Assessment of Bermudagrass as a Feedstock for Ethanol

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It is critical to determine appropriate dedicated bioenergy feedstock crops for Southern United States. Bermudagrass is considered a good candidate as a feedstock for the conversion of cellulose to ethanol because of its high yields and predominance as a forage and hay crop for the South. There have been significant genetic improvements of rumen digestibility along with increased yields in bermudagrass over the last half century. Preliminary results indicate that the conversion efficiency of bermudagrass to ethanol through simultaneous saccharification and fermentation (SSF) is higher than other grass feedstock including switchgrass. The objective of this study was to evaluate bermudagrass germplasm with variability in rumen digestibility for ethanol production through SSF and enzyme pretreatments.

Methods

Bermudagrass genotypes evaluated for this study were selected from a forage bermudagrass core collection (Anderson, 2005) based on high and low *in vitro* dry matter digestibility measurements (IVDMD). Eight-week old material was hand harvested from a field nursery on and May 4, 2006 at Tifton, GA. Samples were dried, ground to 1-mm particle size with a Wiley mill for analyses.

A low stringency conversion protocol was used to compare genotypes for ethanol production and was correlated with rumen digestibility, neutral detergent fiber, and acid detergent fiber data. Samples were pretreated with 1.75% w/v sulfuric acid (8.5 ml) and treated at 121°C for 1 hour and then inoculated with *Saccharomyces cerevisiae* D5A. Fermentations were sampled after 72 hr for ethanol and remaining sugars, which were measured by HPLC.

A select number of germplasm was also assessed for ethanol production using pretreatment enzymes (esterase and cellulase) followed by fermentation. Whole ground plant material (0.5g d wt per tube in triplicate) from bermudagrass samples were incubated with 1.0 g/tube (4393 IU/g) of Depol 740 L in buffer essentially and then incubated with similarly buffered cellulase (Sigma C-8546) at 400 IU/tube for 72 h. *Escherichia coli* LY01 was added in the fermentation culture to a final optical density (550 nm, OD) of 1.0.

Results

All but two bermudagrass genotypes produced more ethanol with SSF than the switchgrass checks (Figure 1). A great deal of variability is present for ability to convert to ethanol and xylans (Figure 2). The pentose sugars are a further source of ethanol with other fermenting agents. Using a conversion rate of 0.51 mg ethanol/mg of xylan, PI 291614 (259.9 mg), Tifton 85 (252.9 mg) and Tifton 84 (250.1 mg) have the highest potential total ethanol yields and substantially higher than switchgrass (212.5 mg). Ethanol yields and xylan sugars were only moderately correlated with *in vitro* dry matter digestibility and fiber data (Table 1). Using esterase and cellulase pretreatments, followed by fermentation yields of the highly digestible Tifton 85 and Coastcross II were much higher (Table 2).

Figure 1: Ethanol produced (mg/g dry matter) from dilute acid pretreatment and SSF of 50 bermudagrass accessions.

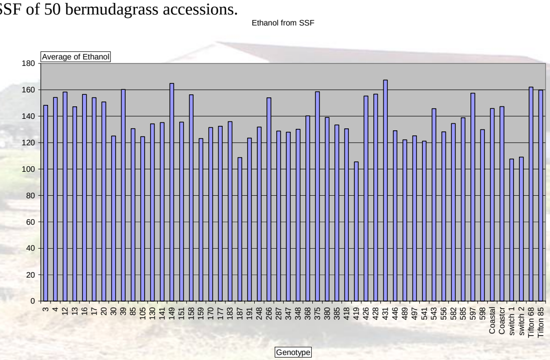
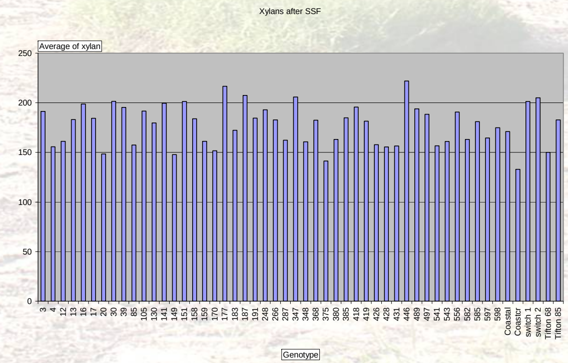


Figure 2: Xylose and arabinose sugar amounts (mg/g dry matter) following dilute acid hydrolysis and SSF of 50 bermudagrass accessions.



References

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Table 1: Pearson correlation coefficients for rumen digestibility (IVDMD), fiber, ethanol and xylans of 50 bermudagrass genotypes.

	IVDMD	NDF	ADF	ADL	Ethanol
Ethanol	0.49**	-0.45**	-0.14	-0.32	
Xylans	-0.67**	0.53**	0.29	0.08	-0.41**
Potential Total Yield	0.02	-0.09	0.07	-0.28	0.75**

** Significant at the p= 0.01 probability level.

Table 2: Percent dry weight (DW) loss, ferulic acid, *para*-coumaric acid and free sugars released in filtrate after pretreatments with commercial esterase and cellulase and subsequent ethanol yields from fermentations for bermudagrass at 4 weeks@

Genotype	% DW loss	Ferulic Acid (mg/g)	P-Coumaric Acid (mg/g)	Xylose (mg/g)	Glucose (mg/g)	Ethanol yields (mg/g)
Coastal	33.1 de	0.44 c	0.31 c	4.7 b	84.0 b	248 b
Tifton 85	41.8 b	0.64 a	0.46 a	9.2 a	112.2 a	276 a
Tifton 44	32.2 e	0.51 b	0.37 b	5.5 b	78.5 b	246 b
C II	38.5 c	0.44 c	0.30 c	6.1 b	112.9 a	282 a

@Values are the sum of subsequent incubations with esterase for 24 h and then cellulase for 72 h.

Conclusions

1. Bermudagrass has excellent quality for conversion to ethanol via fermentation
2. Variability exists within the germplasm to improve feedstock quality through breeding
3. Correlation of ethanol production with IVDMD or fiber is not adequate for indirect selection.
4. Enzymatic pretreatments can enhance potential conversion to ethanol