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Characterization of Cell Wall Composition in Bi-parental Population of Switchgrass

D. D. Serba[1], A. Ziebell[2], R. Sykes[2], B. A. Bahri[3], K. M. Devos[3], E. C. Brummer[1], J. H. Bouton[1] and M. C. Saha[1]

The Samuel Roberts Noble Foundation, Forage Improvement Division, 2510 Sam Noble Parkway, Ardmore, Oklahoma 73401 U.S.A.

Abstract

Enhancing enzymatic hydrolysis of cellulose by minimizing the lignin content will maximize the ethanol yields from plant biomass and reduce the processing cost as well. A full-sib switchgrass mapping population consisting of 251 genotypes developed from a lowland AP13 and upland VS16 cross has been evaluated at three locations (Ardmore and Burneyville, OK and Athens, GA) since 2008. Biomass harvested after senescence in 2009 and 2010 was evaluated for lignin content and sugar release. Biomass samples from all six environments were analyzed at NREL for lignin content, S/G ratio using molecular beam mass spectrometry (MBMS) and sugar release characteristics by co-saccharification. Significant differences were observed among the genotypes and the environments for the traits. The lignin content ranged from 19-27% of the dry biomass while the glucose and xylose release ranged from 120-313 and 123-263 mg g^{-1}, respectively. Regression analysis of the sugar release on the lignin content found that a unit increase in lignin content decreases total sugar release up to 10 mg g^{-1}. Based on the results, 10 genotypes with low average lignin content and above average sugar release value were selected for further evaluation and development of synthetic variety.

Introduction

Switchgrass (*Panicum virgatum* L.) is a warm-season (C4) perennial grass native to the tall grass prairie ecosystem of North America (Moser and Vogel 1995). Switchgrass has recently been identified as a dedicated herbaceous species for cellulosic-based biofuel production (McLaughlin et al., 1999). Switchgrass is an outcrossing, heterozygous species with high genetic diversity and has high potential for improvement (Bouton 2007). One of the priority traits for improvement is the cell wall composition to suit for bioenergy production. It is important to study the level of variability created through hybridization and the inheritance of the traits.

Objectives

1. To study the variability in a bi-parental population for lignin content, S/G ratio and sugar release.
2. To investigate the effect of environment on cell wall composition of switchgrass biomass.

Materials and Methods

A full-sib switchgrass population was developed from AP13 × VS16 cross. A total of 251 F_1 plants from the population, duplicate sets of the parents and Alamo (check) were field-evaluated at three locations (Ardmore and Burneyville, OK and Athens, GA) from 2008 to 2011. Biomass harvested after senescence during 2009 and 2010 was oven dried at ~40^o C for 72 h and milled to 1 mm particle size (mesh size=20) using Thomas Model 4 Wiley® Mill (Thomas Scientific, Swedesboro, NJ). The milled samples were analyzed for lignin content and sugar release characteristics at National Renewable Energy Laboratory (NREL). Lignin content and S/G ratio were estimated by pyrolysis molecular beam mass spectrometry (py-MBMS) (Sykes et al., 2010). The samples were pretreated and saccharified to measure glucose and xylose release (megazymes GOPOD and XDH assay kits were used for quantitation). The hydrothermal pretreatment was performed at 180^o C for 17.5 min.; hydrolysis utilized 70 mg of Ctec2 (Novozymes Cellic® ,Franklinton, NC) per mg biomass for saccharification (50^o C for 70 h).

Statistical analysis was conducted using mixed model on SAS v9.2 (SAS Institute, Cary, NC). Graphical analysis on the data was also performed using R graphics.

Results and Discussion

The lignin and sugar release data were fairly normally distributed (Fig.1a and b). Higher lignin values were observed in 2010 than 2009 across the three locations (Fig. 1a, dark red bars=2010). Conversely, higher sugar yields were obtained in 2009 than 2010 (Fig. 1b).

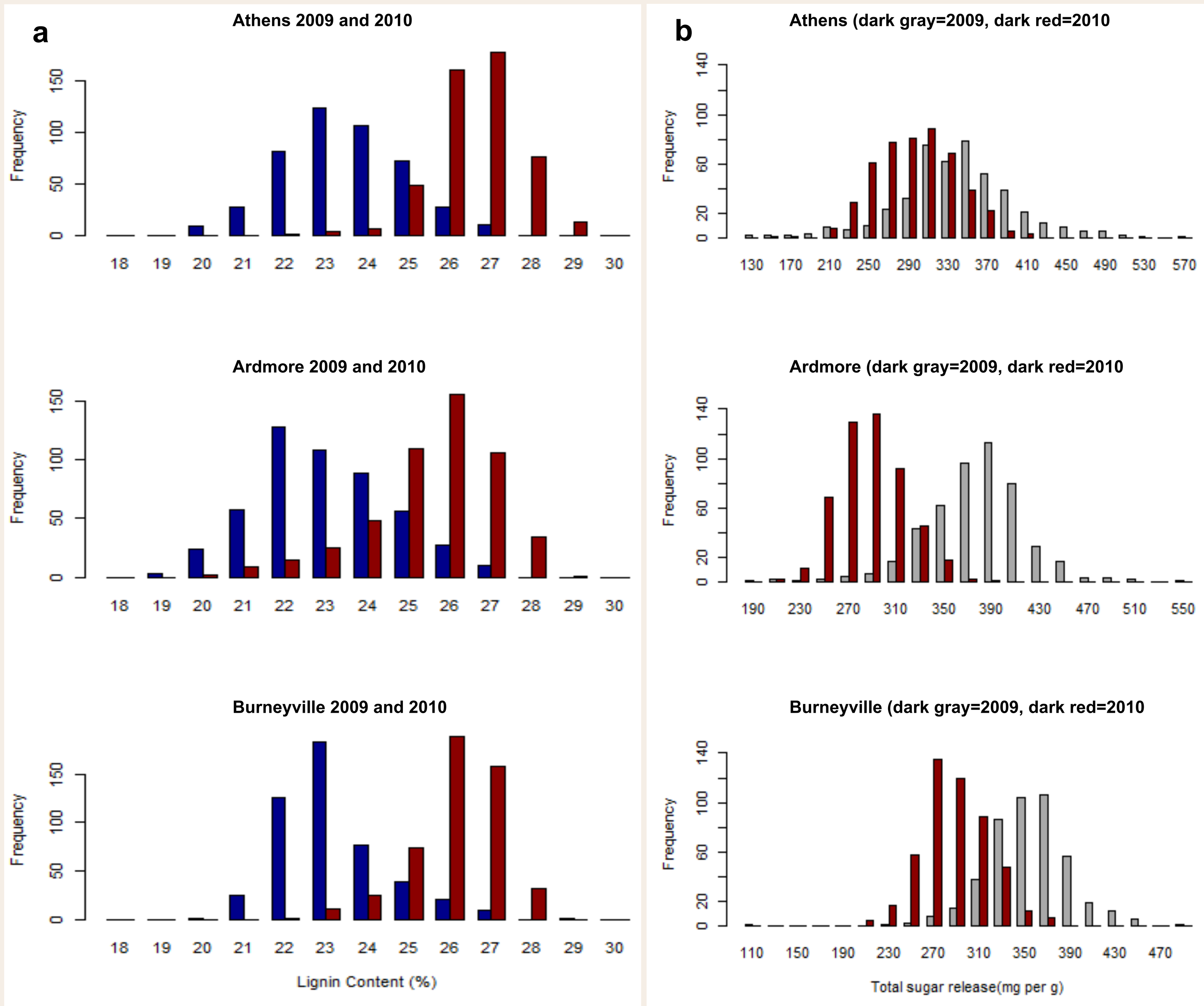

Figure 1. Frequency distribution of (a) lignin content and (b) total sugar release data over three locations and across two years (2009 and 2010)

Mean lignin content per plant ranged from 23.0 to 26.7 across six environments. Lignin content was consistently lower in 2009 than 2010. Age of the plant and/or environment may have influenced the plant composition. Mean glucose and xylose release from the biomass ranged from 140-172 and 145-210 mg g^{-1}, respectively. Total sugar release ranged from 287-383 mg g^{-1}. The mean total sugar and xylose yields were higher in 2009 than 2010 consistently across the three locations. The same is true for glucose yield except at Athens, where similar means were obtained both years. The higher lignin content in 2010 was coupled with a slight increase in S/G ratio (S: syringyl; G: guaiacyl lignin structures) (Table 1).

Table 1. Mean and range performance of AP13 x VS16 population for lignin and sugar release across environments

Statistics	Ardmore		Athens		Burneyville	
	2009	2010	2009	2010	2009	2010
			Lignin (%)			
Mean	23.0	25.6	23.5	26.7	23.2	26.3
Min	21.8	20.4	20.0	20.2	18.7	22.3
Max	29.1	29.3	27.4	28.7	27.5	28.5
Range	8.8	8.5	7.4	7.3	8.9	6.2
SD	1.6	1.5	1.4	1.0	1.3	1.0
			S/G ratio			
Mean	0.63	0.72	0.68	0.75	0.68	0.74
Min	0.62	0.50	0.50	0.60	0.50	0.63
Max	0.87	1.20	0.80	0.90	0.80	0.90
Range	0.30	0.30	0.30	0.25	0.70	0.27
SD	0.06	0.05	0.05	0.05	0.06	0.04
			Glucose (mg g^{-1})			
Mean	172	143	140	141	151	142
Min	64	90	50	99	80	94
Max	215	220	260	204	250	195
Range	170	105	210	151	180	101
SD	26.3	17.8	35.26	24.53	21.95	17.85
			Xylose (mg g^{-1})			
Mean	210	145	203	155	207	146
Min	93	70	80	111	110	109
Max	209	270	380	192	300	195
Range	190	81	300	116	200	86
SD	19.89	13.09	30.13	18.5	18.56	13.36
			Total sugar (mg g^{-1})			
Mean	383	288	344	296	359	287
Min	157	230	130	213	180	204
Max	419	490	570	396	550	378
Range	370	183	440	262	380	174
SD	41.9	27.97	60.81	41.18	37.07	28.82

significantly high differences were observed among genotypes, replications within location, years, and year×location interaction for all the traits (Table 2). The simple regression analysis of the lignin content on sugar release revealed that a unit increase in lignin content may reduce glucose, xylose and total sugar yield from the biomass by as much as 12, 8 and 18 units, respectively (Figure 2). The phenotypic variability explained by the regression analysis ranges from 2-24% for glucose, 4-41% for xylose and 3-40% for total sugar release.

Table 2. Test of fixed effects due to location and year; expected mean squares due to random effects of various sources for lignin content, S/G ratio and sugar release of the population across locations and years

Source	DF	Lignin (%)	S/G ratio	Glucose (mg g^{-1})	Xylose (mg g^{-1})	Total sugar (mg g^{-1})
		Test of fixed effects (F values)				
Year (Y)	1	3701.74**	1282.11**	170.83**	5684.62**	1980.58**
Location (L)	2	3.93	19.83*	4.11	0.2	1.32
Y×L	2	17.22**	26.64**	99.84**	47.67**	81.73**
		Expected mean squares				
Replication (L)	3	33.92**	0.0195**	16345.00**	7955.11**	46556.00**
Genotype (G)	254	3.17**	0.0086**	906.67**	505.68**	2304.61*
G×L	503	1.55†	0.0026*	574.65	374.93†	1616.65
G×Y	254	1.66*	0.026*	622.51	420.95	1789.86
G×Y×L	497	1.36	0.0021*	561.35	405.55**	1623.50†
Residual	1398	1.72	0.0019	534.09	337.58	1475.29

†; *, ** significant at 0.10, 0.05 and 0.01 levels

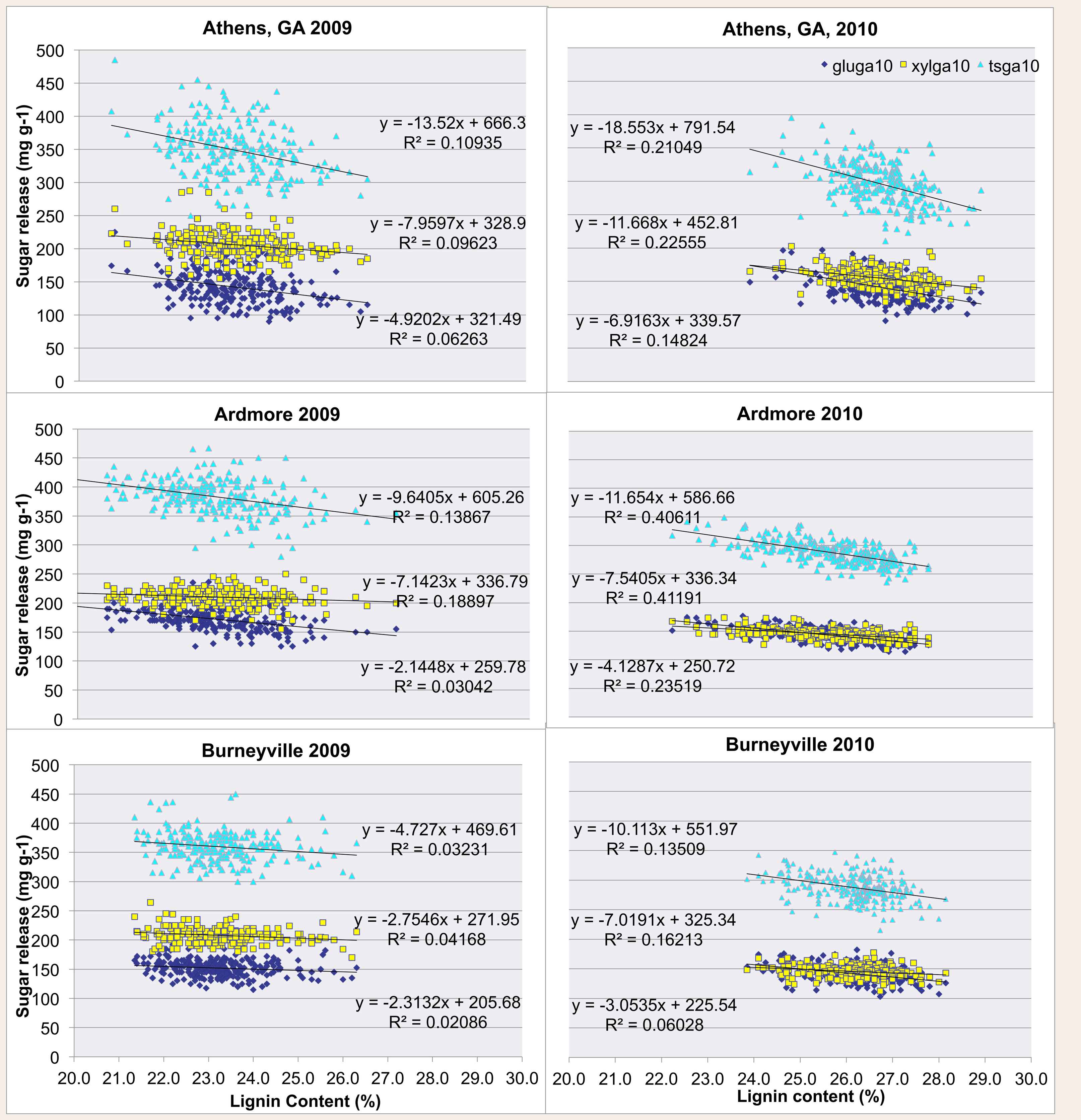

Figure 2. Effect of lignin content on sugar release in AP13 x VS16 Pseudo F_1 mapping population across locations and years

Conclusions

- The bi-parental population is highly variable for both lignin content and sugar yields from the biomass.
- Effect of environment is prevalent on cell wall composition traits as year and location variations were observed.
- The result indicates that sugar yield reduction up to 6% may occur with a unit increase in lignin content of the biomass.

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