1–216 and 2–64. In topcross experiments, four QTLs for whole plant DM content were observed, corresponding to three genomic positions, with no colocalizations with line per se stover DM QTLs. Three QTLs for stover (plant without ear) yield were observed in per se experiments, with alleles increasing yield originating from RIo in two genomic locations, and from WM13 for the QTL with the lowest effect (Table 5). These QTLs did not colocalize with QTLs related to earliness, either estimated through silking date or DM content at silage harvest. Unexpectedly, no QTL for whole plant biomass yield was observed in topcross experiments, despite the large variation observed between RILs. Four QTLs for leaf angle were shown only in the topcross with the modern RFl line. Alleles of the modern RIo line increased leaf erectness. Only one QTL of crude protein content was shown, with increasing content allele originating from RIo.

QTLs for soluble and cell wall carbohydrates

Five QTLs for soluble carbohydrate contents were observed in per se experiments, with increasing alleles originating from WM13 for the two QTLs located in bins 2.04 and 4.05 which had the highest LOD values (Table 6). Four QTLs, corresponding to two genomic positions, were observed for this trait in topcross experiments, with increasing alleles originating from WM13. Two QTLs for starch content were shown in RFl topcross experiments, with increasing alleles in both cases originating from RIo. Eleven QTLs for cellulose and seven QTLs for hemicellulose contents in the cell wall were shown, with all QTLs for hemicelluloses colocalizing with QTLs for cellulose

Table 5 QTL analysis for agronomic traits

Experiment	Trait	Left marker	Markerbin	QTL cM chr-pos	Support interval (cM)	QTL Mbp pos	LOD	R^2	Add value	Line+
RIL per se	Yield	bnlg149	1.00	1–10	2–22	4.0	3.5	9.6	0.39	RIo
RIL per se	Yield	bnlg1505	3.05	3–114	96–120	209.6	3.3	8.9	0.37	WM13
RIL per se	Yield	umc1231	9.05	9–98	90–102	149.4	5.4	14.1	0.43	RIo
RIL per se	DMatter	umc1118	1.11	1–216	210–224	291.3	3.9	10.3	0.60	WM13
RIL per se	DMatter	bnlg381	2.04	2–64	56–70	65.3	7.9	19.9	1.06	RIo
RIL per se	DMatter	umc1066	7.01	7–26	16–36	16.8	3.4	9.1	0.57	RIo
RIL × RFl	DMatter	bnlg1429	1.02	1–48	42–60	16.2	4.0	10.6	0.47	RIo
RIL × RFl	DMatter	bnlg252	4.05	4–88	82–94	114.4	11.4	27.5	1.01	RIo
RIL × Ia153	DMatter	bnlg252	4.05	4–94	84–104	157.8	3.4	9.2	0.57	RIo
RIL × Ia153	DMatter	bnlg1209	9.04	9–68	64–78	108.0	4.0	10.6	0.58	RIo
RIL per se	Silking	bnlg1179	1.01	1–22	14–28	7.3	3.9	10.5	0.97	RIo
RIL per se	Silking	phi037	1.08	1–140	124–152	235.1	3.7	10.0	1.09	WM13
RIL per se	Silking	umc1066	7.01	7–38	28–48	71.0	4.9	13.1	1.41	WM13
RIL × RFl	Silking	umc1269	1.01	1–24	20–30	10.8	4.2	11.3	0.46	RIo
RIL × Ia153	Silking	phi037	1.08	1–148	126–162	247.4	2.8	7.6	0.65	WM13
RIL × RFl	Leaf angle[a]	umc2048	3.10	3–164	158–166	222.5	3.0	8.3	0.75	RIo
RIL × RFl	Leaf angle[a]	bnlg1208	5.04	5–76	72–90	154.2	4.0	10.8	0.95	RIo
RIL × RFl	Leaf angle[a]	phi033	9.01	9–40	30–50	20.6	3.0	8.0	0.78	RIo
RIL × RFl	Leaf angle[a]	umc1231	9.05	9–88	76–96	105.7	5.3	14.0	1.21	RIo
RIL per se	CProtein	phi119	8.02	8–12	0–24	52.4	3.6	10.3	0.34	RIo

QTL positions in cM and Mbp; allele from line + increased trait additive value; trait legends as in Table 1

[a] Leaf angle data from only two locations

Table 6 QTL analysis for soluble carbohydrate and starch contents

Experiment	Trait	Left marker	Marker Bin	QTL cM chr-pos	Support interval (cM)	QTL Mbp pos	LOD	R^2	Add value	Line+
RIL per se	Sol carbohyd	bnlg1179	1.01	1–18	6–24	6.1	3.9	10.5	0.82	RIo
RIL per se	Sol carbohyd	bnlg381	2.04	2–60	56–70	41.2	4.3	11.4	0.77	WM13
RIL per se	Sol carbohyd	bnlg1893	2.09	2–168	160–168	234.9	3.1	8.9	0.63	RIo
RIL per se	Sol carbohyd	bnlg252	4.05	4–84	72–92	85.6	5.8	15.0	0.97	WM13
RIL per se	Sol carbohyd	bnlg1079	10.03	10–76	68–90	126.3	3.7	9.9	0.72	RIo
RIL × RFl	Sol carbohyd	bnlg1831	2.06	2–80	78–92	163.2	3.6	9.7	0.25	WM13
RIL × RFl	Sol carbohyd	bnlg252	4.05	4–88	82–94	114.4	10.2	24.9	0.52	WM13
RIL × Ia153	Sol carbohyd	bnlg381	2.04	2–74	64–82	125.6	3.8	10.1	0.27	WM13
RIL × Ia153	Sol carbohyd	bnlg252	4.05	4–92	84–100	143.3	6.3	16.1	0.35	WM13
RIL × RFl	Starch	umc1165	2.02	2–10	4–16	4.5	3.2	8.5	0.70	RIo
RIL × RFl	Starch	bnlg252	4.05	4–86	76–96	100.0	4.0	10.5	0.90	RIo

Legends as in Tables 1 and 5

contents (Table 7). These colocalizations illustrated the fact that the two carbohydrates are the two major complementary components of the cell wall, and that a higher content of one component corresponds to a lower content of the other. This is corroborated by the observed allelic effects, with WM13 alleles most often increasing cellulose content and decreasing hemicellulose content.

QTLs for lignin content, lignin structure, and *p*-hydroxycinnamic acids

Four ADL/NDF QTLs were shown in per se experiments, two of which explained nearly 15 % of the observed phenotypic variation in this lignin trait. Two were shown in topcross experiments, one of which explained nearly

Table 7 QTL analysis for cell wall carbohydrate contents

	Trait	Left marker	Marker bin	QTL cM chr-pos	Support interval (cM)	QTL Mbp pos	LOD	R^2	Add value	Line+
RIL per se	Cell/NDF	bnlg1556	1.07	1–126	112–136	216.5	3.1	8.3	0.28	WM13
RIL per se	Cell/NDF	bnlg131	1.11	1–230	224–234	296.6	3.9	10.6	0.27	WM13
RIL per se	Cell/NDF	bnlg1904	3.04	3–60	50–76	26.8	3.4	9.3	0.32	WM13
RIL per se	Cell/NDF	umc1963	4.04	4–74	68–80	33.0	6.2	16.1	0.37	RIo
RIL per se	Cell/NDF	phi119	8.02	8–14	4–24	59.0	4.8	13.6	0.41	WM13
RIL × RFl	Cell/NDF	umc1269	1.01	1–30	22–42	12.8	3.0	8.1	0.13	RIo
RIL × RFl	Cell/NDF	bnlg1556	1.07	1–132	126–150	223.8	7.0	17.9	0.21	WM13
RIL × RFl	Cell/NDF	bnlg1505	3.05	3–100	88–118	189.2	4.2	11.2	0.23	WM13
RIL × Ia153	Cell/NDF	bnlg1179	1.01	1–20	14–34	6.7	3.0	8.1	0.13	RIo
RIL × Ia153	Cell/NDF	phi037	1.08	1–138	128–152	232.0	2.9	7.9	0.13	WM13
RIL × Ia153	Cell/NDF	umc1572	6.02	6–26	20–34	101.0	3.6	9.7	0.14	RIo
RIL per se	Hcell/NDF	bnlg1556	1.07	1–128	120–154	218.6	3.1	8.4	0.34	RIo
RIL per se	Hcell/NDF	umc1118	1.11	1–216	210–224	291.3	3.5	9.5	0.32	RIo
RIL per se	Hcell/NDF	umc1963	4.04	4–76	72–86	38.3	7.7	19.5	0.48	WM13
RIL per se	Hcell/NDF	phi119	8.02	8–12	4–20	52.4	7.1	19.4	0.60	RIo
RIL × RFl	HCell/NDF	phi037	1.08	1–142	124–152	238.2	4.4	11.6	0.22	RIo
RIL × RFl	HCell/NDF	bnlg1505	3.05	3–98	84–114	186.3	3.8	10.1	0.26	RIo
RIL × Ia153	HCell/NDF	umc1572	6.02	6–24	20–34	98.8	3.1	8.4	0.15	WM13

Legends as in Tables 1 and 5

Table 8 QTL analysis for cell wall lignin contents

Experiment	Trait	Left marker	Marker bin	QTL cM chr-pos	Support interval (cM)	QTL Mbp pos	LOD	R^2	Add value	Line+
RIL per se	ADL/NDF	umc1118	1.11	1–216	208–224	291.3	3.3	8.8	0.11	WM13
RIL per se	ADL/NDF	umc2195	2.03	2–48	42–58	21.0	3.5	9.4	0.12	WM13
RIL per se	ADL/NDF	umc1963	4.04	4–78	74–86	43.6	5.5	14.3	0.14	RIo
RIL per se	ADL/NDF	phi119	8.02	8–14	4–24	59.0	5.2	14.7	0.18	WM13
RIL × RFl	ADL/NDF	bnlg381	2.04	2–72	66–82	113.5	7.7	19.5	0.08	WM13
RIL × Ia153	ADL/NDF	umc1555	2.03	2–56	46–58	26.3	3.1	8.4	0.04	WM13
RIL per se	KL/NDF	umc1165	2.01	2–14	8–28	5.4	4.1	11.0	0.17	WM13
RIL per se	KL/NDF	bnlg381	2.04	2–72	68–78	113.5	19.9	43.0	0.51	WM13
RIL per se	KL/NDF	umc1746	3.01	3–20	12–28	5.3	6.0	15.7	0.22	RIo
RIL per se	KL/NDF	umc2261	3.04	3–74	68–84	136.2	4.6	12.1	0.20	WM13
RIL per se	KL/NDF	phi93225	3.08	3–158	152–166	227.1	6.7	17.3	0.26	RIo
RIL per se	KL/NDF	umc1066	7.01	7–32	12–46	43.9	3.1	8.5	0.19	WM13
RIL per se	KL/NDF	bnlg1074	10.04	10–82	66–92	133.0	4.6	12.4	0.22	RIo
RIL × RFl	KL/NDF	bnlg381	2.04	2–64	56–74	65.3	3.2	8.5	0.11	WM13
RIL × Ia153	KL/NDF	umc1225	5.08	5–152	146–152	215.8	3.0	8.5	0.10	RIo

Legends as in Tables 1 and 5

20 % of the phenotypic variation (Table 8). Colocalization between per se and topcross QTLs was only observed in bin 2.03, and only after crossing with the old Ia153 line. In per se experiments, seven KL/NDF QTLs were simultaneously mapped, but without any QTL colocalization between QTLs for the two lignin traits. Two KL/NDF QTLs were shown in topcross experiments in two different genomic positions. Four per se KL/NDF QTLs and one topcross QTL had an increasing allele originating from WM13, including one per se QTL in bin 2.04 which explained 43 % of the observed genetic variation. This last QTL colocalized not only with a

Table 9 QTL analysis for lignin monomeric composition

Experiment	Trait	Left marker	Marker bin	QTL cM chr-pos-	Support interval (cM)	QTL pos Mbp	LOD	R^2	Add value	Line+
RIL per se	pHb	bnlg149	1.00	1–6	0–14	3.1	5.4	14.5	0.06	Rio
RIL per se	pHb	umc1774	1.10	1–196	168–208	283.6	3.3	8.8	0.05	WM13
RIL per se	pHb	bnlg1138	2.06	2–82	78–92	173.6	3.6	9.5	0.04	WM13
RIL per se	pHb	bnlg252	4.05	4–80	72–90	56.7	3.1	8.4	0.04	Rio
RIL per se	Va	phi119	8.02	8–16	6–26	65.5	5.1	14.3	0.22	WM13
RIL per se	Va	umc1231	9.05	9–98	90–102	149.4	4.8	12.6	0.16	Rio
RIL per se	Sg	bnlg1138	2.06	2–88	78–96	185.0	5.8	15.1	0.24	WM13
RIL per se	Sg	bnlg1208	5.04	5–80	76–90	162.5	10.4	25.5	0.30	WM13
RIL per se	Sg	phi119	8.02	8–4	0–14	26.1	4.4	12.4	0.20	WM13

Legends as in Tables 1 and 5

Table 10 QTL analysis for cell wall *p*-hydroxycinnamic acid contents

Experiment	Trait	Left marker	Marker bin	QTL cM chr-pos	Support interval (cM)	QTL pos Mbp	LOD	R^2	Add value	Line+
RIL per se	pCA	umc1774	1.10	1–204	194–216	286.5	3.9	10.5	0.32	WM13
RIL per se	pCA	bnlg1138	2.06	2–84	78–94	177.4	3.2	8.6	0.27	WM13
RIL per se	pCA	bnlg1904	3.04	3–58	48–76	24.2	3.8	10.2	0.34	WM13
RIL per se	pCA	umc1963	4.04	4–76	72–86	38.3	4.7	12.3	0.31	RIo
RIL per se	EsterFA	bnlg2046	8.05	8–36	24–46	129.1	3.3	8.8	0.08	RIo
RIL per se	EtherFA	umc2261	3.04	3–70	56–76	79.6	3.8	10.2	0.03	WM13
RIL per se	5-5diFA	bnlg1831	2.06	2–80	66–82	163.3	3.5	9.4	0.004	RIo
RIL per se	5-5diFA	umc2261	3.04	3–74	66–84	136.2	3.6	9.7	0.004	RIo
RIL per se	5-5diFA	phi119	8.02	8–10	4–18	45.8	9.9	26.0	0.009	RIo
RIL per se	8-O-4diFA	bnlg1831	2.06	2–80	78–86	163.3	5.0	13.1	0.007	RIo
RIL per se	8-O-4diFA	umc2261	3.04	3–74	66–84	136.2	3.2	8.6	0.006	RIo
RIL per se	8-O-4diFA	dupssr28	4.08	4–116	106–126	213.8	3.5	9.4	0.007	WM13
RIL per se	8-O-4diFA	phi119	8.02	8–14	10–24	59.0	9.1	24.1	0.013	RIo

Legends as in Tables 1 and 5

topcross KL/NDF QTL but also with a topcross ADL/NDF QTL.

Nine QTLs related to the monomeric composition of lignin were shown in per se experiments, out of which six explained more than 10 % of the phenotypic trait variation (Table 9). One syringaldehyde QTL located in bin 5.04 thus explained a little more than 25 % of this trait variation with the increasing allele originating from WM13. Thirteen QTLs of *p*-hydroxycinnamic acid content were detected, including four QTLs of *p*-coumaric acid and nine QTLs of ferulic and diferulic acids (Table 10). Three QTLs of *p*-coumaric acid colocalized with QTLs of *p*-hydroxybenzaldehyde, in bin 1.10, 2.06, 4.04/05, likely illustrating again oxidation of part of *p*-coumaric acid into *p*-hydroxybenzaldehyde during the alkaline nitrobenzene attack. The two QTLs for 5-5diFA and 8-O-4diFA, both of which each explained nearly 25 % of the phenotypic trait variation, colocalized in bin 8.02 with QTLs of vanillin and syringaldehyde. The latter occurred with reverse allelic effects as allele increasing diferulate release decreased aldehyde releases. In the same position, a QTL for vanillin release after nitrobenzene oxidation has also been shown in the F838 × F286 RIL progeny (Barrière et al. 2008). All diferulate QTLs, except one located in bin 4.08, had increasing alleles originating from the modern RIo line. Only one QTL for esterFA and one for etherFA were observed, in bin 8.05 and 3.04 with increasing alleles originating from RIo and WM13, respectively.

QTLs for cell wall digestibility

In per se experiments, nine QTLs for cell wall digestibility, including six IVNDFD QTLs and three DINAGZ QTLs, were detected in only six positions as all the DINAZ QTLs

Table 11 QTL analysis for cell wall digestibility traits

Experiment	Trait	Left marker	Marker bin	QTL cM chr-pos	Support interval (cM)	QTL pos Mbp	LOD	R^2	Add value	Line+
RIL per se	IVNDFD	bnlg1831	2.06	2–80	78–92	163.3	3.1	8.3	0.52	RIo
RIL per se	IVNDFD	umc2261	3.04	3–72	68–82	107.9	7.1	18.1	0.86	RIo
RIL per se	IVNDFD	umc1320	3.08	3–140	132–156	220.0	6.2	16.0	1.02	WM13
RIL per se	IVNDFD	bnlg252	4.05	4–84	74–90	85.6	6.2	16.1	0.87	WM13
RIL per se	IVNDFD	umc1225	5.08	5–148	140–152	259.7	3.7	10.4	0.63	WM13
RIL per se	IVNDFD	bnlg1074	10.04	10–98	86–108	140.8	3.3	9.1	0.57	WM13
RIL × RFl	IVNDFD	bnlg381	2.04	2–66	56–78	77.3	3.0	8.0	0.25	RIo
RIL × RFl	IVNDFD	umc1482	5.04	5–82	74–92	171.8	4.7	12.3	0.28	RIo
RIL × RFl	IVNDFD	bnlg2190	10.06	10–108	100–108	147.1	3.1	8.7	0.23	WM13
RIL × Ia153	IVNDFD	phi037	1.08	1–156	144–180	259.7	3.6	9.7	0.36	WM13
RIL per se	DINAGZ	umc2261	3.04	3–72	66–76	107.9	8.9	22.3	1.01	RIo
RIL per se	DINAGZ	umc1320	3.08	3–138	126–156	219.4	4.1	11.0	0.88	WM13
RIL per se	DINAGZ	bnlg252	4.05	4–82	74–88	71.1	9.8	24.1	1.10	WM13
RIL × RFl	DINAGZ	bnlg252	4.05	4–82	72–92	71.1	2.6	5.9	0.21	WM13
RIL × RFl	DINAGZ	bnlg1189	4.07	4–104	98–118	183.5	3.1	8.3	0.24	WM13
RIL × Ia153	DINAGZ	bnlg252	4.05	4–92	80–104	143.3	2.7	7.0	0.23	WM13

Legends as in Tables 1 and 5

colocalized with IVNDFD QTLs (Table 11). Four of the cell wall digestibility QTLs explained nearly 10 % of the observed phenotypic variation and five of the latter QTLs explained more than 15 % of the variation, up to nearly 25 %. Alleles increasing per se cell wall digestibility originated from WM13 in four genomic locations and from RIo in two locations, corroborating the higher cell wall digestibility of the older line. Seven cell wall digestibility QTLs were observed in topcross experiments, out of which only two were observed after crossing with the old Ia153 line. Alleles increasing cell wall digestibility originated five times from WM13 and two times from RIo. A significantly high negative interaction was shown between DINAGZ QTLs in position 4–82 and 4–104 (additive value −0.23) in the RFl topcross. Conversely to per se experiments, no colocalizations were shown between QTLs for each of the two cell wall digestibility traits in topcross experiments, corroborating the fact that these two traits illustrated partly different genetic components of cell wall degradability. Colocalizations between cell wall digestibility QTLs in per se and topcross experiments were shown only in bin 4.05. Colocalizations of cell wall digestibility QTLs and other cell wall traits displayed nearly similar patterns in bins 2.06 and 3.04/05, with in the same support intervals QTLs for 5-5diFA and 8-O-4diFA QTL, and no QTL for ADL/NDF. An etherFA QTL was also observed in bin 3.04/05 while *p*-hydroxybenzaldehyde and syringaldehyde QTLs were also shown in bin 2.06. In these two locations, both alleles increasing cell wall degradability and diFA release originated from RIo. The topcross IVNDFD QTL located in bin 5.04 colocalized with the Sg QTL explaining a quarter of the variation for this trait, with the WM13 allele increasing Sg content and decreasing IVNDFD.

Putative candidate gene underlying lignin content and lignin structure QTLs

In bin 2.06, lignin structure and cell wall degradability QTL support intervals overlapped the position of the ZmMYB31 (GRMZM2G050305) transcription factor. However, while this gene was likely a relevant candidate for pCA, pHb, and Sg QTLs, its involvement in diFA variation is more questionable and a second still unknown candidate gene should be considered in a slightly upstream position. In bin 3.08, the two cell wall degradability QTLs and the KL/NDF QTL were in similar positions to a zinc finger CCCH AtC3H14-like encoding gene (GRMZM2G157927). The QTL also colocalized with a NAC transcription factor (GRMZM2G058518) orthologous to *Arabidopsis* NAC SND3. In bins 4.04/05, the lignin content, lignin structure, and cell wall degradability QTLs were in close positions with two NAC genes, ZmNAC115 (GRMZM2G048826) and a VNI1/VNI2-like ZmNAC (GRMZM2G125777). In addition, a third NAC, ZmNAC143 (GRMZM2G069047), was also located in the upstream part of QTL support intervals. In bin 8.02, lignin structure and content QTLs colocalized with a ZmMYB (GRMZM2G119693) and with an ortholog (GRMZM2G031827) of an *Arabidopsis* CCCH zinc finger encoding gene (AtU2AF35b or

At5g42820). The isolated ADL/NDF QTL located in bin 2.02 was in a very close position to an ortholog of AtMYB58 (GRMZM2G038722), but three NAC genes were also located in this QTL support interval including an ortholog of SND1 (GRMZM2G178998), an ortholog of VND7 (AC212859.3_FGP008), and an ortholog of VNI (GRMZM2G176677). In bin 5.05, the QTL for Sg release after nitrobenzene oxidation, which explained 25 % of the variation for this trait, and the topcross IVNDFD QTL, colocalized with an ortholog of *Arabidopsis* XND1 (XYLEM NAC DOMAIN1) NAC transcription factor (GRMZM2G094067). Finally, in bin 10.04, the cell wall degradability and KL/NDF QTLs were surrounded by two ZmMYB. In the upstream part of QTL support intervals was located a ZmMYB ortholog of AtMYB103 (GRMZM2G173633) and in the downstream part of the QTL support intervals was located a ZmMYB ortholog to AtMYB58 (GRMZM2G097638).

Putative candidate genes underlying ferulate QTLs

The esterFA QTL located in bin 8.05 was in a similar position to two PF02458 Arabinose-CoA-acyltransferase genes (GRMZM2G050072 and GRMZM2G050270) putatively involved in the catalytic transfer of a feruloyl group onto an arabinoxylan chain (Mitchell et al. 2007). In bin 3.04, the QTL for etherFA release colocalized closely with a similar QTL with a high R^2 value (19.6 %) that has been shown in the F838 × F286 RIL progeny (Barrière et al. 2008), but without any available candidates. Similarly, no relevant candidates were available for any of the diFA QTLs shown.

Putative candidate genes underlying cell wall carbohydrate QTLs

Cellulose chains are synthesized at the plasma membrane by large cellulose synthase (CesA) complexes, using UDP-D-glucose as a precursor. QTLs for Cell/NDF colocalized with CesA10 (GRMZM2G445905) in bin 1.07, CesA5 (GRMZM2G111642) and CesA6 (GRMZM2G113137) in bin 1.11, and CesA11 (GRMZM2G055795) in bin 3.05. An ortholog of the fragile fiber *Arabidopsis* FRA2 gene, involved in cellulose micro-fibril deposition and organization (Zhong et al. 2002; Burk and Ye 2002), was also located in the downstream part of the Cell/NDF QTL in bin 3.05 (GRMZM2G054715). Hemicellulose polysaccharides are formed from UDP-D-glucose in the Golgi apparatus (Dennis and Blakeley 2000). UDP-D-xylose is thus produced from UDP-D-glucose in a set of two reactions catalyzed by UDP-D-glucose dehydrogenases (G6DH) and UDP-D-GlcA decarboxylases. The QTL for hemicelluloses in bin 1.08 colocalized with one of the three G6DH (GRMZM2G058244) genes shown in the maize genome (Barrière et al. 2009).

Discussion

QTLs and agronomic trait variation in the RIo × WM13 progeny

Maize yield improvement is correlative of changes in physiological processes and morphological traits. It is currently considered that modern maize genotypes are later flowering, but have faster grain filling, a more upright leaf habit and a greater leaf area duration inducing an increased interception of seasonal incident radiation, an increased kernel number, and a more active root system with a greater uptake of water and nutrients. The greater leaf angle in the topcross with the modern RFl line illustrated the more erected plant habit in modern germplasm. Alleles of the modern RIo line increased leaf erectness for all four QTLs shown in topcross experiments. In addition, only one QTL of crude protein content was shown, with increasing content allele originating from RIo, a result that could indicate and corroborate a more effective nitrogen uptake in modern lines, despite the slightly higher crude protein content observed in the older WM13 line. The higher transfers of photosynthetic products to the ear in modern lines could be illustrated by QTLs shown in bin 4.05, with RIo allele increasing starch content and decreasing soluble carbohydrate content. The QTL for soluble carbohydrates located in bin 1.01 colocalized with a QTL of silking date, with both increasing alleles originating from RIo. This probably illustrates in recent lines a longer period of photosynthetic production in later flowering plants. Modern genotypes are also characterized by a greater standability, a greater tolerance to biotic and abiotic stresses, and a faster recovery capacity after stresses. Their greater tolerance to stress than older genotypes, and their superiority, was even more pronounced under difficult environmental conditions (Tollenaar et al. 1994, 1995; Tollenaar and Wu 1999). It can then be assumed that the greater GE interactions observed in the topcross of the RIo × WM13 progeny with the old Ia153 line were a consequence of a greater environmental stress susceptibility of the older genetic background.

QTLs and cell wall trait variation in the RIo × WM13 progeny

Changes in cell wall traits were also shown between old and modern genotypes, as has been illustrated by the evolution of cell wall digestibility value of registered hybrids across eras of breeding (Barrière et al. 2004a).

Several differences related to lignin content and consequences on cell wall digestibility were thus shown, based on investigations in the RIo × WM13 progeny. Three ADL/NDF QTLs out of four and four cell wall degradability QTLs out of six had increasing alleles originating from WM13. Simultaneously, only one colocalization was observed between ADL/NDF and IVNDFD QTLs in bin 4.05, with allele increasing ADL/NDF originating from RIo. In the F838 × F286 RIL progeny, five IVNDFD QTLs out of seven colocalized with ADL/NDF QTLs (Barrière et al. 2008), three IVNDFD QTLs out of four colocalized with ADL/NDF QTLs in the F288 × F271 RIL progeny (Roussel et al. 2002), and this was the case for seven QTLs out of eight in the F7025 × F4 RIL progeny (INRA Lusignan and Génoplante unpublished data). Moreover, only one allele increasing ADL lignin content originated from RIo, in bin 4.04, while the reverse situation would be expected, based on the slightly higher lignin content in RIo, as well as on the average higher lignin content in modern Iodent-related lines. In a similar way, correlations between cell wall degradability traits and ADL/NDF content were negative, but these correlations were lower than previously observed in maize RIL experiments (Méchin et al. 2001; Roussel et al. 2002; Barrière et al. 2008, 2010). This difference, which could also be due to differences in environmental conditions, could more likely be related to an evolution in cell wall quality over decades of breeding, with a suspected increasingly negative impact of lignin content on cell wall degradability. This set of results would indicate different structures of trait relationships between the oldest and the most recent investigated dent lines, probably related to breeding efforts for higher yield and stalk standability. Differences in cell wall degradability between RIo and WM13 are likely not mainly related to ADL lignin content. In addition, lack of colocalizations observed in most cases between QTLs for ADL/NDF and KL/NDF confirmed the fact that the two ways of determining lignin content in maize samples highlighted different parts of the polymer, with different underlying genetic mechanisms. When applied to grasses, the acid detergent solution can dissolve half and often more of the lignins, while the Klason method is commonly considered to give a more representative estimate of the whole lignin polymer (Hatfield and Fukushima 2005). ADL could thus be considered as the core lignin part. Moreover, ADL tended to be more consistently correlated with cell wall digestibility than Klason lignin, even if QTL colocalizations between cell wall digestibility and ADL/NDF were not so frequent in this RIL progeny.

Relationships between lignin structure and cell wall degradability shown in the RIo × WM13 progeny corroborated previous results shown in RIL or lines collections, even if investigations on the impact of lignin structure on the susceptibility of the cell wall to enzymatic hydrolysis did not allow drawing a definite conclusion (Méchin et al. 2000; Grabber et al. 1997, 2009). However, in agreement with the observed negative correlations and QTL colocalizations, an increased proportion of *p*-coumaryl and sinapyl alcohol in the lignin polymer likely could contribute to the lowering of cell wall degradability, corroborating previous results (Riboulet et al. 2008b). A higher proportion of syringyl alcohol in lignins might thus indicate a higher proportion of mature secondary wall in tissues, while a higher proportion of *p*-coumaryl alcohol increases the possibilities of condensed linkages between monomers. Similar to the proportion of syringyl alcohol in lignins, pCA content is likely a relevant indicator of the intensity of secondary tissue lignification. The correlation between cell wall degradability and pCA content was thus shown to be negative and often higher than the correlation with lignin content (Fontaine et al. 2003; Riboulet et al. 2008b).

Transcription factors as candidate genes involved in lignin-related trait variation

No colocalizations between cell wall-related QTLs and genes involved in monolignol biosynthesis were shown in the FR × WM13 progeny, nor with peroxidases or laccases involved in monolignol polymerization. Conversely, most observed QTL colocalizations occurred with transcription factors regulating secondary wall biosynthesis or assembly of the MYB, NAC, and to a lesser extent zinc finger CCCH families. No colocalizations were also shown with WRKY transcription factors, more likely involved in biotic and abiotic lignin responses, nor with genes involved in lignified-tissue patterning, such as ATHB8 HDZIP III or COV1-like genes.

The regulation of phenylpropanoid biosynthesis was the first role identified for a plant R2R3-MYB transcription factor (Paz-Ares et al. 1987; Tamagone et al. 1998), and R2R3-MYB were also the first transcription factors shown to be involved in the regulation of secondary wall compound biosynthesis. In the RIo × WM123 progeny, colocalizations between cell wall-related QTLs and MYB transcription were observed in four genomic locations. Lignin structure and content QTLs colocalized in bin 8.02 with a ZmMYB which is orthologous to AtMYB46 and EgMYB2, which are both activators of lignification and secondary wall biosynthesis (Goicoechea et al. 2005; Zhong et al. 2007b, 2008). The cell wall degradability and KL/NDF QTLs located in bin 10.04 were in similar positions as a ZmMYB ortholog of AtMYB103, of which over expression increased secondary wall thickening in *Arabidopsis* fibers (Zhong et al. 2008). These latter QTLs also colocalized with a ZmMYB orthologous to AtMYB58

which is a transcriptional activator of monolignol biosynthesis genes in the SND1-mediated network (Zhou et al. 2009). Similarly, the isolated ADL/NDF QTL located in bin 2.02 was in a very close position to another ortholog of AtMYB58. In bin 2.06, colocalizations of the lignin QTL occurred with a MYB having a reverse regulatory effect. ZmMYB31 is a R2R3-MYB orthologous to EgMYB1, which is a repressor of lignification (Legay et al. 2007) ZmMYB31 is also orthologous to a barley Hv5 MYB which is one of three MYB genes expressed in cells flanking secondary xylem or phloem differentiating barley tissues (Wissenbach et al. 1993). Moreover, ectopic expression of ZmMYB31 in *Arabidopsis* was shown to down-regulate several genes involved in monolignol biosynthesis (Fornalé et al. 2006, 2010).

The first demonstrations of the involvement of NAC transcription factors in secondary wall assembly were likely the description of NST1 and NST2 roles in secondary wall thickening (Mitsuda et al. 2005) and VND6 and VND7 roles in vessel xylem formation (Kubo et al. 2005). Several colocalizations were thus shown between cell wall-related QTLs and orthologs of *Arabidopsis* NAC genes which have a priority importance in secondary wall lignification. These "master" NAC genes have been shown to regulate in *Arabidopsis*, the expression of several transcription factors and/or genes involved in secondary cell wall biosynthesis (Zhong et al. 2008; Zhong and Ye 2009). The lignin content, lignin structure, and cell wall degradability QTLs in bins 4.04/05 were in close positions with ZmNAC115. ZmNAC115 is orthologous to the *Arabidopsis* master NAC transcription factor VND7, which is in *Arabidopsis* specific to sclerenchyma cells (Zhong et al. 2008; Yamaguchi et al. 2011). These latter QTLs also colocalized with the ZmNAC143 gene located in the upstream part of QTL support intervals. This gene is orthologous to the SND1 gene of *Arabidopsis*, a master NAC transcription factor activating the developmental program of secondary wall biosynthesis (Zhong et al. 2007a, 2008). A NAC transcription factor orthologous to *Arabidopsis* SND3, which is highly expressed in fiber and xylem tissues and involved in the SND1/NST1-mediated transcriptional regulation of secondary wall biosynthesis (Zhong et al. 2008) colocalized with the lignin QTL in bin 3.08. Finally, the lignin QTL located in bin 2.02 colocalized with both an ortholog of SND1 and an ortholog of VND7.

Colocalizations were also shown with NAC having other roles in secondary wall assembly. In bins 4.04/05 and 2.02, lignin QTLs colocalized with VNI1/VNI2-like ZmNAC. AtNAC082/VNI1 and AtNAC083/VNI2 are two NAC proteins interacting with VND transcription factors (VNI for VND interacting). The role of VNI proteins has only been shown in *Arabidopsis* for VNI2 which interacts with VND family proteins and regulates xylem cell specification as a transcriptional repressor (Yamaguchi et al. 2010). In bin 5.05, the cell wall-related QTLs colocalized with an ortholog of *Arabidopsis* XND1 NAC transcription factor. XND1 (XYLEM NAC DOMAIN1) was considered to affect tracheary element growth through regulation of secondary wall synthesis and programmed cell death, but seemingly did not affect phloem development in *Arabidopsis* (Zhao et al. 2008).

In addition, colocalizations were also shown in bin 3.08 with an ortholog of the zinc finger AtC3H14 encoding gene which has been shown with a similar role in the cell wall assembly as the master NAC genes. A member of this AtC3H14 zinc finger protein family was thus shown to activate all of the *Arabidopsis* secondary wall phenolic-related genes that were tested (Ko et al. 2009).

Biased estimates of cellulose and hemicellulose contents

As previously observed (Riboulet et al. 2008b), correlations between cell wall degradability traits and cellulose content were negative, while they were positive with hemicellulose content. However, this fact might be partly related to the solubilization processes used with the Goering and Van Soest (1970) method, which could lead in maize to a loss of more accessible cellulose in the "hemicellulose" residue and a loss of an acido-soluble part of lignins in the "cellulose" residue. The so-called cellulose fraction will thus be deprived of its more accessible part and will also be contaminated with a soluble lignin part. In addition, only one QTL position for both cellulose and hemicellulose contents, in bin 1.07, was simultaneously found in RIL per se and topcross experiments. This lack of colocalization possibly illustrated a different hierarchy of genetic factors involved in cell wall carbohydrate deposition in inbred lines and hybrids. In all cases, underlying determinants could be involved either in cellulose or in hemicellulose biosynthesis.

Ferulate cross-linkage and cell wall degradability variation

Specific results related to relationships between cell wall digestibility and ferulate linkages were also shown from the RIo × WM13 progeny experiments. The three QTLs for 5-5diFA colocalized with 8-O-4diFA QTLs, and alleles increasing diferulate releases originated from RIo (bins 2.06, 3.04, and 8.02). The extra 8-O-4diFA QTL in bin 4.08 had an increasing allele originating from WM13. The two diferulate QTLs, in bins 2.06 and 3.04, colocalized with IVNDFD QTLs for which the increasing allele similarly originated from RIo. The latter is in agreement with

previous observations, both in RIL progenies and in lines collection (Barrière et al. 2008, 2009; Riboulet et al. 2008b), showing that a greater release of diferulates was unexpectedly related to higher cell wall degradability. In fact, the extent to which released diferulates reflect total diferulate cross linkages is presently not known (Grabber et al. 2004). Rather than a higher content in the cell wall and a higher intensity of cross linkages, a higher release of diferulates could correspond as well to a higher accessibility of the breakage points and thus to an easier release, whatever the real content. The diFA release is more representative of cell wall component accessibility than an estimate of the quantity of diFA cross linkages in the cell wall (Riboulet et al. 2008b; Barrière et al. 2008). Similarly to diferulates, it is not known how well etherified ferulate reflects total ferulate and diferulate cross linking in cell walls. Released etherified ferulate may only account for as little as 15 % of total cross linking because C–C, 8-O-4 styryl ether, and biphenyl ether coupling of ferulate and diferulates to lignin have not yet been determined due to the limitations and complexity of current solvolytic methods (Grabber et al. 2000). However, the correlation between etherFA content and cell wall degradability was always shown to be negative, both in maize and in other grasses (Casler and Jung 1999; Méchin et al. 2000; Lam et al. 2003; Riboulet et al. 2008b; Jung and Phillips 2010). Moreover, in the only case of colocalization between etherFA and IVNDFD QTLs (bin 3.04), the allele increasing etherFA content (WM13 allele) decreased cell wall degradability, as it was previously shown (Barrière et al. 2008). Cross linkages through ester-ether-FA bridges significantly impede cell wall carbohydrate degradation to an equal degree or even to a greater extent than lignin content. The role of ferulate cross linking was indeed "tentatively estimated to account for nearly one half of the inhibitory effects of lignin on cell wall fermentation" (Grabber et al. 2009). In addition, ferulate cross linkages were considered to be also involved in stalk stiffness (Grabber et al. 1995; Grabber et al. 2000; MacAdam and Grabber 2002). Consequently, these latter were assumed to have negative effects on tissue friability and silage intake (Barrière et al. 2009). Intake (and milk production) was shown greater for cows fed diets containing W23sfe silages, with a mutation inducing lower etherFA cross linkage in the cell wall, than for those fed W23 silage (Jung et al. 2011). A similar result has also been shown in comparison between DK265 and control hybrids, while DK265 is highly suspected to also have lower etherFA cross linkages (Barrière et al. 2004b). However, breeding for lower levels of cross linkages is currently hindered by limitations in measuring all ferulate and diferulate cross links by analytic methods. An accurate method for estimating total ferulate and diferulate cross linking, whatever the lignin content and structure, is needed both to understand effects of these linkages on stiffness and degradability and to provide relevant breeding traits to maize breeders.

Candidate genes underlying cell wall ferulate QTLs

The colocalization between two PF02458 arabinose-CoA-acyltransferase genes and the esterFA QTL located in bin 8.05 greatly reinforce the probable involvement of members of the CoA-dependent acyltransferase PF02458 (Finn et al. 2008) gene family in the feruloylation of arabinoxylan chains. Down-regulations of orthologs of these genes in rice have been associated with a reduction in plant FA content (Piston et al. 2010). Moreover, these members of the PF02458 family have been identified as acyltransferase genes specifically expressed in grasses in contrast to dicotyledons in which this particular function was supposed to be missing (Mitchell et al. 2007). No candidate genes were shown either for the etherFA QTL or any of the diFA QTLs. This fact illustrates how little is known about ferulate and diferulate biosynthesis pathways in grasses. In addition to QTL investigations, the sfe low ferulate mutant (Jung and Phillips 2010) is one of the rare available tracks towards the elucidation of cross-linkage assembly in grasses.

Conclusion

In addition to the observation of original relationships between cell composition and cell wall degradability, the search for QTLs in the progeny of the modern RIo line and the old WM13 has contributed to expand the list of genomic areas involved in maize cell wall traits, especially in bins 2.06, 3.08, and 8.02. Based on investigations in RIo × WM13 and five RIL progenies (Méchin et al. 2001; Roussel et al. 2002; Barrière et al. 2008, 2010; Riboulet et al. 2008a; Inra Lusignan and Génoplante network unpublished data), 50 QTLs were demonstrated for ADL/NDF corresponding to 23 positions, while 53 QTLs were demonstrated for IVNDFD also corresponding to 23 positions. Positions in bin 8.02 for ADL/NDF and in bins 2.06 and 5.08 for IVNDFD were nevertheless specific to the RIo × WM13 progeny. Similarly, specific-QTL positions were shown for lignin monomeric composition and *p*-hydroxycinnamic acid contents (bins 2.06, 5.04, 8.02). The observed differences in cell wall structure and composition cannot yet be related with certainty to the fact that one parent has not been involved in the recent selection processes. However, the RIo × WM13 progeny has confirmed that investigating old germplasm contributed to a better understanding of the cell wall assembly and gave

new information for breeding lines with improved quality for silage and biofuel production. In addition, several major QTL clusters were located in vicinity of centromers, a fact that could be considered as somehow unexpected because of the lower gene density in these chromosomal areas (Schnable et al. 2009). However, similar results have been shown in other maize QTL investigations. This fact might result from a greater power of QTL detection in the centromere regions as a consequence of the lower recombination rate in these areas. Due to the limited resolution of most QTL analyses, linked genes with small individual effects would then appear as a single major QTL, especially in these chromosomal regions with high gene density relatively to recombination (Schön et al. 2010; Larièpe et al. 2012). The possible existence of such polygenic blocks gathering several cell wall-related genes could explain the occurrence of colocalizations of QTLs for several cell wall traits driven by different genomic determinants. An upstream regulation factor could, however, have a similar effect.

Based on the observed colocalizations, differences in lignin or cell wall degradability traits are likely more related to variation of genes involved in the regulation of lignin biosynthesis and secondary wall deposition than to variation in genes involved in monolignol biosynthesis or polymerization. In addition, the genetic determinants of traits might also be genes of still unknown functions or miRNA that were not currently considered as putative candidates. Several QTL positions were indeed without any known candidates. However, the development of plants with improved cell wall degradability and high agronomic value for both ruminant feeding and biofuel production requires understanding the respective effects of lignin content, lignin monomeric composition, and *p*-hydroxycinnamic acid linkages on cell wall mechanical quality and degradability. A systematic inventory of the major cell wall-related QTLs, based on a collection of RIL progenies with diverse genetic backgrounds, followed by a positional cloning of genetic determinants, will allow the identification of mechanisms underlying trait variation and then an efficient marker-assisted selection.

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