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Cell Wall Composition and Underlying QTL in an F_1 Pseudo-Testcross Population of Switchgrass

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Abstract Natural genetic variation for reduced recalcitrance can be used to improve switchgrass for biofuel production. A full-sib switchgrass mapping population developed by crossing a lowland genotype, AP13, and upland genotype, VS16, was evaluated at three locations (Ardmore and Burneyville, OK and Watkinsville, GA). Biomass harvested after senescence in 2009 and 2010 was evaluated at the National Renewable Energy Laboratory (NREL) for sugar release using enzymatic hydrolysis and for lignin content and syringyl/guaiacyl lignin monomer (S/G) ratio using pyrolysis molecular beam mass spectrometry (py-MBMS). Glucose and xylose release ranged from 120 to 313 and 123 to 263 mg g^{-1}, respectively, while lignin content ranged from 19 to 27 % of the dry biomass. Statistically significant differences were observed among the genotypes and the environments for the cell wall composition traits. Regression analysis showed that a unit increase in lignin content reduced total sugar release by an average of 10 mg g^{-1}. Quantitative trait loci (QTL) analysis detected 9 genomic regions underlying sugar release and 14 for lignin content. The phenotypic variation explained by the individual QTL identified for sugar release ranged from 4.5 to 9.4 and for lignin content from 3.8 to 11.1 %. Mapping of the QTL regions to the switchgrass genome sequence (v1.1) found that some of the QTL colocalized with genes involved in carbohydrate processing and metabolism, plant development, defense systems, and transcription factors. The markers associated with QTL can be implemented in breeding programs to efficiently develop improved switchgrass cultivars for biofuel production.

Keywords Lignin content · Quantitative trait loci · Sugar release · Glucose · Xylose · Recalcitrant

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Introduction

Utilization of renewable energy resources could decrease the negative environmental impacts caused by burning non-renewable fossil fuels. Second-generation, lignocellulosic feedstocks such as switchgrass (*Panicum virgatum* L.) [1] could produce fuel with less impact on food security than grain crops like corn (*Zea mays* L.). Switchgrass is a perennial warm season (C4) bunchgrass native to North America [2] and a predominant species of the tallgrass prairie ecosystems naturally grown from northern

Mexico to southern Canada east of the Rocky Mountains [3]. Switchgrass is an allogamous species with abundant genetic variation that can be harnessed to improve biomass yield and cell wall composition traits for biofuel conversion [4, 5].

Lignocellulosic biomass is naturally recalcitrant to saccharification, limiting the production of liquid fuels. The plant cell wall is a complex structure consisting of cellulose microfibrils embedded in a network of hemicellulose and lignin. The presence of lignin in plant cell walls obstructs access of the hydrolysis and fermentation enzymes to the structural carbohydrates. This limits sugar recovery from cellulose and hemicellulose [6] and, consequently, impacts ethanol yield from biomass feedstock [7, 8]. Genotypic variation for lignin content and the ratio of syringyl to guaiacyl units (S/G ratio) were shown to significantly affect amenability of *Populus* (*Populus trichocarpa* Torr. & Gray) biomass feedstocks to hydrolysis [9]. An increase in dilute acid hydrolysis rate was observed with decreasing S/G ratios, which may be due to reduced covalent cross-linking [9]. Compared to woody feedstock species such as pine and eucalyptus, switchgrass had faster hydrolysis rates and higher sugar yields with dilute acid and ionic liquid pretreatment [10] due to its inherent low S/G ratio.

Research at the Department of Energy (DOE) Bioenergy Science Center (BESC) is focused on reducing recalcitrance of plant cell walls, mostly through transgenic approaches. Genetic modification of the cell wall composition may not only reduce recalcitrance and enhance amenability of lignocellulose to hydrolysis into simple sugars for fermentation but will also significantly enhance the economics and efficiency of the conversion processes [11]. Reduction of lignin content has been achieved through downregulation of genes involved in the phenylpropanoid pathway or overexpression of gene transcription suppressors [12–15]. However, some transgenic switchgrass plants with significantly reduced lignin content have also exhibited abnormal growth and developmental characteristics [12, 13, 15–17] which may limit their utility as a feedstock.

In addition to reducing switchgrass recalcitrance via a transgenic approach, due attention should be given to the assessment of the natural genetic variation that exists for lignin content and sugar release efficiency, their ultimate relationship, and the genomic regions underlying these traits. Quantitative trait loci (QTL) underlying cell wall composition could be manipulated using genetic markers, and marker-assisted breeding could be used to rapidly and efficiently develop improved biofuel-ready switchgrass cultivars. Our objective of this study was to identify QTL for cell wall composition traits such as lignin content, S/G ratio, and sugar (glucose and xylose) release in a segregating switchgrass population derived by crossing an individual genotype from each of the two main switchgrass ecotypes (lowland and upland).

Materials and Methods

Mapping Population and Field Evaluations

An F_1 pseudo-testcross population was developed by crossing the lowland ecotype AP13 (female) with the upland ecotype VS16 (male) and has been previously described [18]. Field experiments were conducted at Ardmore and Burneyville in OK, and Watkinsville, GA as described in detail in Serba et al. [19]. Briefly, each genotype was clonally propagated and grown in four replicates at each of the three locations. The field experiments at Ardmore and Burneyville were transplanted on July 19, 2007 and May 08, 2008, respectively, using a randomized complete block design (RCBD) arranged in a honeycomb [20]. At Watkinsville, the experiment was laid out in RCBD with four replications. Two replicates were transplanted in the field on September 25, 2007, while the remaining two were transplanted adjacent to the first two on April 30, 2008. Detailed crop management practices such as seedbed preparation, fertilizer application, weed control, and interplant cultivation were as described previously [19]. Biomass was harvested after complete senescence in the fall in all the environments. Harvesting was conducted manually using hedge trimmers.

Cell Wall Composition Analysis

Total shoot biomass, including stem, leaf, and panicle harvested after senescence, was sampled from two replications in 2009 and 2010 in both locations in OK and in 2010 in GA, for a total of five location-year environments. Samples were oven dried at approximately 40 °C for 72 h and milled to a 1-mm particle size (mesh size 20) using a Thomas Wiley® Mill Model 4 (Thomas Scientific, Swedesboro, NJ, USA). The milled samples were analyzed for lignin content and sugar (glucose and xylose) release estimates at the National Renewable Energy Laboratory (NREL), Golden, CO, USA.

Sugar release estimates were generated using a high-throughput screening method that involved hydrothermal pretreatment and an enzymatic hydrolysis [21–23]. Biomass was treated with alpha-amylase (Spirizyme Ultra—0.25 %) and beta-glucosidase (Liquozyme SC DS—1.5 %) in 0.1 M sodium acetate (24 h, 55 °C, pH 5.0) to remove possible starch content using 16 mL enzyme solution per 1 g biomass. This was followed by an

ethanol (190 proof) Soxhlet extraction for an additional 24 h to remove other soluble compounds. After drying the samples overnight, 5 mg (±0.5 mg) was weighed in triplicate into one of 96 wells in a solid Hastelloy microtiter plate. Water was added (250 μL); the samples were sealed with silicone adhesive, Teflon tape, and heated to 180 °C for 17.5 min. Once cooled, 40 μL of buffer-enzyme stock (8 % CTec2 (Novozymes) in 1 M sodium citrate buffer) was added. The samples were then gently mixed and left to statically incubate at 50 °C for 70 h. After 70-h incubation, an aliquot of the saccharified hydrolysate was diluted and tested using Megazymes (Wicklow, Ireland) glucose oxidase/peroxidase (GOPOD) and xylose dehydrogenase (XDH) assays. For quantitation, results were calculated using mixed glucose/xylose solutions, and the amount of glucose and xylose released to liquid was measured using a colorimetric assay. Sugar release data were reported in milligrams of sugar released per gram of biomass residues. The average of the triplicates for each sample was used as a data point for a statistical analysis.

Lignin content and S/G lignin monomer analysis were performed using pyrolysis molecular beam mass spectrometry (py-MBMS), using the protocol described by Sykes et al. [24]. Approximately 4 mg of the cell wall residue per sample was loaded into 80-μL stainless steel cups and pyrolyzed at 500 °C in a quartz reactor using a frontier py2020 autosampler. The resulting pyrolysis vapors exit through a 250-μm crystal orifice and expand into a vacuum, quenching the reaction. A molecular beam is formed when a portion of the pyrolysis vapor stream is pulled through a 1-mm stainless steel skimmer, and then, the beam is ionized using electron impact (EI) ionization at −17 eV. Ions travel through a series of focusing lenses and are then bent 90° to travel through a single quadrupole to be detected as a mass spectrum. The relative intensities of the mass spectra peaks identified for lignin precursors were summed to estimate total lignin content [24]. S/G ratio was determined by dividing the sum of the intensity of the syringyl peaks by the sum of the intensity of the guaiacyl peaks.

Statistical Data Analysis

Normality of the composition trait data was checked with the UNIVARIATE procedure using a Q-Q plot of residuals. All the traits fit a normal distribution. Analysis of variance was conducted to test the effects of environment, genotype, and genotype × environment interaction on cell wall composition using the mixed procedure of the SAS statistical software program version 9.3 (SAS Institute, Cary, NC, USA). Replication and environments (location-year combination) were considered to be random, and genotypes were considered fixed effects. Least square means of genotypes across environments were computed and used for QTL analysis.

Broad sense heritability (H^2) for all traits in the combined dataset was calculated from the variance component generated by PROC VARCOMP in SAS. Heritability estimates were calculated using the following formula:

$$H^2 = \frac{\text{genotypic variance}}{\left[\text{genotypic variance} + \left(\frac{\text{gei variance}}{n}\right) + \left(\frac{\text{error variance}}{nr}\right)\right]}$$

where "gei" is genotype × environment interaction, "r" is the number of replications, and "n" is the number of environments.

QTL Detection

QTL analysis for sugar release, lignin content, and lignin S/G ratio was conducted using across environments, least square means data for the 188 progeny plants for which marker genotype data was available. Because we did not detect significant GXE interactions for the lignin content and sugar release traits, we analyzed data across environments to obtain genotypic means for QTL analysis. The QTL analysis was conducted using WinQTL Cartographer v2.5 [25]. A three-step QTL analysis was conducted starting with a non-parametric single-marker analysis [26] to establish marker-trait associations. Then, simple interval mapping [27] was conducted using a linear model for F_1 progenies in an outcrossing species [28] for estimating relationships between phenotypic values

Table 1 Effects of various sources of variance on sugar release, lignin content, and S/G ratio for AP13 × VS16 F_1 pseudo-testcross population combined across five environments (Ardmore and Burneyville, OK in 2009 and 2010 and Watkinsville, GA in 2010)

Source	Mean squares				
	Total sugar release	Glucose release	Xylose release	Lignin content	S/G ratio
Genotype (G)	1727**	705**	360*	3.24**	0.01**
Environment (E)	916,727**	79,163**	498,007**	1350.16**	1.01**
G × E	1298 ns	443 ns	296 ns	1.41 ns	0.003**

ns not significant

*$0.01 \le p \le 0.05$; **$0.001 \le p \le 0.01$ probability levels, respectively

Table 2 Population mean and heritability of sugar release, lignin content, and S/G ratio for AP13 × VS16 F_1 pseudo-testcross population combined across five environments (Ardmore and Burneyville, OK in 2009 and 2010 and Watkinsville, GA in 2010)

Trait	Population			Parents		H^2
	Min.	Max.	Mean ± SE	AP13 ± SE	VS16 ± SE	
Glucose release	141	172	150 ± 0.5	153 ± 1.3	168 ± 1.9	0.27
Xylose release	145	211	172 ± 0.7	169 ± 1.7	180 ± 2.2	0.54
Lignin content	23.0	26.7	24.9 ± 0.1	23.6 ± 0.6	26.7 ± 0.8	0.34
S/G ratio	0.63	0.74	0.7 ± 0.0	0.7 ± 0.01	0.73 ± 0.03	0.38

SE standard error

and putative QTL positions. Finally, the QTL was confirmed with composite interval mapping (CIM) [29, 30]. CIM was performed with forward and backward stepwise regressions at a threshold of $p < 0.05$ for automatic cofactor selection, a window size of 10, and a 1.0 cM walking speed along the linkage groups (LGs). Permutation analysis repeated 1000 times was

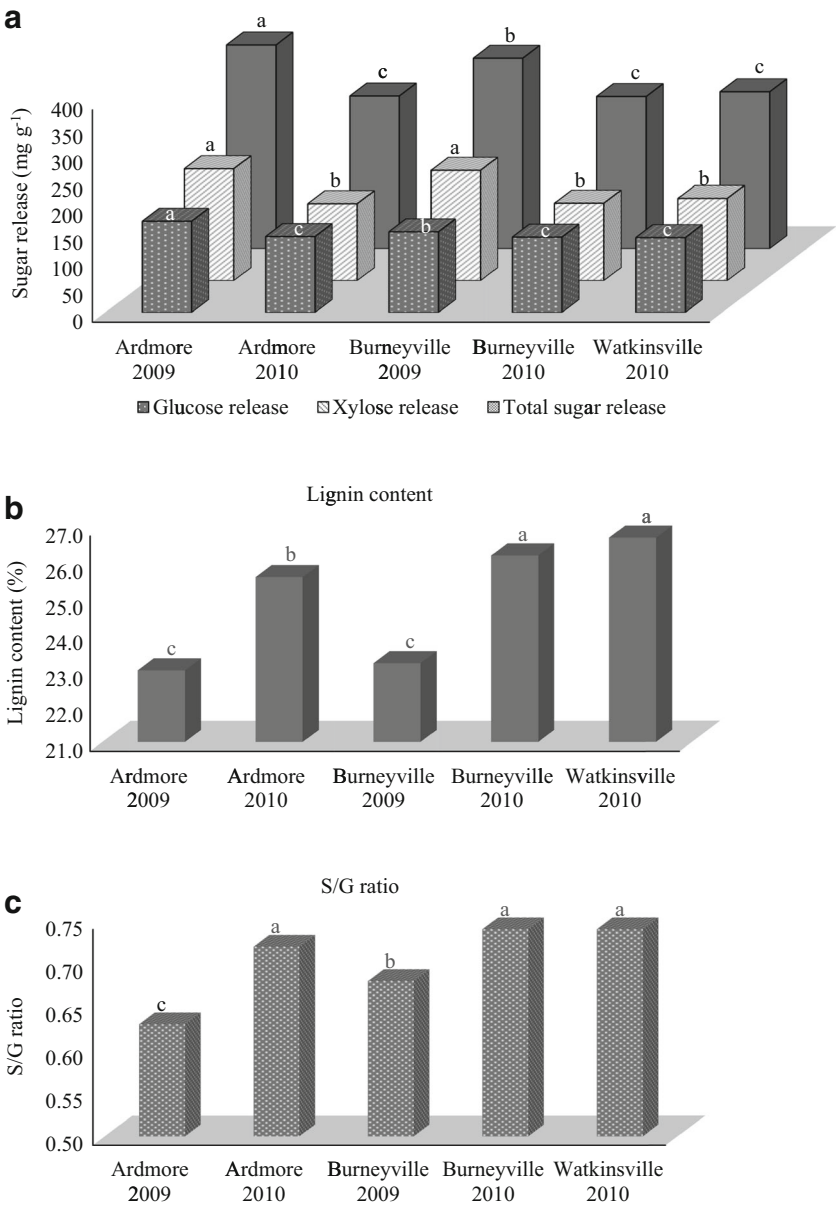

Fig. 1 Mean sugar release (**a**), lignin content (**b**), and S/G ratio (**c**) for the AP13 × VS16 population across five environments (Ardmore and Burneyville, OK in 2009 and 2010 and Watkinsville, GA in 2010). Means with the same letter within a given trait are not significantly different ($p < 0.05$)

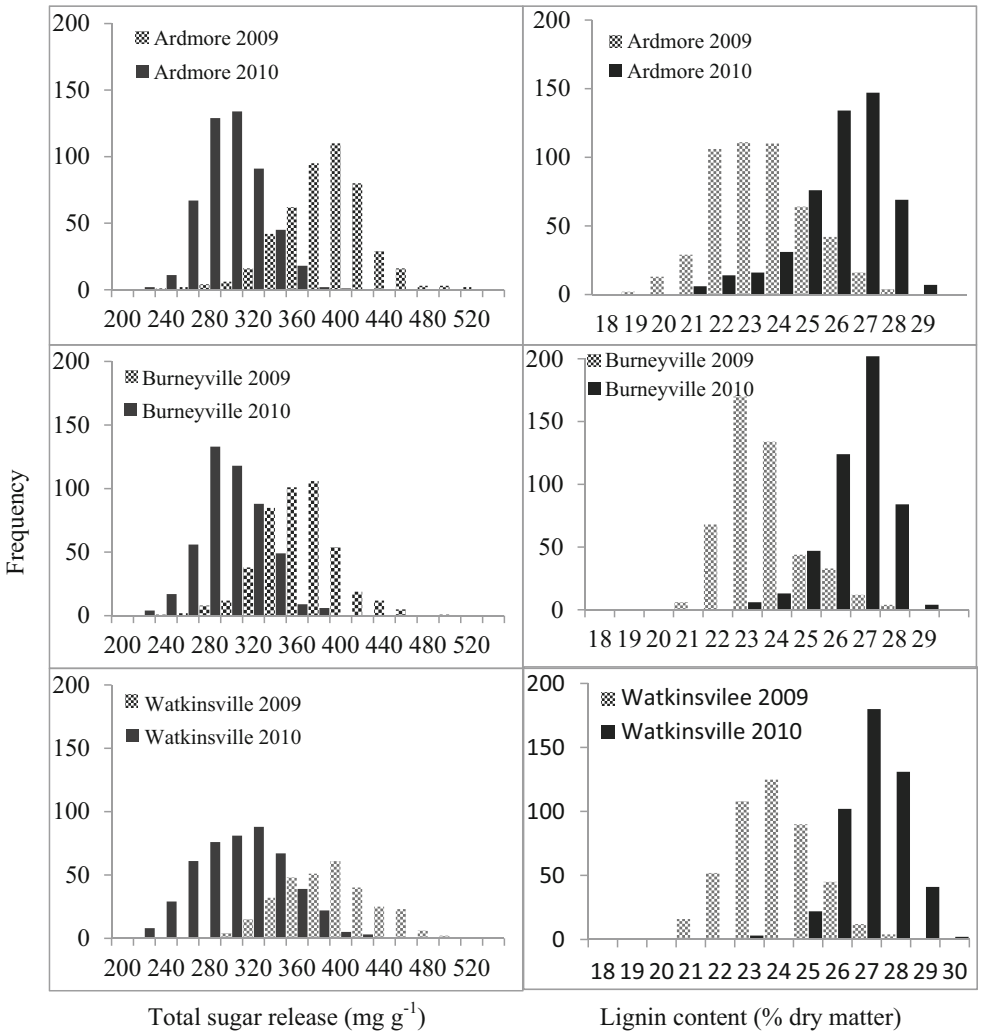

Fig. 2 Frequency distribution of sugar release and lignin content in the AP13 × VS16 F_1 pseudo-testcross mapping population evaluated across five environments (site-year combination)

used to set the genome-wide threshold of significance ($p < 0.05$) for QTL at 2.4 for glucose release, 2.7 for xylose release, and 3.1 for lignin content. We considered a threshold log of odds (LOD) minus 0.5 for a "putative QTL." Main effect QTL were validated by inclusive composite interval mapping (IciMapping) using the QTL in biparental population (BIP) functionality [31]. Epistatic QTL were rescanned using the QTL-by-environment interaction in biparental population functionality of the IciMapping software. QTL designations consisted of abbreviations for the trait names (Glu = glucose, Xyl = xylose, and Lig = lignin content) followed by the LG name (1 to 9) and sub-genome ("a" or "b") and a serial number when there were two or more QTL on the same LG.

Localization of QTL Flanking Markers in the Switchgrass Genome Sequence

To determine the physical location of QTL and identify putative candidate genes underlying the QTL, flanking marker sequences were used as queries in a BLASTn search [32] against the *P. virgatum* AP13 v1.1 genome sequence assembly (*P. virgatum* v1.1, DOE-JGI, http://www.phytozome.net/panicumvirgatum, accessed November 20, 2015). Genes annotated in the region spanned by the QTL flanking markers plus 50 kbp at either side were selected as candidate genes, and their functional annotation was recorded. Most of the QTL identified in this study had LOD scores <3.0. Thus, we delimited putative QTL intervals using the criterion "threshold LOD minus 0.5" with whiskers at LOD scores "threshold minus 1."

Results and Discussion

Population Performance

Statistically significant differences were observed among genotypes and environments (years and locations) for the cell wall composition traits investigated (Table 1).

Genotype-by-environment interaction effects were only significant for S/G ratio. The two parents significantly differed in sugar release and lignin content but were similar in their S/G ratio (Table 2). All traits segregated in the progeny and the prevalence of genetic variation among genotypes of the population for the cell wall traits guarantee that significant improvements can be achieved through selection. Since we are mapping in an F_1 pseudo-testcross population, it is the variation present within each of the heterozygous parents that is pertinent.

The average total sugar (glucose and xylose) release across five environments ranged from 287 to 384 mg g^{-1}, with an overall average of 322 mg g^{-1}. The highest average sugar release was observed at Ardmore in 2009 and the lowest at Burneyville in 2010 (Fig. 1a). The mean lignin content across the five environments ranged from 23.0 to 26.7 % (Fig. 1b). The mean S/G ratio ranged from 0.63 to 0.74 (Fig. 1c), indicating that switchgrass lignin is mainly composed of the guaiacyl (G) monomers derived from coniferyl alcohol. A low concentration of syringyl (S) units, derived from sinapyl alcohol, is in line with results from previous analyses of lignin composition in grasses [10].

Mean sugar release was lower in 2010 than in 2009 across locations, concomitant with and probably due to an increase in lignin content, which was 12 % higher in 2010 than in 2009 (Fig. 2). The S/G ratio of lignin composition was higher in 2010 than in 2009. This could be favorable for biofuel processing because a higher concentration of S lignin relative to G lignin increases sugar release or the efficiency of biomass conversion [8, 33]. However, a small reduction in S/G ratio was found to improve dilute acid hydrolysis in *Populus* [9]. Variation in lignin content across years could be caused by environmental factors that affected plant growth (plant height and biomass yield were also higher in 2010 than in 2009 [19]) or by biotic and abiotic stresses that are known to affect lignification [34]. It is also possible that the differences in lignin content across years are due to changes in the leaf to stem ratio because stems have a higher lignin content and a higher S/G ratio than leaves [35–37]. Future assessments of the natural variation in lignin content across environments should also consider the relative ratio of leaves to stems in harvested biomass.

The broad sense heritability for cell wall composition traits was estimated from a combined analysis of variance components (Table 2) at 0.27 for glucose release and 0.54 for xylose release. The heritability of lignin content was 0.34 and of S/G ratio 0.38. The moderate heritability estimates obtained for these traits indicate a preponderance of genetic control of the phenotypic variation. These heritability estimates are similar to those made for biomass yield (0.29–0.65) and related traits (plant height <0.27, tillering 0.28–0.48, etc.) in other populations of switchgrass [38–40].

Impact of Lignin Content on Sugar Release

A concomitant reduction in total sugar release was observed with an increase in lignin content (Fig. 3a). Lignin is a complex phenolic polymer that has an important role in structural integrity of the stems [41]. Lignification and cross-linkage of lignin with other cell wall components form a natural barrier against the penetration of disparaging enzymes through the cell wall [42]. This barrier negatively impacts sugar release from cellulose and hemicellulose [43]. We found that a 1 %

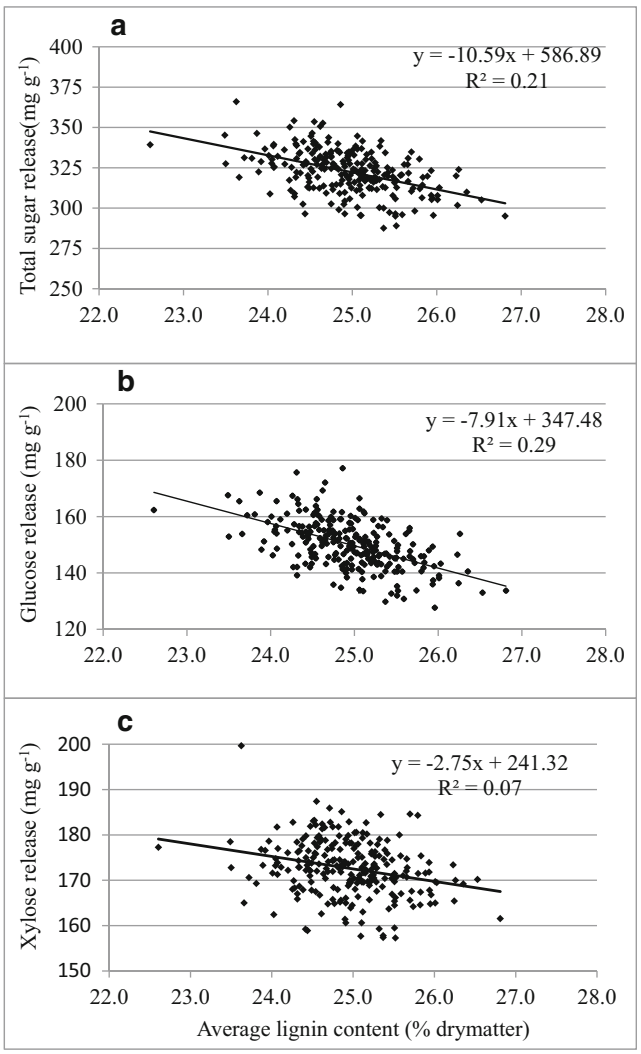

Fig. 3 Simple linear regression of lignin content to sugar release in AP13 × VS16 F_1 pseudo-testcross mapping population. Data were averaged across five environments; **a** total sugar release, **b** glucose release, and **c** xylose release

Table 3 Main effect QTL detected for sugar release and lignin content in AP13 × VS16 F_1 pseudo-testcross mapping population tested across five environments (Ardmore and Burneyville, OK in 2009 and 2010 and Watkinsville, GA in 2010)

Map	Trait	QTL	LG	Position (cM)	LOD	PVE (%)	Additive effect[a]
Female (AP13)	Glucose	Glu3af	IIIa-f	42.5	2.4	5.7	4.2
		Glu3bf	IIIb-f	88.5	2.5	4.9	3.9
		Glu7bf	VIIb-f	66.1	2.2	5.0	3.9
	Xylose	Xyl3af	IIIa-f	68.4	2.2	5.2	2.7
	Lignin	Lig1bf	Ib-f	4.8	2.2	3.8	0.2
		Lig2bf	IIb-f	60.9	2.4	4.5	−0.3
		Lig3af1	IIIa-f	63.4	2.8	5.6	−0.3
		Lig3af2	IIIa-f	79.6	3.0	6.2	−0.3
		Lig4bf	IVb-f	5.6	2.7	5.1	−0.3
		Lig7af	VIIa-f	38.3	5.8	11.1	0.4
		Lig7bf	VIIb-f	64.1	3.2	6.2	−0.3
		Lig9bf	IXb-f	34.6	2.1	3.9	0.2
Male (VS16)	Glucose	Glu4am	IVa-m	78.2	2.0	4.5	3.7
		Glu5am	Va-m	13.9	2.5	5.5	4.1
		Glu9am	IXa-m	86.5	2.6	6.4	−4.4
	Xylose	Xyl2bm	IIb-m	52.0	4.4	9.4	6.2
		Xyl5am	Va-m	28.1	3.0	6.4	3.1
	Lignin	Lig2bm	IIb-m	37.4	2.7	5.8	−0.3
		Lig3am1	IIIa-m	33.6	4.2	9.5	0.4
		Lig3am2	IIIa-m	59.3	2.8	5.2	0.3
		Lig5bm	Vb-m	72.8	2.1	4.4	0.3
		Lig9am1	IXa-m	3.4	4.3	8.6	−0.4
		Lig9am2	IXa-m	119.4	2.1	4.1	0.2

[a] Additive effect: mg g^{-1} for sugar release and % for lignin content

increase in lignin content reduced sugar release by 10.6 mg g^{-1} (Fig. 3). The impact of lignin content was higher for glucose release than for xylose release (Fig. 3b, c). From the regression analysis, we estimated that a 1 % increase in lignin content brought about a 7.9 mg g^{-1} reduction in glucose release and a 2.75 mg g^{-1} reduction in xylose release. Interestingly, in a study of natural variants of *Populus*, Studer et al. [8] found that the strong negative impact of lignin content on glucose release was observed only for pretreated samples with a low S/G ratio (<2.0). Xylose release, on the other hand, did not correlate with lignin content. Since the S/G ratio is low in switchgrass, our observation that lignin content predominantly affects glucose release efficiency agrees with those observations. Decreased lignin content due to downregulation of 4-coumarate:coenzyme A ligase (4CL), a gene in the phenylpropanoid biosynthetic pathway, resulted in an increase in glucose but not xylose release [15].

QTL Mapping

A total of nine QTL for sugar release and 14 QTL for lignin content were detected in the female and male maps (Table 3, Fig. 4). Three QTL each for glucose release were detected in the female and male maps, while one and two xylose release QTL were detected in the female and male maps, respectively. No QTL was detected for S/G ratio at threshold LOD (1.97) determined by genome-wide 1000 permutation test minus 0.5 as for other traits. Most of the QTL for sugar release and lignin content that mapped to the same LG had opposite additive effects. For instance, on LG VIIb-f, Glu7bf had a positive additive effect on sugar release and Lig7bf had a negative additive effect on lignin content.

The individual QTL identified for lignin and sugar release each explained less than 10 % of the phenotypic variation in the population, except for Lig7bf, which explained 11.1 % (Table 3). The relatively low phenotypic variation explained (PVE) by individual QTL indicates that these traits are controlled by several QTL with small effects. Therefore, improvement in feedstock quality can occur by accumulating positive alleles at multiple loci (Fig. 5). All the additive effects of the glucose and xylose release QTL mapped in the female and male maps were positive, except Glu9am which had an additive effect of −4.4 mg g^{-1}. The additive effects of most of the lignin concentration QTL detected in the female map were negative, while they were positive for QTL detected in the male map.

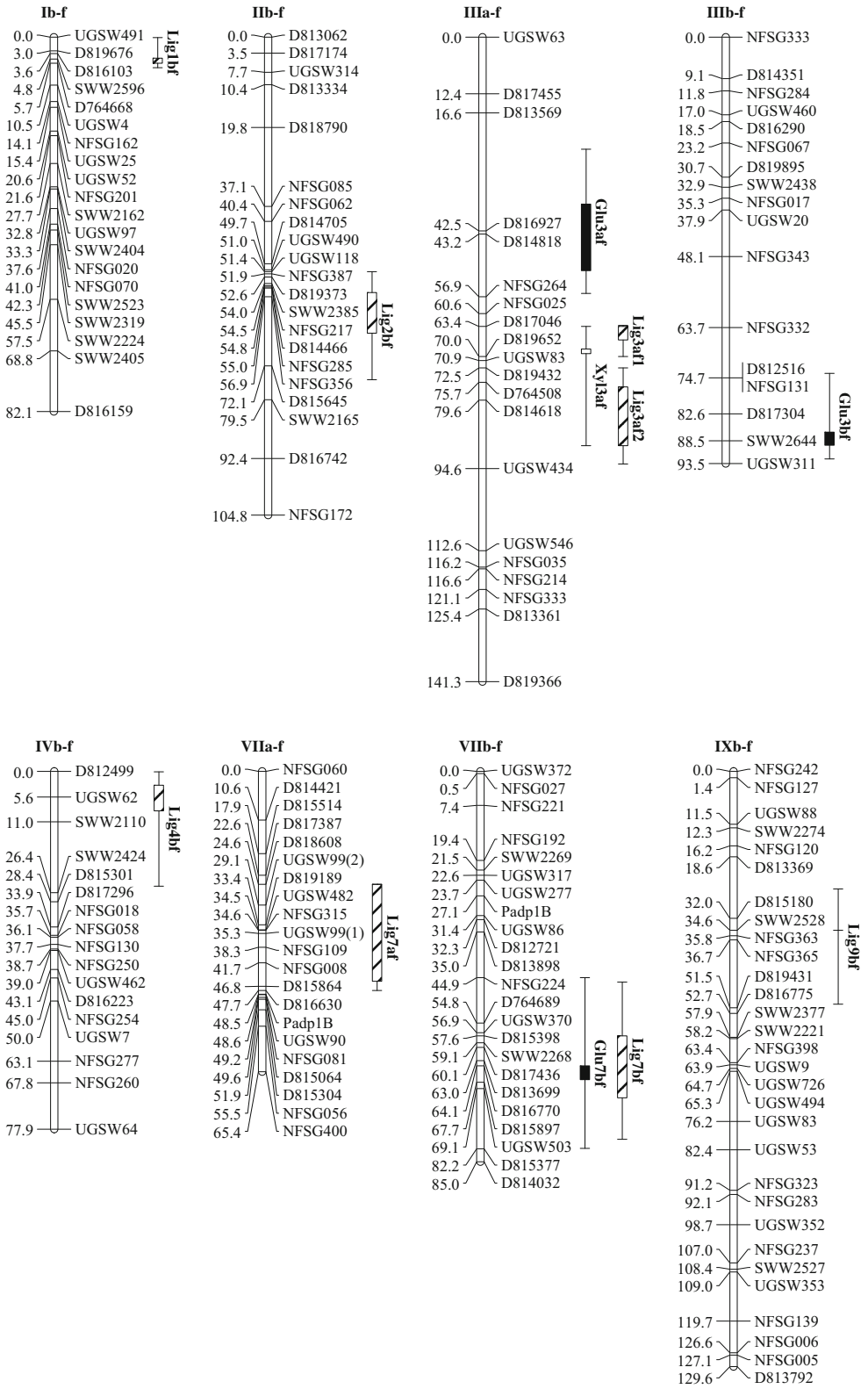

Fig. 4 Main effect QTL for sugar release and lignin content detected with overall LSM across five environments in AP13 (female, lowland) and VS16 (male, upland) linkage maps. QTL with LOD values equal or greater than 2.0 are presented. QTL for glucose and xylose release, and lignin content were named as Glu, Xyl, and Lig, respectively, followed by the LG name on which the QTL was detected (serial numbers were included when there were more than one QTL on a LG). The *box* on the QTL chart indicates a threshold LOD minus 0.5, and the *whiskers* on one or both ends of the QTL are a threshold LOD minus 1.0

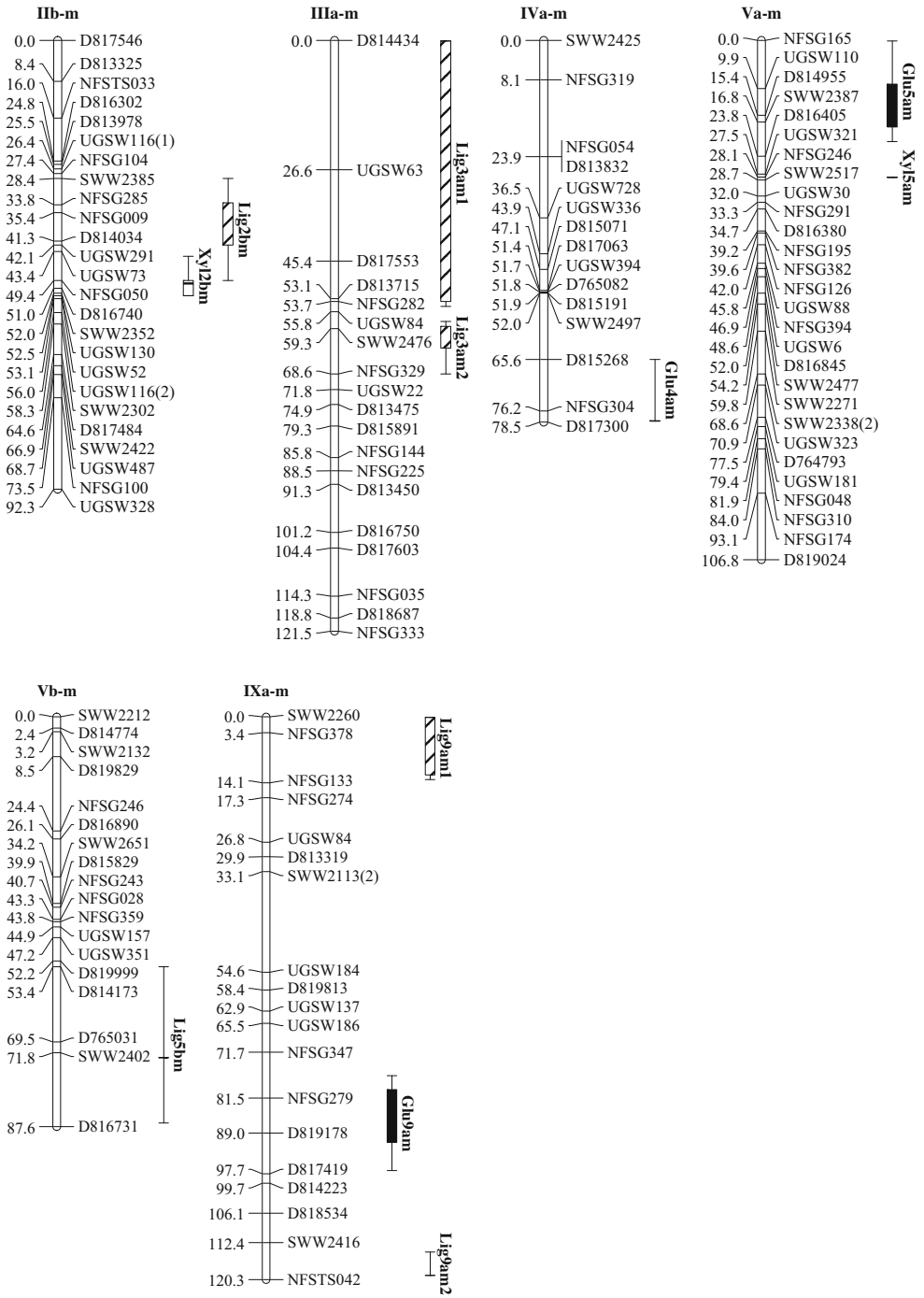

Fig. 4 (continued)

In addition to the main effect QTL, 12 epistatic QTL for glucose release, 9 for xylose, and 11 for lignin concentration were detected (Table 4). In general, the epistatic QTL for the sugar release traits accounted for more of the phenotypic variation than the main effect QTL detected for the traits. The epistatic QTL for glucose release had 8.9 to 18.6 % PVE with the additive by additive effect ranging from −17.6 to 13.5 mg g^{-1}. The nine epistatic QTL detected for xylose release had PVE from 8.9 to 17.1 %. The additive by additive effect of the xylose epistatic QTL ranged from −12.6 to 8.3 mg g^{-1}. Similarly, the epistatic QTL detected for lignin concentration had PVE ranging from 7.6 to 12.2 %. The additive by additive effect of the epistatic QTL detected for lignin ranged from −7.8 to 8.0 g kg^{-1}. All epistatic QTL for lignin except those on LGs Ib-f/IIa-f, IIb-f/VIIIb-f, and IIIa-f/IXb-f in the female and IIIa-m/IVa-m in the male had positive additive effects (Table 4).

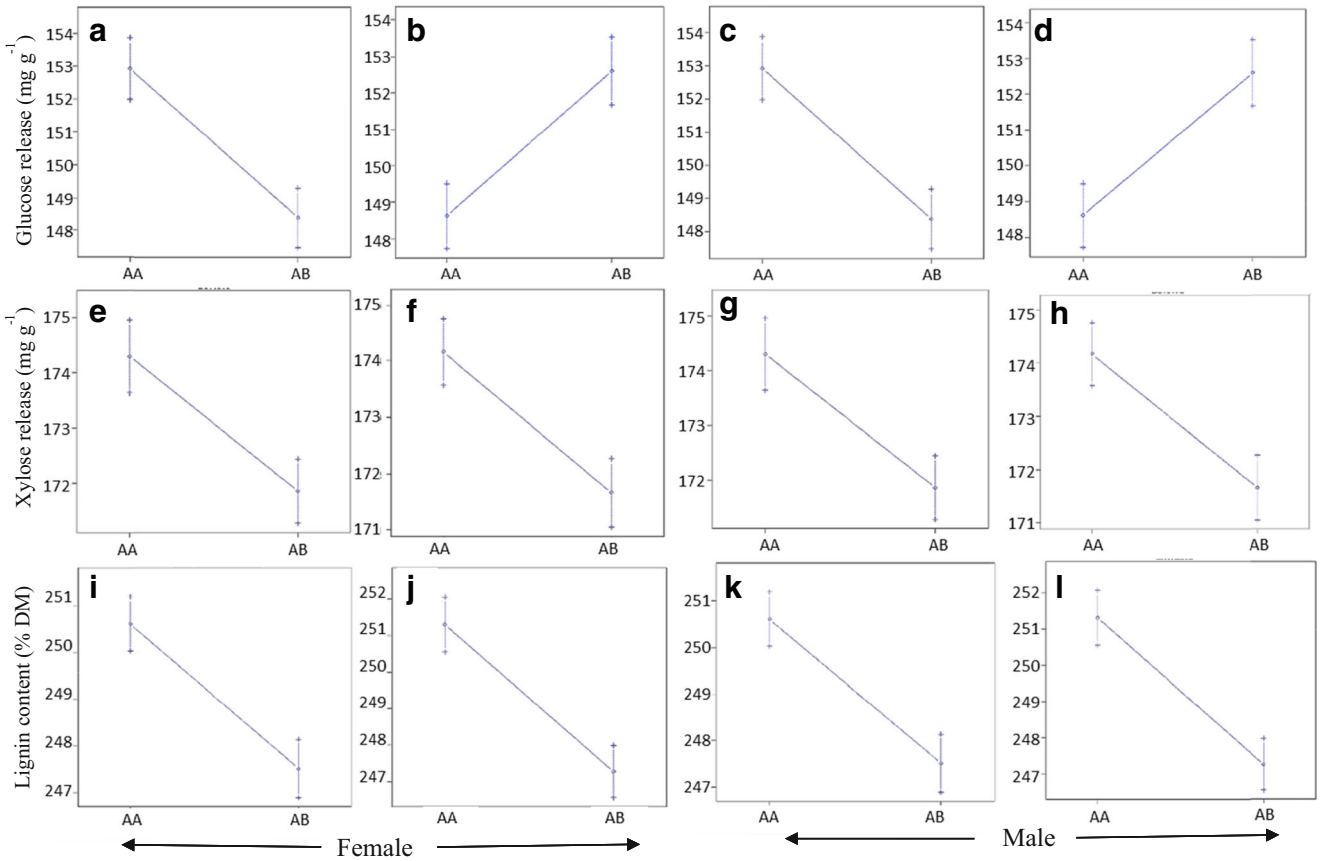

Fig. 5 Allelic effect plot of selected QTL for glucose release (**a**–**e**), xylose release (**f**–**h**), and lignin content (**i**–**l**) in the AP13 and VS16 maps. *AA* and *AB* represent the allelic states of the markers close to the peak of the QTL

The importance of epistatic effect QTL has been documented for various traits in rice [44–48], wheat [49–51], and soybean [52], demonstrating that both main effect and epistatic QTL are underlying various quantitative traits in plants. The negative correlation of lignin content and sugar release observed in this population suggests that some segregants or natural variants with reasonably low lignin content and above average sugar release may be identified through selection.

Co-mapping of QTL for Yield and Quality

Several of the QTL detected for cell wall composition traits co-mapped with biomass yield and plant height QTL reported previously [19]. One xylose release QTL on IIIa-f mapped very close to a biomass yield QTL but had a contrasting allelic effect. Similarly, a glucose release QTL that co-mapped with plant height on LG IVa-m had contrasting effects on glucose release and plant height. Three lignin QTL, namely Lig3af, Lig9bf, and Lig3am, co-mapped or closely mapped with biomass yield QTL, and two lignin QTL (Lig1bf and Lig7af) comapped with plant height. As expected, alleles that increased biomass yield or height also increased lignin content. Exceptions were the alleles at QTL Lig9bf and Lig3am which increased lignin content and had a reducing effect on biomass yield. As the breeding targets for a new cultivar have increased biomass with reduced lignin content, selection of beneficial alleles at Lig9bf and Lig3am will be important. Where QTL for biomass and cell wall composition co-map and the direction of the effect is the same (higher biomass, higher lignin), it will be hard to uncouple them to improve both yield and recalcitrance simultaneously. In this case, the focus of breeding should be on regions where QTL do not co-map for those two target traits. On the other hand, lignin content and biomass yield are positively correlated, placing a daunting challenge on breeding to combine both traits to produce high yielding genotypes with improved biomass conversion [53].

Candidate Genes for Cell Wall Composition

The linkage maps of switchgrass used for the QTL detection have been constructed using SSR, STS, and DArT markers. A BLASTN analysis of the genomic and EST sequences of the markers flanking the QTL was conducted against the *P. virgatum* AP13 v1.1 assembly, and the regions between 50 kbp upstream of the left and downstream the right flanking markers were searched for possible candidate genes with functional relationships to the QTL. Most of the QTL regions

Table 4 Epistatic effect QTL detected for sugar release and lignin content in AP13 × VS16 F_1 pseudo-testcross mapping population tested across five environments (Ardmore and Burneyville, OK in 2009 and 2010 and Watkinsville, GA in 2010)

Map	Trait	LG1	Pos1	LMarker1	RMarker1	LG2	Pos2	LMarker2	RMarker2	LOD	PVE (%)	AA
Female (AP13)	Glucose	IIIb-f	75	NFSG131	D817304	IVb-f	60	UGSW7	NFSG277	3.0	10.7	9.9
		IIb-f	65	NFSG356	D815645	Va-f	55	UGSW321	NFSG256	3.2	10.5	−10.3
		IIIa-f	65	D817046	D819652	Vb-f	5	D819275	NFSG246	3.1	10.1	10.4
		IVb-f	40	UGSW462	D816223	VIa-f	40	D818983	NFSG265	3.1	15.7	11.7
		VIa-f	60	D819209	UGSW50	VIa-f	80	UGSW329	UGSW56	4.6	18.6	−17.6
		IVa-f	15	D819834	UGSW462	VIIIa-f	0	D817694	D815577	4.1	12.0	12.5
		IVb-f	40	UGSW462	D816223	VIIIa-f	70	UGSW128	NFSG266	3.8	17.7	13.5
		Va-f	30	UGSW169	NFSG165	IXa-f	60	NFSG137	SWW2175	3.3	8.9	9.6
		IIIa-f	25	D813569	D816927	IXa-f	85	D814746	NFSG238	3.3	14.7	−12.6
		IIIa-f	105	UGSW434	UGSW546	IXb-f	0	NFSG242	NFSG127	3.9	14.1	13.1
	Xylose	IIa-f	85	NFSG082	D764605	Va-f	5	SWW2125	SWW2240	3.1	10.6	−7.4
		IIIa-f	95	UGSW434	UGSW546	Va-f	95	D814066	D819990	3.3	10.6	8.1
		IIb-f	0	D813062	D817174	VIa-f	5	NFSG145	NFSG410	3.1	8.9	−6.6
		VIa-f	70	UGSW50	UGSW329	VIa-f	85	UGSW329	UGSW56	4.7	17.1	−12.6
		IIb-f	40	NFSG085	NFSG062	VIIIa-f	5	D817694	D815577	3.9	12.0	−8.1
		IVa-f	15	D819834	UGSW462	VIIIa-f	5	D817694	D815577	3.3	12.2	8.3
		VIa-f	10	NFSG145	NFSG410	IXb-f	20	D813369	D815180	3.4	9.8	7.4
		IIb-f	55	NFSG285	NFSG356	IXb-f	100	UGSW352	NFSG237	3.3	10.2	−7.2
	Lignin	Ib-f	10	D764668	UGSW4	IIa-f	5	SWW2373	NFSG307	3.3	9.4	−0.64
		IVa-f	30	UGSW175	UGSW481	IVb-f	0	D812499	UGSW62	3.1	11.7	0.7
		Ib-f	75	SWW2405	D816159	VIb-f	45	UGSW393	D815557	3.5	10.2	0.8
		VIIb-f	25	UGSW277	Padp1B	VIIIa-f	25	D813050	D814743	3.1	10	0.78
		VIIb-f	5	NFSG027	NFSG221	VIIIb-f	20	D814579	D816233	3.8	10.1	0.7
		IIb-f	50	D814705	UGSW490	VIIIb-f	55	UGSW103	D819029	3.1	8.8	−0.68
		VIa-f	80	UGSW329	UGSW56	IXa-f	115	SWW2369	D816125	3.9	12.1	0.77
		IIIa-f	100	UGSW434	UGSW546	IXb-f	5	NFSG127	UGSW88	3.1	12.2	−0.78
Male (VS16)	Glucose	IIb-m	15	D813325	NFSTS033	IVa-m	70	D815268	NFSG304	3.5	14.7	−11.3
		IXa-m	60	D819813	UGSW137	IXb-m	15	NFSG242	D812600	3.1	14.0	11.7
	Xylose	Vb-m	0	SWW2212	D814774	IXa-m	65	UGSW137	UGSW186	4.1	10.3	−7.3
	Lignin	IIIa-m	105	D817603	NFSG035	IVa-m	10	NFSG319	NFSG054	3.4	7.8	−0.68
		IIa-m	30	D817257	SWW2545	Vb-m	5	SWW2132	D819829	3.1	10.4	0.74
		IIIb-m	15	NFSG131	NFSG332	IXb-m	105	D764952	NFSG039	3.4	7.6	0.73

LG linkage group, *Pos* position in respective LG, *AA* additive by additive epistatic effect

harbored genes involved in cell wall modifications (Table 5). Among candidate genes, we identified for glucose main effect QTL detected on LGs IIIa-f, VIIb-f, IVa-m, Va-m, and IXa-m were hexokinases, which are enzymes that phosphorylate six-carbon sugars and form hexose phosphate [54]. An auxin-induced transcription factor that functions as a repressor of early auxin response genes at low-auxin concentrations [55] was also colocalized with glucose QTL. The MYB family transcription factors, transferase family proteins, and other transcriptional regulators were also identified in the vicinity of glucose main effect QTL. QTL for xylose release on LGs IIIa-f, IIb-m, and Va-m were found within 50 kbp of putative candidate genes such as glutathione *S*-transferases, glycosyltransferases, CDP-alcohol phosphotidyltransferases, oligosaccharyltransferases, and phosphotransferases. Glutathione *S*-transferase is involved in the reduction of organic hydroperoxides formed during oxidative stress [56]. Glycosyltransferase establishes natural glycosidic linkages, including the biosynthesis of polysaccharides [57]; CDP-alcohol phosphotidyltransferase is involved in phospholipid biosynthesis; oligosaccharyltransferase is a membrane protein complex that transfers a 14-sugar oligosaccharide from dolichol to nascent protein, and phosphotransferase catalyzes phosphorylation reactions (moving sugars to the cell).

Lignin QTL colocalized with genes encoding phosphoglycerate mutase, a key catalyst of glycolysis [58] that plays an

Table 5 Selected sugar release and lignin content QTL colocalized genes in the switchgrass genome sequence v1.1 assembly

LG	QTL	QTL flanking marker	Position (cm)	§Swg chr	Genome coordinate (start position)	Gene in 50 kbp (either side)	Gene annotation	Function
Ib-f	Lig1bf	UGSW491	0	01a	5598907	Pavir.Aa00497	Phosphoglycerate mutase	Glycolysis
Ib-f	Lig1bf	SWW2596	4.8	01a	8097987	Pavir.Aa00725	Glycosyl hydrolase family 47 domain contain protein	Carbohydrate metabolism
IIb-f	Lig2bf	NFSG356	56.9	02a	56259914	Pavir.Ba02948	HOTHEAD precursor	Cellular interaction
IIIa-f	Lig3af1	NFSG264	56.9	03a	9450012	Pavir.Ca00807	Hexokinase	Phosphorelation
IIIa-f	Lig3af1	UGSW83	70.9	09a	58734357	Pavir.Ia02847	Glutathione *S*-transferase	Oxidative stress (ROS scavenging)
IIIa-f	Lig3af2	UGSW434	94.6	03a	42120396	Pavir.Ca02525	MYB family transcription factor	Transcription regulation
IVb-f	Lig4bf	SWW2110	11	04b	49514187	Pavir.Db02381	Calcium-dependent protein kinase isoform AK1	Signaling and phosphorylation
VIIa-f	Lig7af	NFSG109	38.3	07a	24619214	Pavir.Ga01944	2-Oxoglutarate dehydrogenase E1 component	Carbohydrate metabolism
VIIa-f	Lig7af	NFSG008	41.7	07a	25927298	Pavir.Ga02020	Chloroplastic group IIA intron splicing facilitator	Nucleic acid binding
VIIb-f	Lig7bf	SWW2268	59.1	07b	37646288	Pavir.Gb02537	Auxin-induced protein 5NG4	Phytohormone signaling and metabolism
VIIb-f	Lig7bf	UGSW503	69.1	07b	46216662	Pavir.Gb02471	Transferase family protein	Stress response
IXb-f	Lig9bf	SWW2528	34.6	09b	66125263	Pavir.Ib03140	Citrate transporter protein	Substrate carrier protein
IXb-f	Lig9bf	NFSG363	35.8	09b	65896745	Pavir.Ib04323	Protein kinase domain containing protein	Signaling and phosphorylation
IIb-m	Lig2bm	UGSW291	42.1	02b	28701538	Pavir.Bb01370	Fasciclin-like arabinogalactan protein 8 precursor	Cell wall modification and cell growth
IIb-m	Xyl2bm	SWW2352	52	02a	21008158	Pavir.Ba01601	Glycosyltransferase family 8	Stress response
IIIa-m	Lig3am1	UGSW63	26.6	03a	1256523	Pavir.Ca00067	Phosphatidate cytidylyltransferase	Metabolism and signaling
IIIa-m	Lig3am2	UGSW84	55.8	03b	12361681	Pavir.Cb00653	Terpene synthase	Metabolism
IVa-m	Glu4am	NFSG304	76.2	04a	49554446	Pavir.Da02398	MYB family transcription factor	Transcription regulation
Va-m	Glu5am	UGSW110	9.9	05a	58995560	Pavir.Ea03609	Transcriptional regulator	Transcription regulation
Va-m	Glu5am	SWW2387	16.8	05a	56198452	Pavir.Ea03421	MYB family transcription factor	Transcription regulation
Va-m	Xyl5am	SWW2517	28.7	05a	54093837	Pavir.Ea03130	CDP-alcohol phosphatidyltransferase	Phospholipid biosynthesis
Va-m	Xyl5am	UGSW30	32	05b	64711095	Pavir.Eb03105	Oligosaccharyl transferase	Protein glycosylation
Vb-m	Lig5bm	SWW2402	71.8	05b	7144138	Pavir.Eb00474	Flavonol synthase/flavanone 3-hydroxylase	Flavonoid biosynthesis
IXa-m	Lig9am1	NFSG378	3.4	09b	602040	Pavir.Ib00050	1,3-beta-glucan synthase domain containing protein	Cell wall modification and cell growth
IXa-m	Lig9am2	SWW2416	112.4	09a	1854647	Pavir.Ia00210	Kelch motif family protein	Protein-protein interaction

Flanking markers with LOD score >threshold −1 were included

§swg chr switchgrass chromosome (pseudo-molecule), *muc* multiple unanchored contigs

important role in vegetative growth and stomatal movement [59] (Table 5). A glycosyl hydrolase, a MYB family transcription factor which plays a regulatory role in plant developmental processes and defense responses [60], 2-oxyglutarate dehydrogenase, a citrate transporter protein, a protein kinase, a fasciclin-like arabinogalactan protein, a terpene synthase, a 3-hydroxyacyl CoA dehydrogenase, a flavonol synthase, a 1,3-beta-glucan synthase, and a kelch motif family protein were also identified in lignin QTL regions. The phenylpropanoid pathway includes about ten genes involved in lignin biosynthesis [61, 62]. Several lignin pathway genes have been mapped in the vicinity of lignin content QTL in maize [63]. It is difficult to conclude that the identified genes play a role in the control of the cell wall composition traits mapped in the AP13 × VS16 population until further validation is conducted.

Conclusions

This population derived from a cross between an upland and a lowland genotype exhibited substantial variation for cell wall composition traits of importance for bioenergy production. The QTL analysis revealed six main effect QTL for glucose release which collectively explained about 32 % of the phenotypic variation of the trait. Three main effect QTL detected for xylose release explained a total of 21 % of the phenotypic variation. Fourteen main effect QTL for lignin content accounted for a total of 84 % of the phenotypic variation. Most of the sugar release and lignin content QTL colocalized with genes functioning in carbohydrate processing and metabolism and other functions related to plant growth and development including cell wall biosynthesis.

Selection of favorable alleles at this limited number of QTL will lead to a significant improvement in the cell wall composition traits in switchgrass that are relevant to improved feedstock production. Both parents carried alleles for lower lignin and higher sugar release, suggesting that both ecotypes can contribute to desirable breeding targets. The cell wall composition traits had no defined relationship with the yield traits reported in the same population [19], which may facilitate simultaneous improvement for both yield and quality. The markers or candidate genes that are tightly associated with the QTL peaks can provide a high level of selection accuracy for the target traits in breeding programs. Future isolation of the actual genes underlying the QTL will require either fine-mapping or validation of strong candidates using molecular approaches.

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