

# Quantitative Trait Loci and Comparative Genomics of Cereal Cell Wall Composition<sup>1</sup>

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Quantitative trait loci (QTLs) affecting sugar composition of the cell walls of maize (*Zea mays*) pericarp were mapped as an approach to the identification of genes involved in cereal wall biosynthesis. Mapping was performed using the IBM (B73 × Mo17) recombinant inbred line population. There were statistically significant differences between B73 and Mo17 in content of xylose (Xyl), arabinose (Ara), galactose (Gal), and glucose. Thirteen QTLs were found, affecting the content of Xyl (two QTLs), Ara (two QTLs), Gal (five QTLs), Glc (two QTLs), Ara + Gal (one QTL), and Xyl + Glc (one QTL). The chromosomal regions corresponding to two of these, affecting Ara + Gal and Ara on maize chromosome 3, could be aligned with a syntenic region on rice (*Oryza sativa*) chromosome 1, which has been completely sequenced and annotated. The contiguous P1-derived artificial chromosome rice clones covering the QTLs were predicted to encode 117 and 125 proteins, respectively. Two of these genes encode putative glycosyltransferases, displaying similarity to carbohydrate-active enzyme database family GT4 (galactosyltransferases) or to family GT64 (C-terminal domain of animal heparan synthases). The results illustrate the potential of using natural variation, emerging genomic resources, and homeology within the Poaceae to identify candidate genes involved in the essential process of cell wall biosynthesis.

As a defining feature of plants, the cell wall is important to all aspects of their biology. Furthermore, plant cell walls provide fuel, fiber, and food to all human societies. Cell walls are a complex composite of polysaccharides, proteins, and lignin. The polysaccharide components can be classified into three broad categories: pectins, hemicelluloses, and cellulose. In both monocotyledons and dicotyledons, the most abundant polysaccharide in the majority of tissues is the simple polymer cellulose. In contrast, the hemicelluloses are chemically and physically more complex and their monomer composition varies between species and between tissues and cell types within an individual plant (e.g. Fincher, 1992; Doblin et al., 2001). The cell walls of the commelinoid monocotyledons, which includes the grasses and cereals (family Poaceae), differ significantly in composition from other plants in having a larger amount of arabinoxylan and a unique hemicellulose, mixed-

linked glucan ( $\beta$ 1,3- $\beta$ 1,4-glucan, also known as cereal  $\beta$ -glucan; Carpita and Gibeaut, 1993; Carpita, 1996). Thus, the several stages of hemicellulose biosynthesis (precursor synthesis, polymerization, secretion, and incorporation into the wall) must differ significantly among plant species and be dynamically regulated processes within a particular plant.

A full understanding of the biosynthesis of the cell wall remains a major unsolved problem in plant biology. Biochemical approaches to identification of the enzymes and genes involved have been hindered by the lability of the enzymes and our ignorance of their biosynthetic mechanisms. Although substantial progress has recently been made on the identification and function of cellulose synthases and the corresponding genes (called *CESA*) and on several non-processive glycosyl transferases such as xylosyl, galactosyl, and fucosyl transferases, little is known about the genes and enzymes involved in synthesis of the backbones of the hemicellulosic polymers (Arioli et al., 1998; Edwards et al., 1999; Perrin et al., 1999; Fagard et al., 2000; Taylor et al., 2000; Faik et al., 2002; Peng et al., 2002; Vanzin et al., 2002).

A genetic approach to cell wall biosynthesis has been successful in identifying *CESA* genes in different species and also genes involved in hemicellulose precursor biosynthesis (Reiter et al., 1993; Bonin et al., 1997; Arioli et al., 1998). Wall compositional differences in T-DNA-tagged plants of *Arabidopsis* have also been examined (Gardner et al., 2002). The exploitation of natural variation is an alternative ge-

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netic approach to these mutagenesis-based approaches. Traits such as fruit size, flowering time, morphology, and light responsiveness differ among lines, ecotypes, or accessions of a particular plant species, and a number of these have been successfully analyzed genetically. Because they are typically inherited in a quantitative manner, they are more challenging to analyze, and isolation of the responsible genes is more difficult. Nonetheless, in recent years a number of quantitative trait loci (QTLs) have been identified and isolated (Frery et al., 2000; Yano et al., 2000; El-Assal et al., 2001; Maloof et al., 2001). Contrary to some expectations, the QTLs underlying natural variation have turned out to be variant alleles of genes that play a central role in the trait under study, and not minor, secondary genes with an indirect role (Millar, 2001). Thus, the identification of genes controlling natural quantitative differences in cell wall properties might lead to the identification of critical, primary genes involved in hemicellulose biosynthesis.

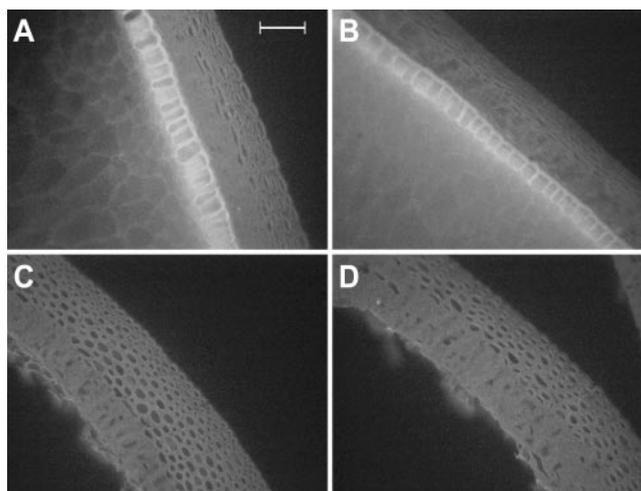
A number of important agronomic properties of plants are influenced by the properties of their cell walls; for example, nutrient absorption and digestibility by humans and animals, wheat (*Triticum aestivum*) bread making quality, barley (*Hordeum vulgare*) brewing quality, and insect resistance (e.g. Martin and Bamforth, 1980; Brice and Morrison, 1982; Hedin et al., 1993; Lundvall et al., 1994; Courtin and Delcour, 1998). The inheritance of the cell wall properties underlying some of these traits has been studied in several species including soybean (*Glycine max*), wheat, barley, and maize (*Zea mays*; e.g. Powell et al., 1985; Jung and Buxton, 1994; Lundvall et al., 1994; Saulnier et al., 1995; Lempereur et al., 1997; Stombaugh et al., 2000). These traits typically exhibit a genotype by environment interaction effect and have complex patterns of inheritance indicative of control by many genes. QTLs affecting mixed-linked glucan content in oat (*Avena sativa*) and barley, fiber content in maize, and ratio of Ara to Xyl in wheat flour have been detected (Han et al., 1995; Lübberstedt et al., 1997, 1998; Martinant et al., 1998; Kianian et al., 2000; Méchin et al., 2000). However, the specific genes underlying the QTLs identified in any of these studies, due to the complexity of the relevant plant genome, the lack of adequate genomic resources, or both.

Substantial progress has been made in recent years in the development of genetic and genomic resources for many plants, including cereals such as rice (*Oryza sativa*) and maize. This opens the possibility of using natural variation to identify genes involved in a complex process such as wall biosynthesis, with the ultimate goal of elucidating the underlying biochemical mechanisms of hemicellulose backbone synthesis. Here, we have exploited high-throughput cell wall analysis and advanced maize genetic resources to identify QTLs affecting the sugar monomer compo-

sition of cell walls. The results illustrate the feasibility of identifying candidate genes involved in hemicellulose biosynthesis when QTL analysis is combined with synteny between rice and maize and with the available partially annotated rice genome.

## RESULTS

Several criteria were considered for defining a model plant and tissue for the analysis of natural genetic variation of cell wall composition. A major consideration is the existence of rich genetic and genomic resources, which is satisfied for maize in light of recent advances in the development of intermated recombinant inbred lines (RILs) such as the IBM population derived from B73 and Mo17, a large number of genetic markers, and synteny with the completely sequenced genome of rice (Davis et al., 1999, 2001; Goff et al., 2002; Lee et al., 2002; Song et al., 2002). The tissue to be sampled should be easy to collect, have a low level of starch, and its composition should be insensitive to small differences in rates of growth and development among accessions or ecotypes. The maize pericarp satisfies these criteria. It is predicted to have low starch content and, if sampled from a fully mature cob, variation in growth and development should be minimal. Sufficient amounts of tissue can be sampled from a single ear. The pericarp was removed with forceps from seeds that had been soaked in water. When cross-sections of intact seeds were observed by fluorescence microscopy, three types of cells were visible at the edge (Fig. 1, A and B; Kiesselbach, 1949). The pericarp is the outermost layer and is composed of approximately 10 thick-walled cells that fluoresce blue. The aleurone layer is characterized by relatively large round cells



**Figure 1.** Fluorescent microscopy of cross sections of intact seeds (caryopses; A and B) and excised pericarp (C and D). A, Mo17; B, B73; C, RIL 356; D, RIL 338. The three layers of cells visible in the whole caryopsis cross section, from inside to outside, are endosperm, aleurone, and pericarp. Only pericarp is present in C and D. Bar = 100  $\mu\text{m}$ .

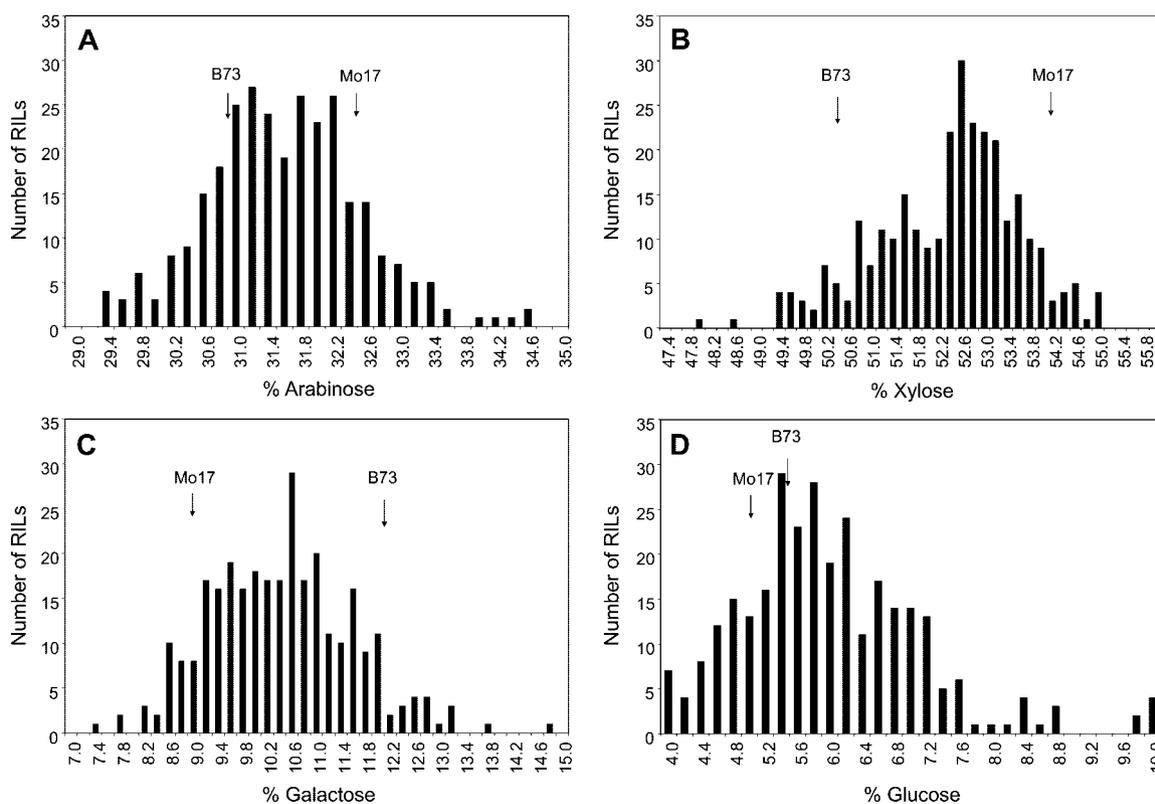
**Table 1.** Mean and range values for relative and absolute quantity of monosaccharides extracted from pericarp cell walls of the IBM mapping population and parents

Monosaccharide	Parents			RILs	
	B73 (relative chromatograph peak area [RCPA])	Mo17 (RCPA)	Average (RCPA)	Minimum (RCPA)	Maximum (RCPA)
Ara	30.98	32.48	31.49	29.26	34.47
Xyl	51.33	54.19	52.26	47.83	54.98
Gal	12.14	8.31	10.32	7.28	14.80
Glc	5.55	5.02	5.92	3.54	11.52
	B73	Mo17	Average	Minimum	Maximum
	<i>mg g<sup>-1</sup></i>				
Ara	156.94	161.37	153.48	113.86	188.50
Xyl	260.20	269.70	254.97	183.85	305.43
Gal	61.51	41.25	50.31	32.59	71.26
Glc	28.13	24.81	28.91	15.91	55.04

that fluoresce bright yellow-green under alkaline conditions. Inside this layer of maternal tissue is the endosperm, composed of large thin-walled cells. Microscopy indicated that the pericarp preparations used in this study contained minimal contamination by aleurone or endosperm walls (Fig. 1, C and D). There were no noticeable differences among B73, Mo17, and two randomly selected RILs from the IBM population in appearance of the pericarp (Fig. 1).

We could not find any previous reports of the sugar composition of maize pericarp. Our analyses indicate that mature maize pericarp contains an av-

erage of 52% Xyl, 32% Ara, 10% Gal, and 5.0% Glc. Rha, Fuc, and Man were present in trace quantities. Arabinoxylan is the major hemicellulose in most cereal cell walls, although there are large differences in the degree of Ara substitution among tissues. Therefore, the high values for Xyl and Ara probably represent a high content of Ara-substituted xylan (arabinoxylan) in the pericarp. The polymeric nature of the pericarp Gal is not known, although in coleoptiles and young seedlings of maize it is mostly nonreducing terminal (Kato and Nevins, 1984; Carpita, 1996). The trace quantities of Rha and Man eliminate galac-

**Figure 2.** Distribution of means of monosaccharide composition in the pericarps of the IBM RIL population. A, Ara; B, Xyl; C, Gal; D, Glc. Parental values are indicated by arrows. RIL effects were significant ( $P < 0.01$ ) for all four sugars.

tomannan and rhamnogalacturanan as significant contributors of Gal. The low Glc content indicates that the pericarp contains only small amounts of starch or other acid-digestible Glc polymers such as mixed-linked glucan, which is relatively abundant in barley aleurone and endosperm walls (Bacic and Stone, 1981a, 1981a).

Summary statistics for the cell wall composition of pericarps from the IBM RIL population (Davis et al., 2001; Lee et al., 2002) are given in Table I. There was a continuous range of values for all measured traits among the RILs (Fig. 2). Some RILs had extreme values for Glc (Fig. 2), possibly due to contamination of the pericarp sample with endosperm starch. Normal distributions were observed for all monosaccharides as illustrated by the histograms (Fig. 2). Significant differences among the RILs for monosaccharide content were detected at the 1% level using analysis of variance. The midparent values (i.e. the values halfway between the two parents) and the median of the RILs for Ara, Xyl, and Gal were very similar, but the median for Glc was greater than the midparent value, and the population was slightly skewed to the right (Fig. 2).

The proportion of the total variance of each source of variation, RIL, replication, and error (RIL by replication interaction) were calculated from the variance components and are summarized in Table II. Although RIL had a significant effect on monosaccharide content, the proportion of the total variation accounted for by RIL for each trait varied. Error had the largest variance component for Ara, Xyl, and Glc. Glc was the only trait where replicate variance was greater than RIL variance. The magnitude of the variance among replicates for Xyl and Gal was negligible. RIL effects accounted for the greatest proportion of the variance for Gal and the least portion for Glc.

Correlation analysis showed a significant and strongly positive relationship between Ara and Xyl content (Table III). Gal content was moderately correlated with Ara and Xyl. No significant correlation at the 5% level was observed between Glc and the other monosaccharides (Table III).

Thirteen QTLs for maize pericarp monosaccharide relative chromatograph peak area (RCPA) were detected. The map location, log of the odds ratio (LOD) score, flanking markers, marker bin, additive effect,

**Table II.** Percentage of the total variance for each source of variation for RCPA of cell wall monosaccharide content of the IBM mapping population

The coefficients of variance for Ara, Xyl, Gal, and Glc were 3.32, 2.33, 6.62, and 28.3, respectively.

Source of Variation	Monosaccharide			
	Ara	Xyl	Gal	Glc
RIL	35.92	41.49	70.84	14.63
Replication	27.38	1.94	0.25	17.61
Error	36.69	56.57	28.90	53.16

**Table III.** Pair-wise Pearson's correlation coefficients of means of absolute monosaccharide content of the IBM mapping population

Monosaccharide	Ara	Xyl	Gal
	<i>mg g<sup>-1</sup></i>		
Ara	–	–	–
Xyl	0.85 <sup>a</sup>	–	–
Gal	0.22 <sup>a</sup>	0.28 <sup>a</sup>	–
Glc	0.07	0.02	0.06

<sup>a</sup> Significant at  $P < 0.01$ .

and percent of the total variance accounted for by each locus are given in Table IV. Eleven of the loci were associated with single monosaccharides. A locus on chromosome 3 was associated with both Ara and Gal content, and a locus on the short arm of chromosome 6 was associated with both Xyl and Glc content. All of the Mo17 alleles had a positive additive effect on Ara and Xyl content. Nine of the 11 B73 alleles had a positive effect on Gal and Glc content. Four of the QTL accounted for 10.0% or more of the total variance. The size of the genetic map interval for the QTLs ranged from 2.53 cM for QTL 1 to 31.4 cM for QTL 2 (Table IV).

Several approaches were taken to identify known nucleic acid sequences that might plausibly account for the observed variation associated with the QTLs. Some genes of known or putative function mapped near some of the QTLs. Other candidates were identified by BLAST search of molecular marker partial nucleic acid sequences. For example, the microsatellite locus *umc1111* maps to bin 1.11, and its flanking sequences have sequence similarity to a gene encoding a sterol-methyltransferase. Sterols have recently been shown to play a role as intermediates in cellulose biosynthesis (Peng et al., 2002). Marker *umc1366*, which maps to bin 9.06 along with Gal and Ara QTLs 10 and 11, is predicted to encode a  $\beta$ 1,3-glucanase, an enzyme with a potential role in degradative cell wall metabolism. A QTL for Glc content maps to bin 3.05 as does Suc phosphate synthase (UDP-Glc-Fru-phosphate glucosyltransferase, encoded by *sps2*; Causse et al., 1995). The nearest flanking marker to the 5.63-cM Gal QTL interval on chromosome 10, *umc1053*, has high sequence homology to a gene encoding a wall-localized invertase (Shanker et al., 1995).

Searching for gene candidates in maize is currently limited by the lack of genomic sequence. Therefore, syntenic regions for the QTLs were identified within the rice genome using MapSearch at the Gramene Web site (<http://www.gramene.org>; Ware et al., 2002). Rice chromosome 1 is syntenic to much of the apical portion of maize chromosome 3 (Gale and Devos, 1998). Because rice chromosome 1 is completely sequenced and annotated, two contiguous segments of DNA could be identified that correspond to QTL 3 (Ara plus Gal), QTL 4 (Ara), and QTL 5 (Gal) on maize chromosome 3 (Table III). A flanking

marker, *mmp36*, of QTL 3 has significant homology to sequences on overlapping rice P1-derived artificial chromosome (PACs) AP002872 and AP002540 (<http://rgr.affrc.go.jp/>). These two PACs map to the short arm of rice chromosome 1. The nearest maize/rice marker on the other flank of the QTL, *umc1392*, is 45 cM proximal to *mmp36* and has homology to rice PAC AP003214 (Fig. 3). Assuming that map distances and physical distances are roughly proportional, but erring on the side of inclusion, AP002540 and five PACs in the direction of *umc1392* (i.e. AP002872, AP003311, AP002483, AP002872, and AP002913) were selected as most likely completely spanning QTL3. Together, these six PACs form a contig of 638 kb and were predicted to encode 117 proteins. A second contig chosen to include the proximal Ara and Gal QTLs 4 and 5 (flanking markers *mmp9* and *umc1449*) includes PACs AP001073, AP001081, AP000837, AP000836, and AP001072, is 658 kb, and is predicted to encode 125 proteins.

The predicted protein sequences were analyzed by standard BLAST against the nonredundant database of the National Center for Biotechnology Information and also against the carbohydrate-active enzyme database (CAZy) of glycosyltransferases (Campbell et al., 1997; Coutinho and Henrissat, 1999). A single candidate glycosyltransferase was found in each contig. One candidate, BAB40110, encodes a predicted member of glycosyltransferase family GT4, which contains a variety of transferases that form alpha-linkages, including Suc synthase, GDP-Man  $\alpha$ -mannosyltransferase,  $\alpha$ -1,3-rhamnosyltransferase, trehalose phosphorylase, and digalactosyldiacylglycerol synthase. A second predicted protein, BAA90366, is a member of family GT64. This family includes plant proteins that are related to the C-terminal domain of animal heparan synthases, which are themselves

bifunctional glycosyltransferases that use UDP-GlcNAc and UDP-GlcA to synthesize polysaccharides with alternating  $\alpha$ 1,4-GlcNAc and  $\beta$ 1,4-GlcA residues. Plant genes related to the N-terminal domain or the C-terminal domain of animal heparan synthetases (families GT47 and GT64, respectively) are abundant, Arabidopsis having at least 44 members and rice more than 30. The function of at least one member of plant family GT47 has been elucidated (pectin  $\beta$ -glucuronyltransferase; Iwai et al., 2002). The precise function of family GT64 in plants is not known, but similarity to the C-terminal domain ( $\alpha$ -GlcNAc transferase) of animal heparan synthetases suggests it could be an  $\alpha$ -glycosyltransferase.

## DISCUSSION

Analysis of the IBM population of RILs led to the identification of 13 QTLs affecting the sugar monomer composition of maize pericarp walls. Using markers that bridge maize and rice, it was possible to identify syntenic regions in rice corresponding to some of the maize QTLs and, thus, identify corresponding candidate rice genes. Due to the nature of QTLs, the regions for searching were rather large. Nonetheless, within this collection, the number of proteins with a plausible role in wall biosynthesis was small. Therefore, it should be practical to study the candidate genes further for a role in wall biosynthesis; for example, by detailed studies on their pattern of expression (e.g. are they expressed in the pericarp?) or biochemical function by heterologous expression (e.g. Faik et al., 2002).

It was fortuitous to find genetically influenced differences in cell wall composition between B73 and Mo17, thereby making the genetic reference map a tool for cell wall discovery. The IBM mapping pop-

**Table IV.** Summary of QTLs affecting sugar composition of maize pericarp

A positive additive effect indicates that the B73 allele was associated with a higher value, whereas a negative effect indicates that the Mo17 allele was associated with a higher value.

QTL	Chromosome Position	QTL Map Position	LOD	Trait	Flanking Markers	Bin	Additive Effect	Variance
								%
1	1	54.52–57.05	4.10	Xyl	php20537-ufg33	1.01	-0.2972	5.56
2	2	67.19–98.23	4.22	Xyl	umc1824-bnl2g277	2.02	-0.3773	16.2
3	3	277.70–284.00	4.37	Ara	mmp144-mmp36	3.04	-0.2263	5.50
3	3	281.00–287.04	7.98	Gal	mmp144-mmc0132	3.04	0.3362	8.54
4	3	328.70–331.70	3.27	Ara	bnlg1816-umc1920	3.04	-0.2215	5.15
5	3	351.55–353.84	3.79	Gal	mmp9-umc1449	3.04	0.2229	3.67
6	3	453.05–459.15	4.53	Glc	umc1167-psr119a	3.05	0.2907	5.45
7	4	332.44–345.10	4.73	Glc	lim446-php10025	4.09	0.3167	6.60
8	5	364.89–365.64	3.44	Gal	umc1155-nbp35	5.05	0.2502	4.51
9	6	182.42–189.44	7.33	Glc	bnlg1174-phi078	6.05	0.3931	10.0
9	6	182.42–189.44	5.37	Xyl	bnlg1174-phi078	6.05	-0.3483	14.5
10	9	368.57–375.66	11.63	Gal	bnl5.09a-bnl14.28	9.06	0.4110	12.4
11	9	381.32–387.22	4.41	Ara	umc1789-umc1675	9.06	-0.2262	5.43
12	10	119.86–125.10	5.63	Gal	psb527d-umc1053	10.04	-0.2864	5.91
13	10	205.96–221.34	6.25	Gal	psb365a-umc1993	10.06	0.4110	6.95



ulation was instrumental in mapping these QTL with high resolution because it has nearly a 4-fold increase in recombination events relative to a conventional RIL population that is not intermated, in addition to a high level of saturation of over 1,800 molecular markers (Lee et al., 2002). The large population size of over 300 RILs and the heritability of the traits also contributed to the fine level of mapping. Several small effect QTLs were identified for each trait. Although all but four of the QTL accounted for less than 10.0% of the total variation, many were mapped to small intervals. The maize genome is estimated to be 2,500 Mb (Laurie and Bennett, 1985), and the IBM genetic linkage map reported at MaizeDB has a total of 6,246 cM (Coe et al., 2002). Therefore, the approximate physical distance of 1 cM is 400 kb. If the maize genome contains 50,000 genes, the gene density is predicted to be 8 genes  $\text{cM}^{-1}$ . Therefore, the individual QTLs identified in this study are predicted to contain 20 to 250 genes.

Continuous and natural genetic variation for cell wall properties has been documented in various cereals and legumes (e.g. Powell et al., 1985; Lundvall et al., 1994; Saulnier et al., 1995; Lempereur et al., 1997; Stombaugh et al., 2000). Environmental effects and genotype by environment interaction effects also influence cell wall composition. The range of significant phenotypic variation within the IBM population and the differences between the parents imply that the quantity of the individual monosaccharides is under the control of several genes, each having a small effect. Our results indicate that a large portion of the total variance of Gal and Xyl content is controlled by at least nine loci. A smaller portion of the Ara and Glc variability can be attributed to genetic effects. However, several QTLs were identified for each trait.

No differences in pericarp cell morphology or pericarp cell counts were observed between samples, and the content of total monosaccharides was also not significantly different within the recombinant population. Therefore, the genetic variation that we observed is likely due to differences in cell wall composition, in either quantity of backbone sugars and/or the degree of their substitution, rather than cell size or shape. The differences in composition could, in turn, be due to any of a number of allelic variations in the genes controlling the multiple steps along the pathway of cell wall biosynthesis, from precursor synthesis to final incorporation into the wall.

Two QTLs were each found to be associated with differences in the levels of two sugars, Ara and Gal on chromosome 3 (QTL 3) and Glc and Xyl on chro-

mosome 6 (QTL 9). The gene underlying QTL3 might be one that affects synthesis of a polysaccharide containing both Ara and Gal, such as the arabinogalactan attached to membrane-associated proteins (arabinogalactan proteins). QTL9 might plausibly be associated with synthesis of xyloglucan, which is present in cereals (Kato et al., 1982; Carpita, 1996). On the other hand, although there was a strong positive correlation between Ara and Xyl content, no QTLs were identified that influence the quantity of those two monosaccharides together. One possible explanation for this is that B73 and Mo17 do not have any genetic differences influencing overall arabinoxylan biosynthesis.

The ultimate goal of the study of natural variation in maize cell wall composition is to identify the exact nucleotide polymorphism that is responsible for each mapped phenotypic variation. Based on current resources, we were able unequivocally to identify only two candidate genes in the genomic regions corresponding to QTLs 3 to 5. Further analyses were limited by the lack of sufficient markers that bridge maize and rice and the incomplete annotation of the rice genome. It is expected that these resources will become available within the next year. The combined use of maize mapping populations and the completed rice genome is also potentially limited by a lack of synteny in critical regions. It is too early to know if this will create insurmountable obstacles.

Additional genetic resources can be used to make further progress. For example, it should be possible to map some of the QTLs to smaller regions using other near-isogenic RILs, such as the BC3-derived near-isogenic lines representing introgression of Tx303 chromosomal regions into the B73 genetic background and BC3-derived near-isogenic lines representing introgression of Oh43 alleles into the Mo17 genetic background (Stuber, 1998).

## MATERIALS AND METHODS

### Genetic Resources

We measured the cell wall monosaccharide profile of the intermated RIL IBM population derived from B73 and Mo17 (Davis et al., 2001; Coe et al., 2002). B73 and Mo17 represent two of the major heterotic U.S. maize (*Zea mays*) germplasm pools, Iowa Stiff Stalk Synthetic and Lancaster Sure Crop, respectively. The  $F_2$  were intermated for five generations and subsequently self-pollinated to make RILs. The RILs (302 total) were used to make a high-density genetic linkage map with >1,850 molecular markers. These data are available at MaizeDB ([www.agron.missouri.edu/maps.html](http://www.agron.missouri.edu/maps.html)). A subset of the population was obtained from the Maize Genetic Consortium Stock Center and the complete population from the University of Missouri Maize Mapping Project. Seed were collected from a single location and year and used directly.

**Figure 3.** Chromosome line graphs and QTL likelihood plots of relative pericarp cell wall monosaccharide content (RPCA) in the IBM population based on composite interval mapping. The horizontal axes in each graph indicate LOD scores, and the vertical lines indicate the empirically derived LOD threshold ( $P < 0.05$ ) for each monosaccharide. The length of the bars indicates an LOD score of 3.0 for each QTL, and the line extensions of each bar, when present, indicate an LOD score of 2.0.

## Wall Analysis

For each replicated sample, two seeds were soaked overnight in 2 mL of tap water in 24-well microtiter plates. The pericarp was removed using forceps. All tissue samples were stored in 70% (v/v) ethanol at 4°C. Only the pericarp was removed from the seed. For microscopy, maize seeds or pericarp were sectioned by hand and mounted on glass slides in 0.1 M ammonium hydroxide (pH 9.8). Slides were observed using an Axiophot Fluorescence Microscope (HBO 100 Hg vapor bulb, Zeiss, Jena, Germany) with a 365- ± 12.5-nm exciter and a 450-nm long-pass filter, using the 10× and 20× objectives. Wall-bound ferulic acid fluoresces green at high pH (Rudall and Caddick, 1994).

Cell wall monosaccharides were analyzed as the alditol acetates after acid hydrolysis as described by Reiter et al. (1993) and Blakeney et al. (1983) with modification. The samples were washed for 1 h at 70°C with 70% (v/v) ethanol, with one change of ethanol after 30 min, washed once with acetone, and dried. Aliquots of each sample (7–15 mg) were hydrolyzed with 1 M H<sub>2</sub>SO<sub>4</sub> for 1 h at 121°C. The released sugars were reduced to alditols by addition of 100 μL of 9 M ammonium hydroxide followed by 1 mL of 2% (w/v) NaBH<sub>4</sub> in dimethyl sulfoxide, and incubated at 40°C for 90 min. The alditols were acetylated by the addition of 250 μL of acetic acid, 250 μL of 1-methylimidazole, and 4 mL of acetic anhydride. After addition of 8 mL of water, the alditol acetates were extracted with 2 mL of dichloromethane and separated using an Agilent 6890 series GC system equipped with an Agilent DB-225 capillary column (Agilent, Palo Alto, CA). Detection was by flame ionization. Quantitation used the integration software in GC ChemStation (Agilent). The temperature program was: 1 min at 160°C, 20°C min<sup>-1</sup> to 200°C, hold for 5 min, 20°C min<sup>-1</sup> to 240°C, hold for 11.5 min, 25°C min<sup>-1</sup> to 150°C, and hold for 1 min.

Peak areas were adjusted relative to an internal inositol standard. Rha, Fuc, and Man were present in maize pericarp in only trace amounts and were not included in the QTL analysis. Sugars are reported as both RCPA of the four principal monosaccharides (Ara, Xyl, Gal, and Glc) and total quantity (milligram of sugar per gram of tissue).

## Statistical Analyses

The seeds were sampled and analyzed within each replication in a completely random manner. Three independent pericarp samples were collected from a pair of seeds for each RIL. Analyses of variance were performed using PROC GLM with inbred as the single random effect (SAS Institute, 2000). Variance components were calculated to estimate the percentage of the total variance originating from RIL, replication, and error using PROC VARCOMP under the assumption that all sources of variation were random.

The map score data and genetic linkage maps were provided by the Maize Mapping Project (University of Missouri, Columbia). Map distances were confirmed using MapMaker version 3.0 (Lander et al., 1987) with an LOD score threshold of 3.0 and a maximum recombination frequency of 0.50. The Kosambi function was used to transform recombination frequencies into centiMorgans. Cosegregation of phenotypic properties and genetic markers was determined using QTL Cartographer version 1.15 (Basten et al., 2001). QTLs were identified using means and a framework genetic linkage map with a marker density of approximately one per 10 cM. Areas where QTLs were identified were saturated with previously mapped markers to a density of one per 0.10 cM and reanalyzed. In both cases, we employed composite interval mapping to increase resolution and reduce background marker effect (Zeng, 1994). LOD thresholds were determined by computing 1,000 permutations for each trait (Churchill and Doerge, 1994). The levels of significance for Ara, Xyl, Gal, and Glc levels at  $P < 0.05$  were LOD 3.22, 3.32, 3.54, and 3.27, respectively, and at  $P < 0.01$ , the LOD were 4.17, 4.17, 4.24, and 4.12, respectively. The graphical representation of the linkage maps and QTLs were prepared using MapChart (Voorrips, 2002).

## Distribution of Materials

Upon request, all novel materials described in this publication will be made available in a timely manner for noncommercial research purposes, subject to the requisite permission from any third party owners of all or parts of the material. Obtaining any permissions will be the responsibility of the requestor.

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