USDA - ARS - National Center for Agricultural Utilization Research

Conversion of Lower Lignin Mutants of *Sorghum bicolor* to Ethanol

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Fermentation Biotechnology Research

Bioenergy – II: Fuels and Chemicals from Renewable Resources
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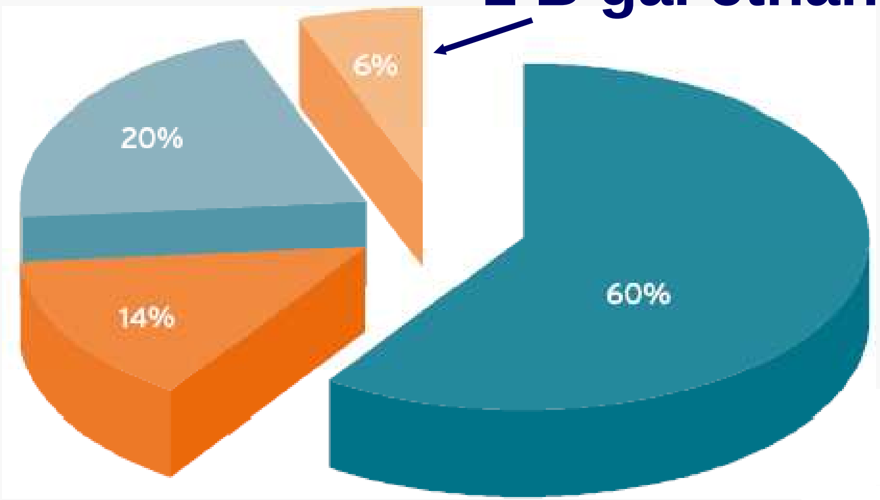
Growing use of corn for ethanol

*Increases in ethanol balanced by increases in crop yields.

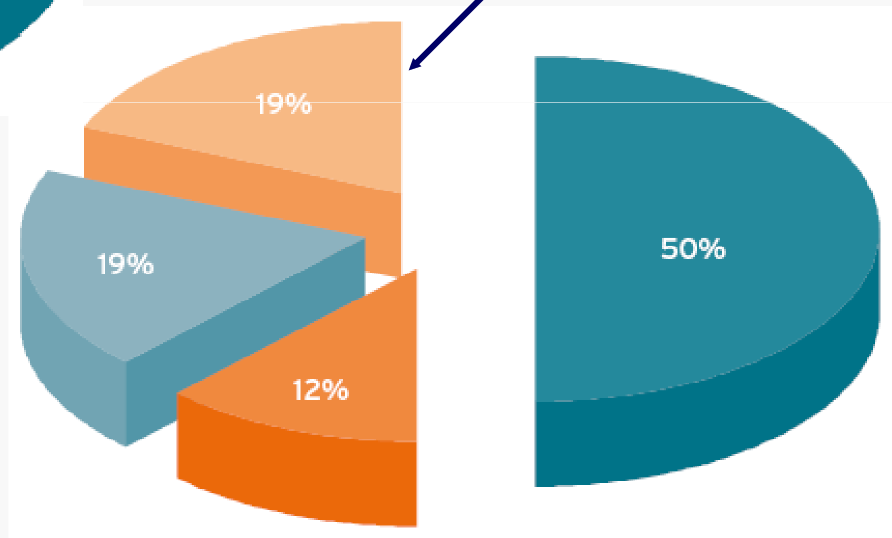
*Only 1% of global arable land used for biofuels crops (2006).

1 B gal ethanol

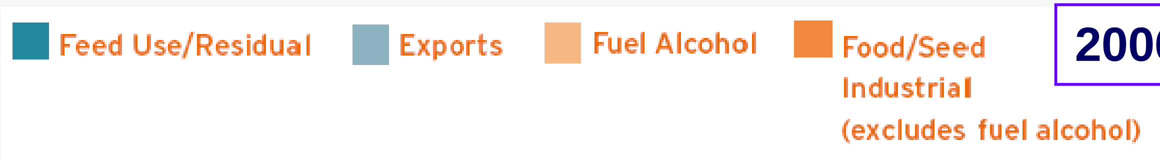


1990/1991 Crop Year

6.5 B gal ethanol



2006/2007 Crop Year



What Grain Alcohol Can Do

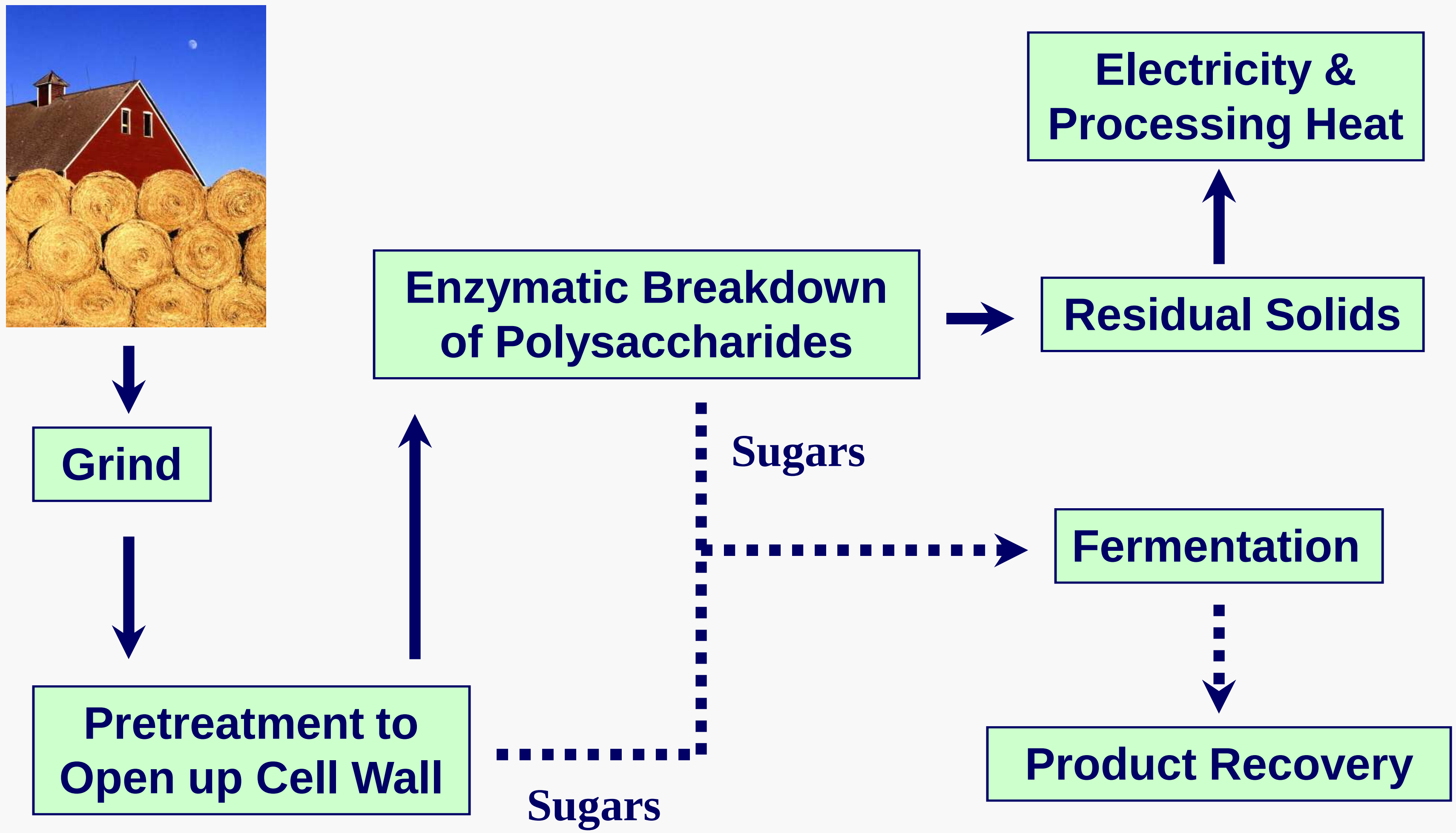
Year	Amt. Ethanol	% of Harvest
2006	5 B	19%
2015	15 B	38%

Data: NCGA & CFA

Herbaceous energy crops can be part of the solution

- Enough energy crops can be grown in US to produce 35+ billion gal/yr of ethanol
- Can be cultivated on marginal farming land, so, no conflict with food production
- Equal to corn ethanol in substituting for oil and uses less natural gas (0.08 BTU/BTU oil & 002 BTU/BTU gas)
- More effective at reducing emissions of green house gases (12% vs. 83%)
- Perennials may add to soil quality and serve as wildlife refuges

Cellulosic Biomass to Fermentable Sugars



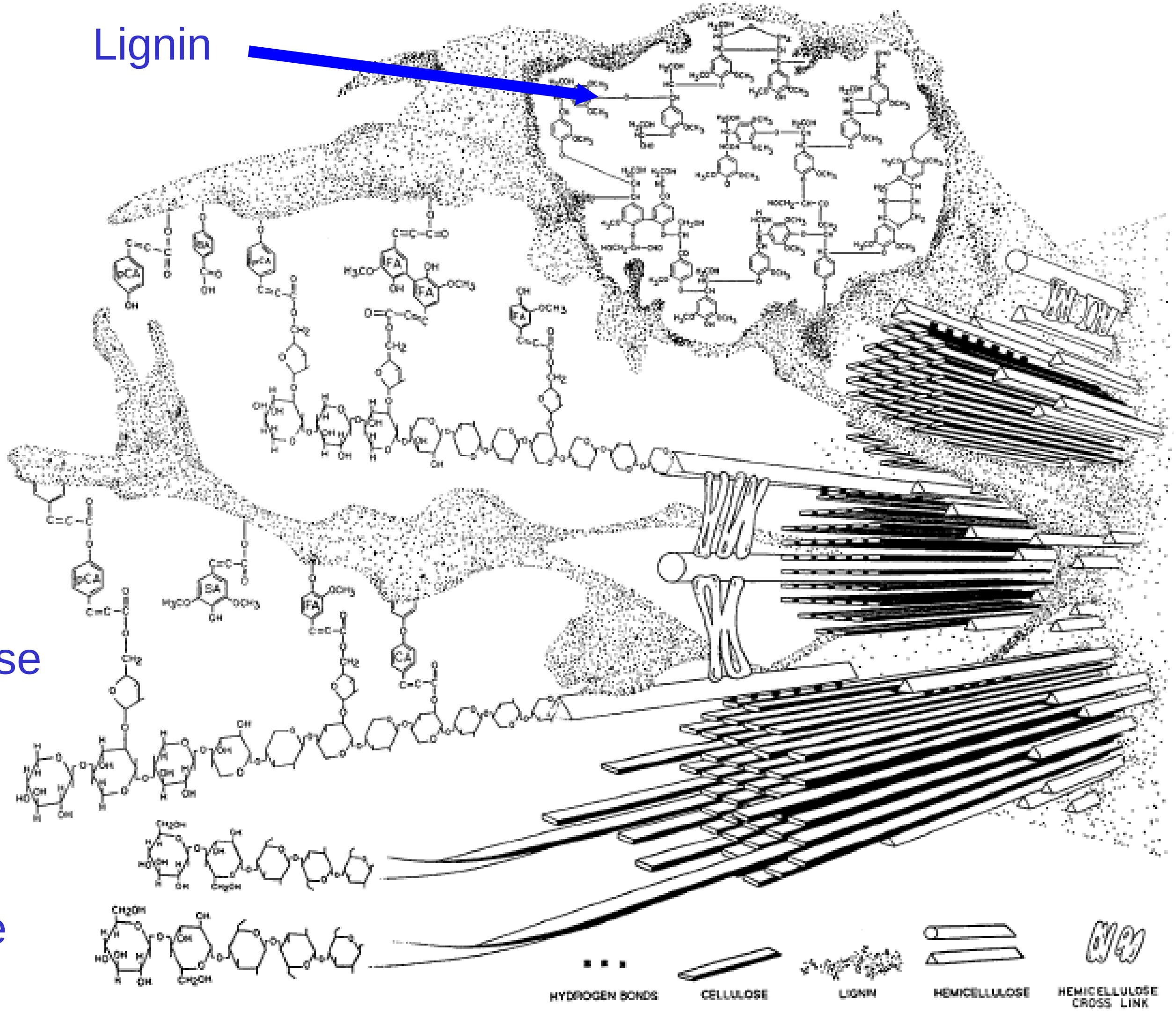
Courtesy of Hans Jung

Grass secondary cell wall model

Lignin

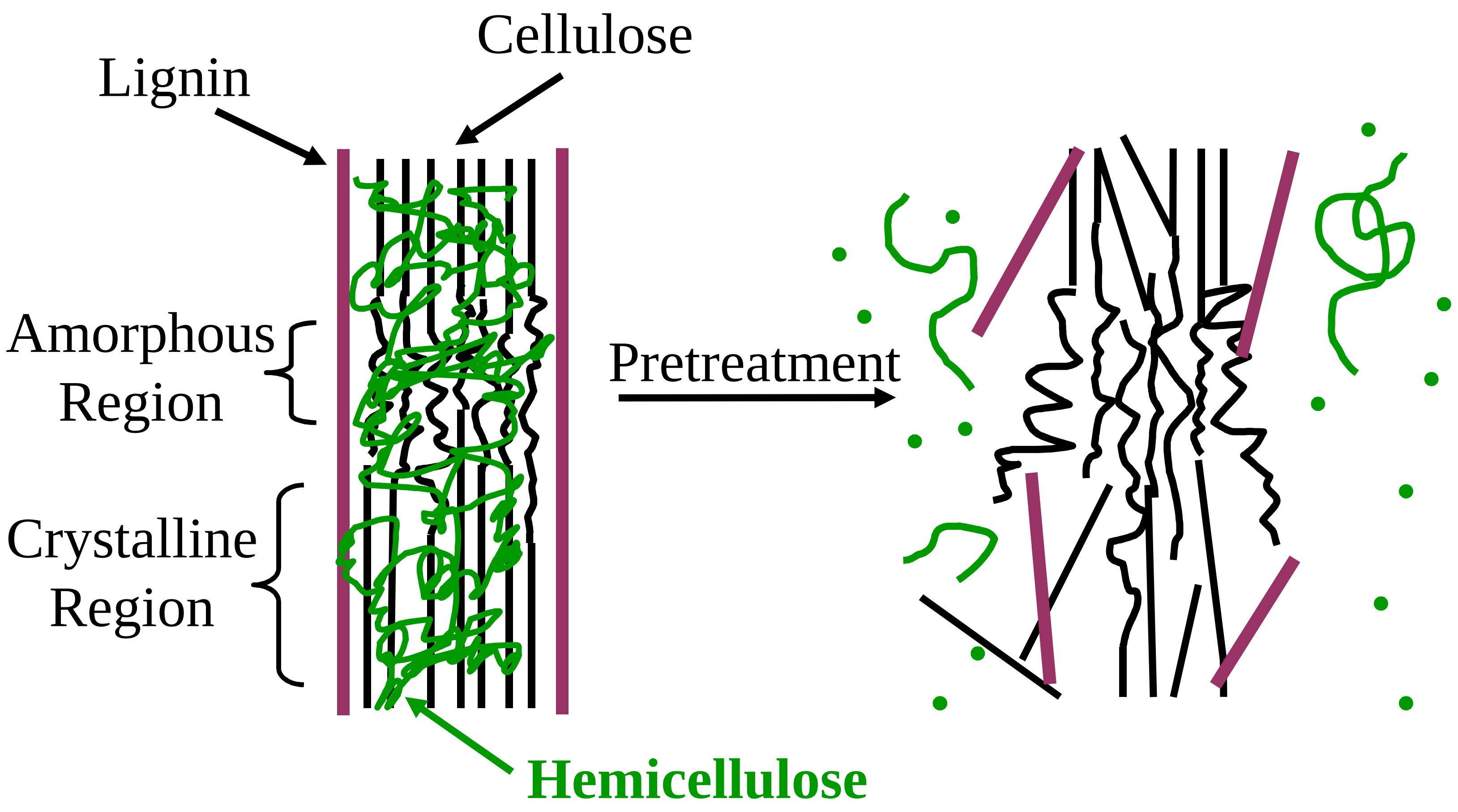
Hemicellulose

Cellulose



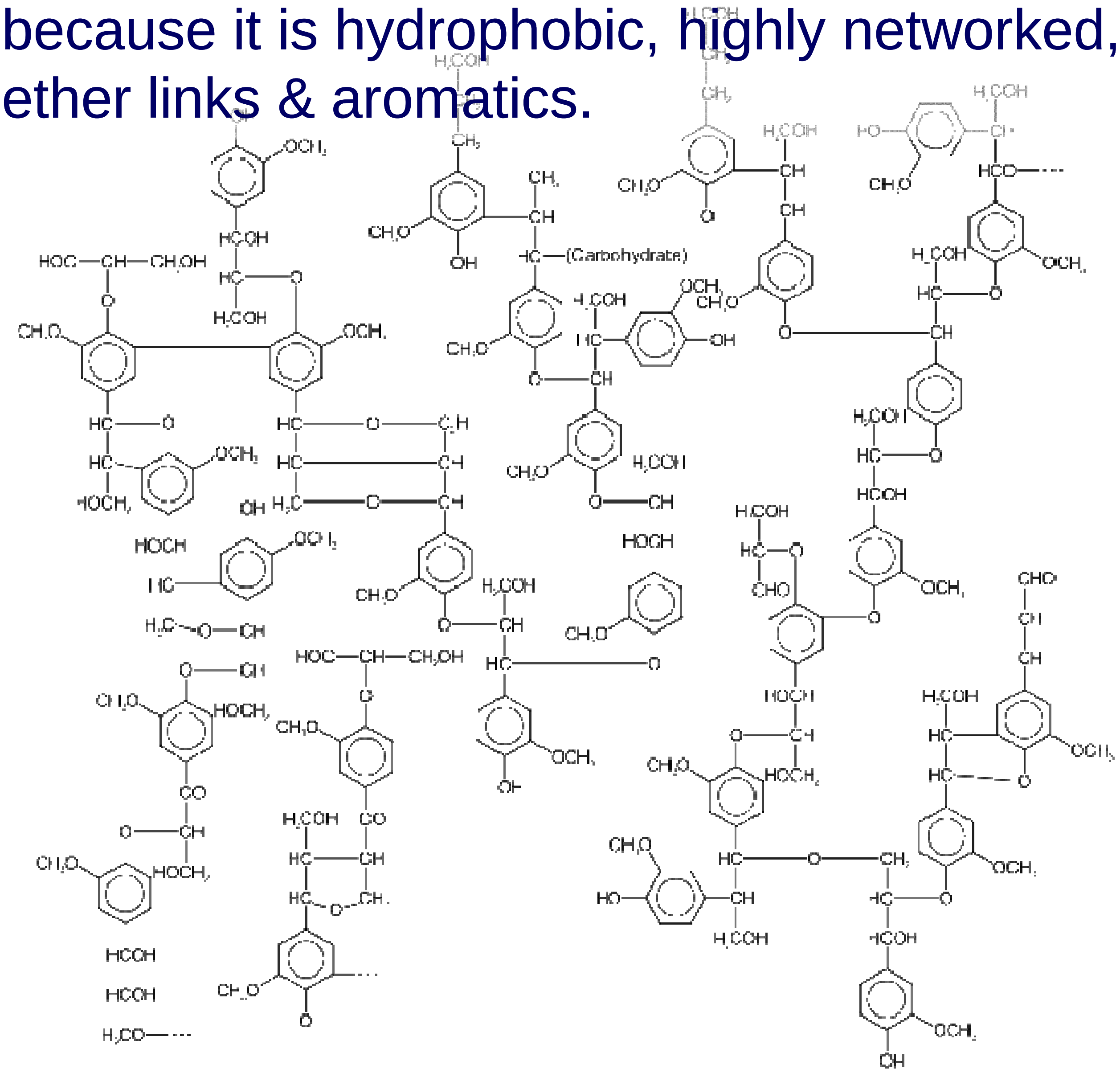
Proc. Okla. Acad. Sci. 72:51-56 (1992)

Ethanol yield largely determined by accessibility of cellulases to cellulose



(adapted from Mosier)

Lignin is particularly hard to remove as a barrier because it is hydrophobic, highly networked, and has ether links & aromatics.



Source: Glazer, A. W., and Nikaido, H. (1995). Microbial Biotechnology. New York: W. H. Freeman, p. 340. (Wikipedia)

Source of reduced lignin biomass for this study

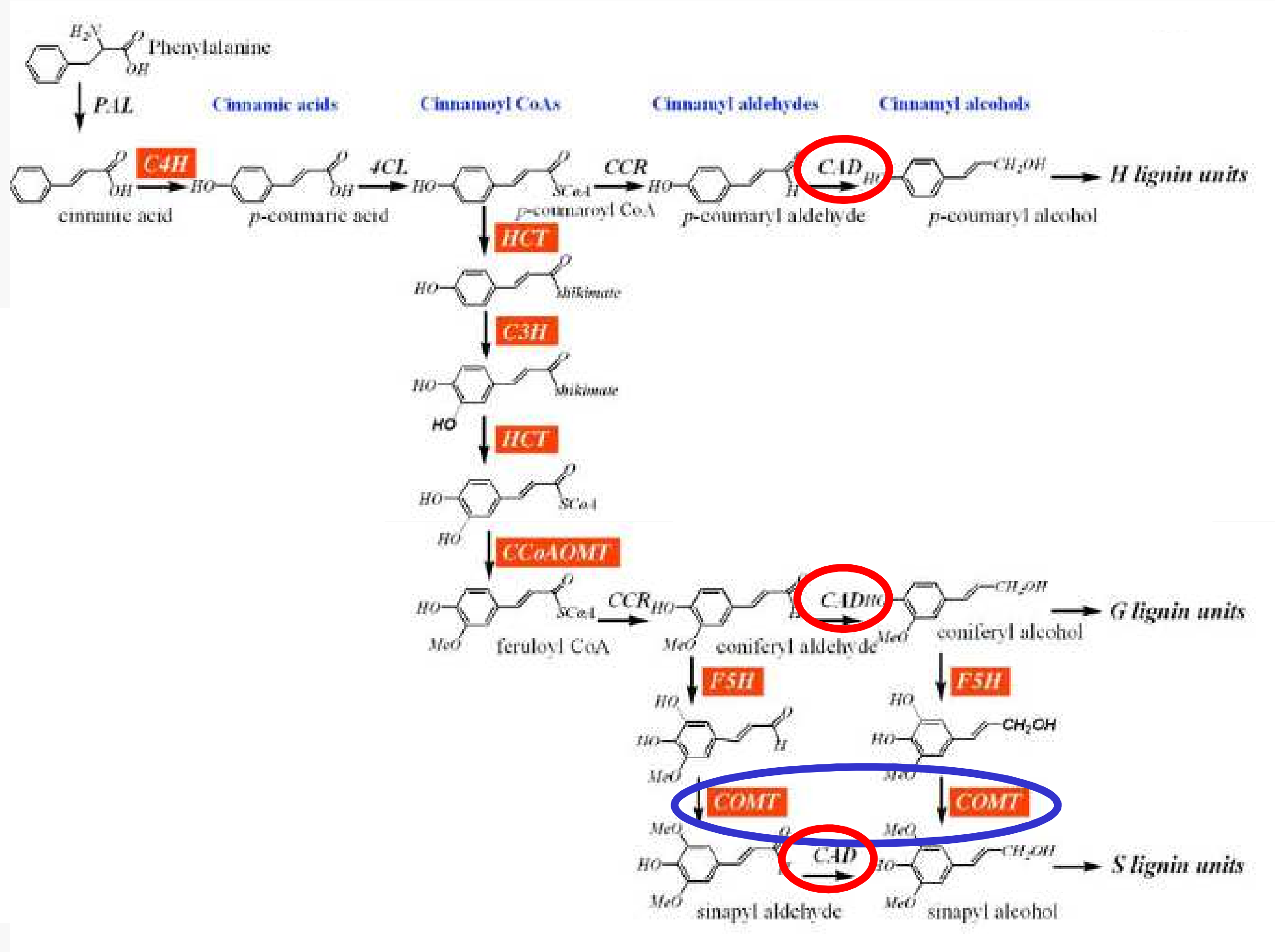
CROP SCIENCE, VOL. 46, JANUARY–FEBRUARY 2006

Registration of Seven Forage Sorghum Genetic Stocks Near-Isogenic for the Brown Midrib Genes *bmr*-6 and *bmr*-12

Seven forage sorghum [*Sorghum bicolor* (L.) Moench] genetic stocks, N592 to N598, (Reg. no. GS-121–GS-127, PI639702–PI639708) near-isogenic to their wild-type counterparts for the brown midrib genes *bmr*-6 and *bmr*-12 were developed jointly by the USDA-ARS and the Agricultural Research Division, Institute of Agriculture and Natural Resources, University of Nebraska, and were released in January 2005.

J.F. PEDERSEN,* D.L. FUNNELL, J.J. TOY,
A.L. OLIVER, AND R.J. GRANT

Lignin Synthesis Pathway in Plants



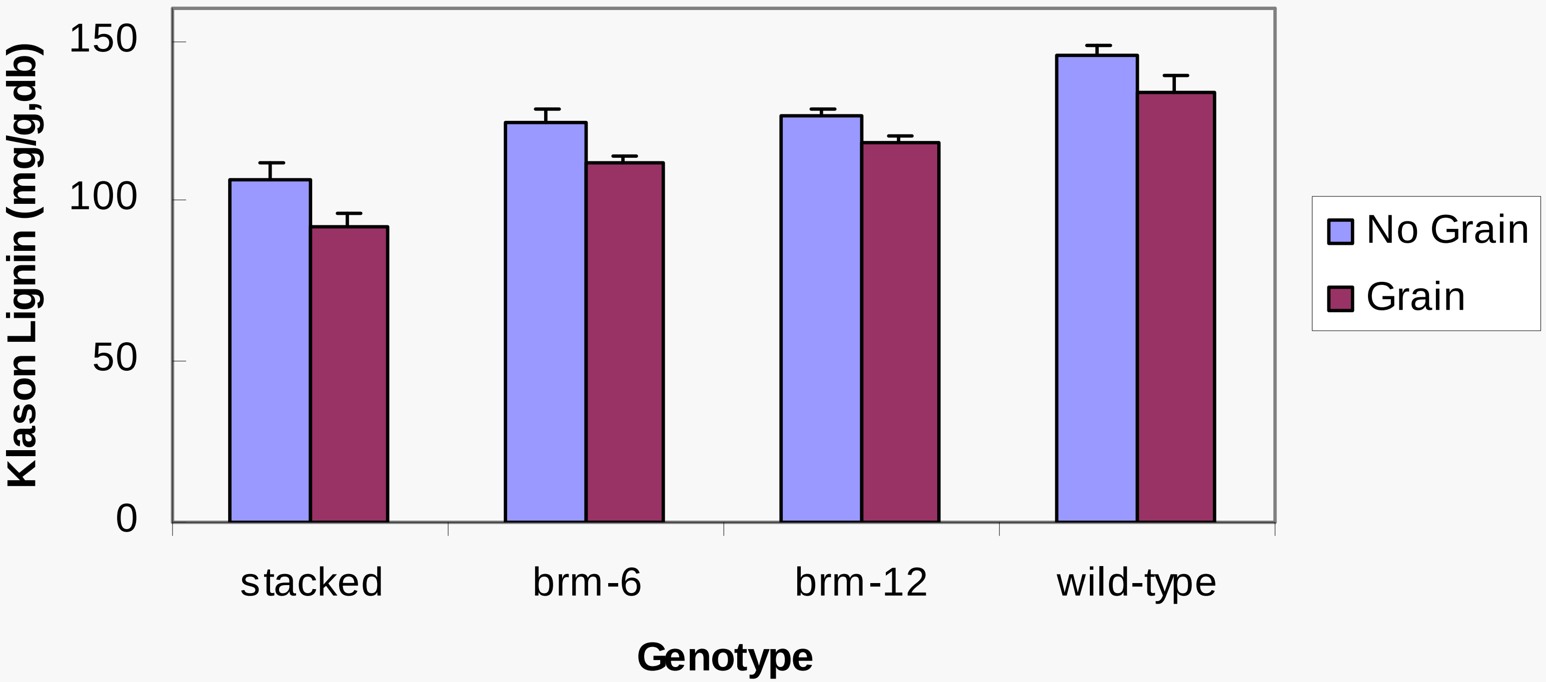
BMR6 = reduced cinnamyl alcohol dehydrogenase (CAD)
BMR12 = reduced caffeate O-methyltransferase (COMT)

(Chen and Dixon, 2007)

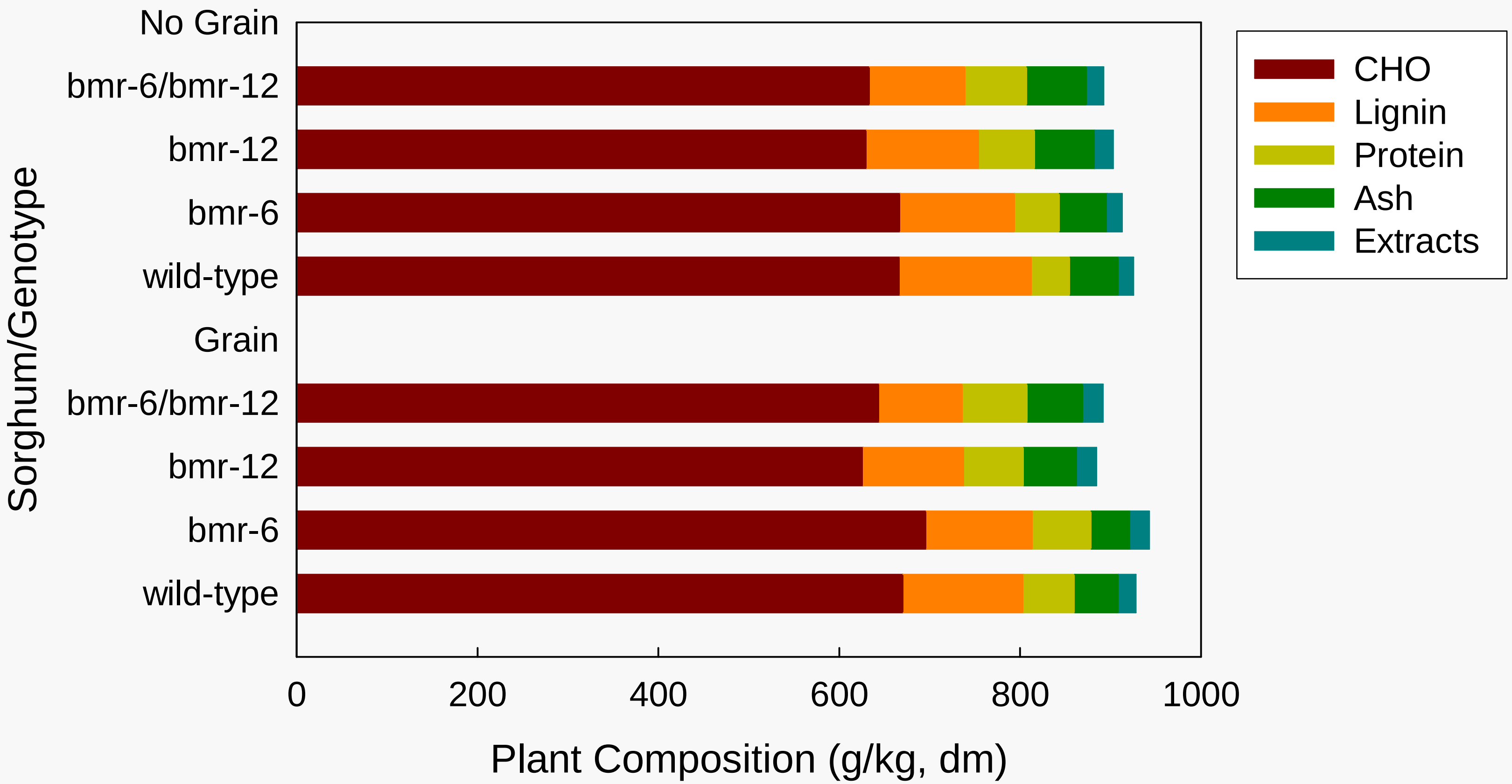
Comparison of lignin contents (%w/w, db)

Genotype	Klason Lignin	ADL
	Sorghum with grain removed	
bmr-12/bmr-6	10.6	1.09
bmr-6	12.4	2.16
bmr-12	12.7	2.03
Wild type	14.6	2.92
	Whole sorghum plant	
bmr-12/bmr-6	9.24	0.79
bmr-6	11.2	1.89
bmr-12	11.8	1.89
Wild type	13.3	2.80

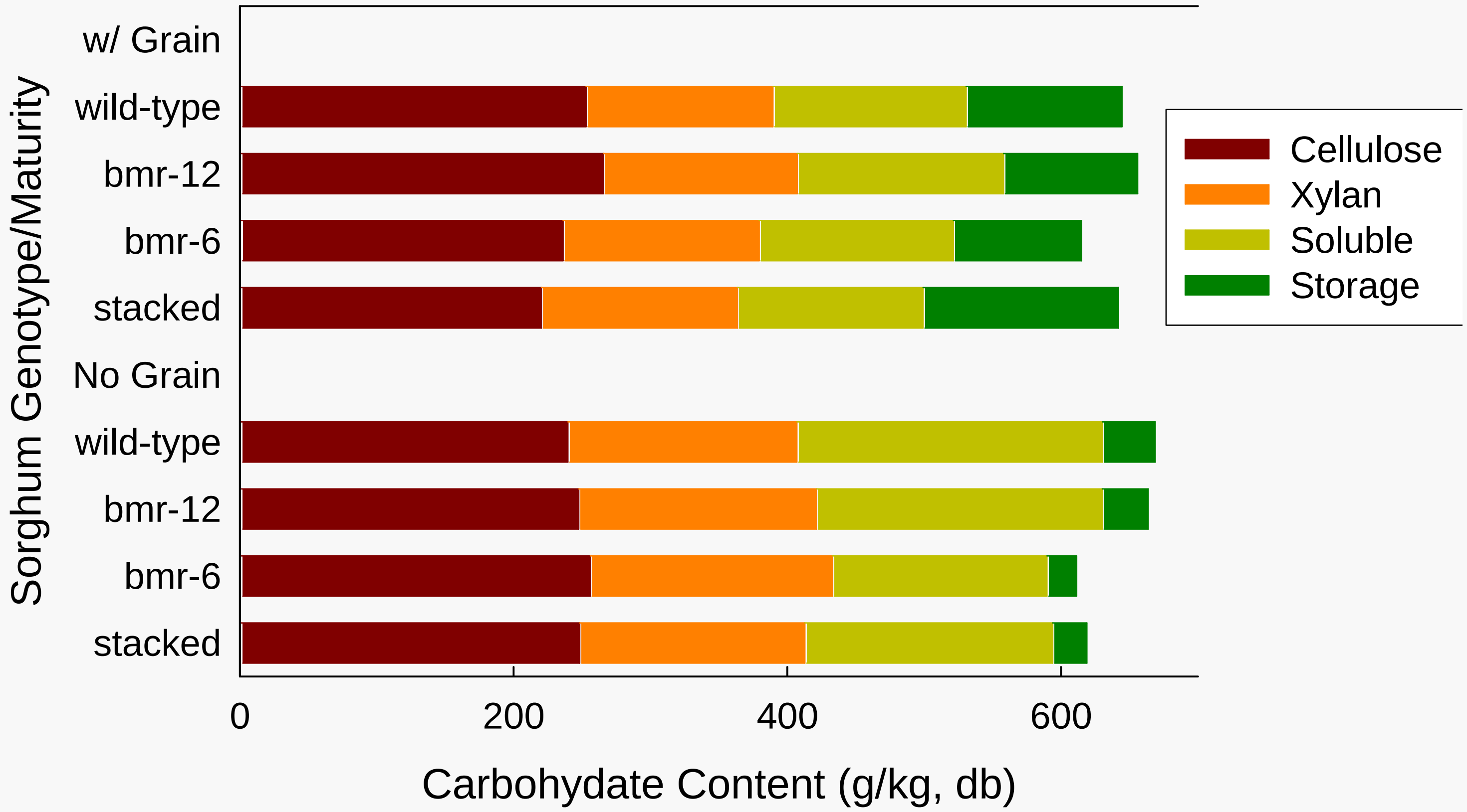
Differences in lignin are significant



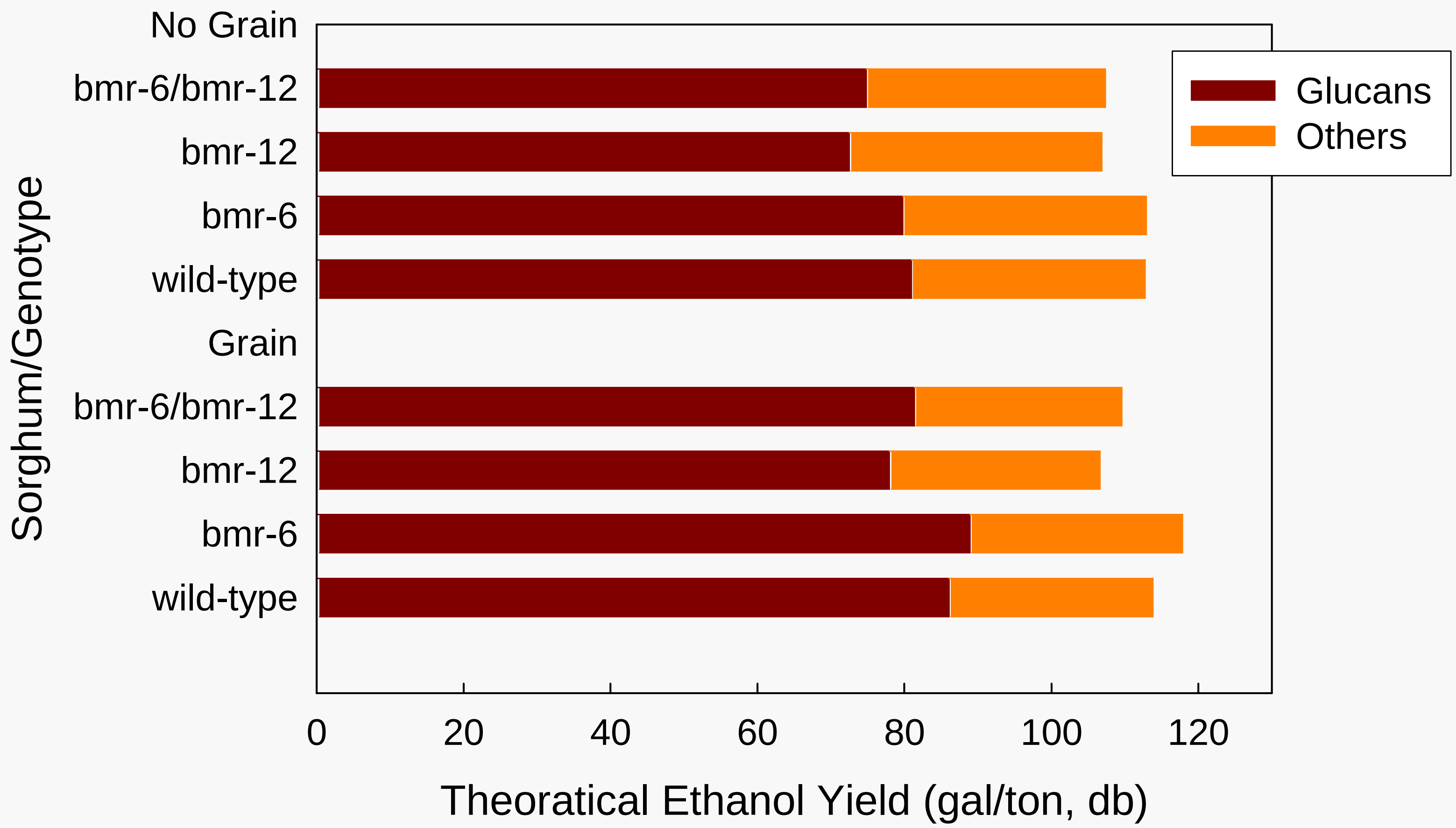
Total Composition



Genotype does not influence carbohydrate contents



Theoretical Ethanol Yield



Digestion Assay

Pretreatment

pH = 1
Temp = 121ºC
Time = 1 hr

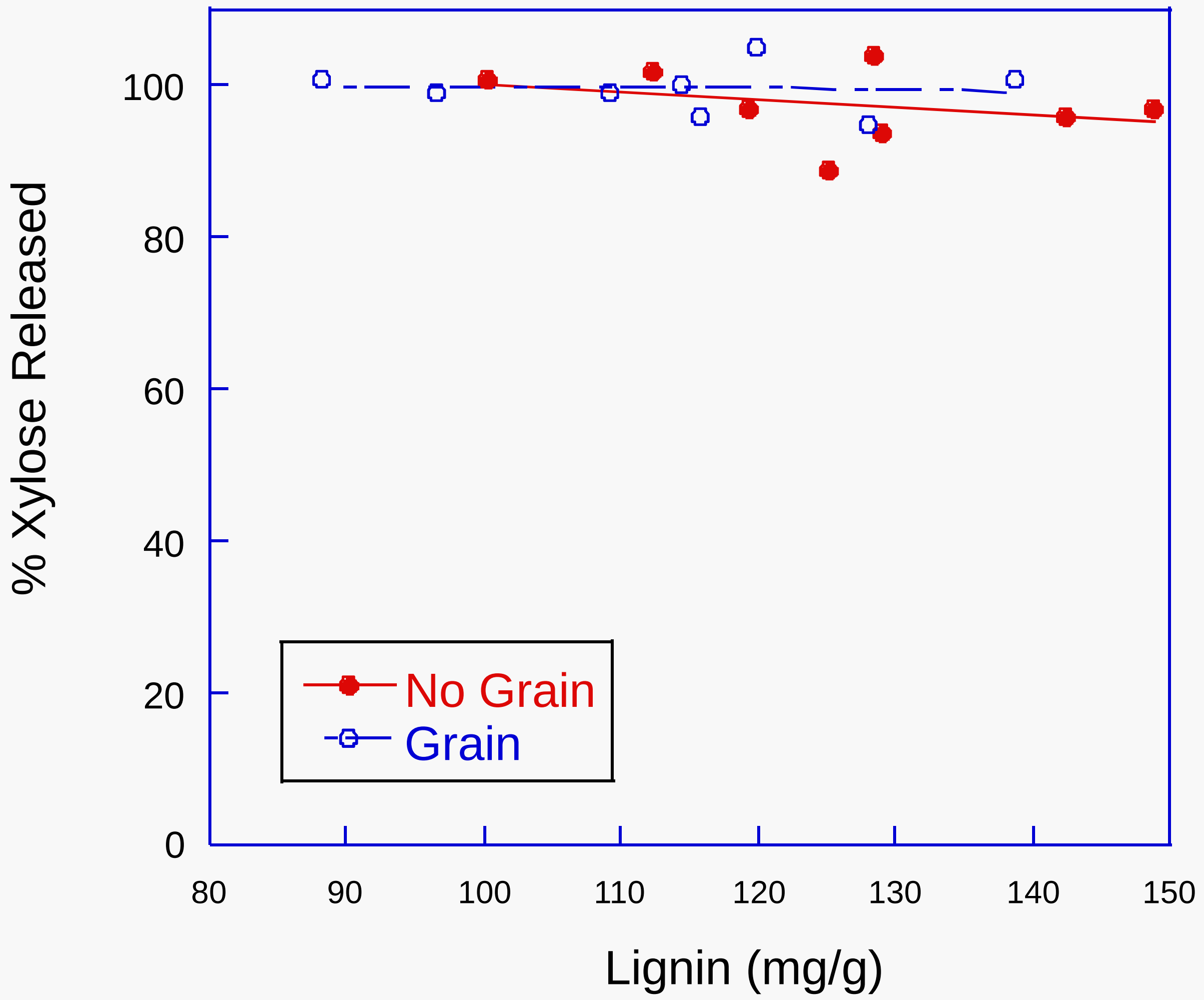
Digestion Assay

Cellulase: 50 FPU/g glucan + 40 U of beta-glucosidase/g glucan
Temperature = 50ºC, pH = 4.8, & 100 rpm
Time = 72 hr

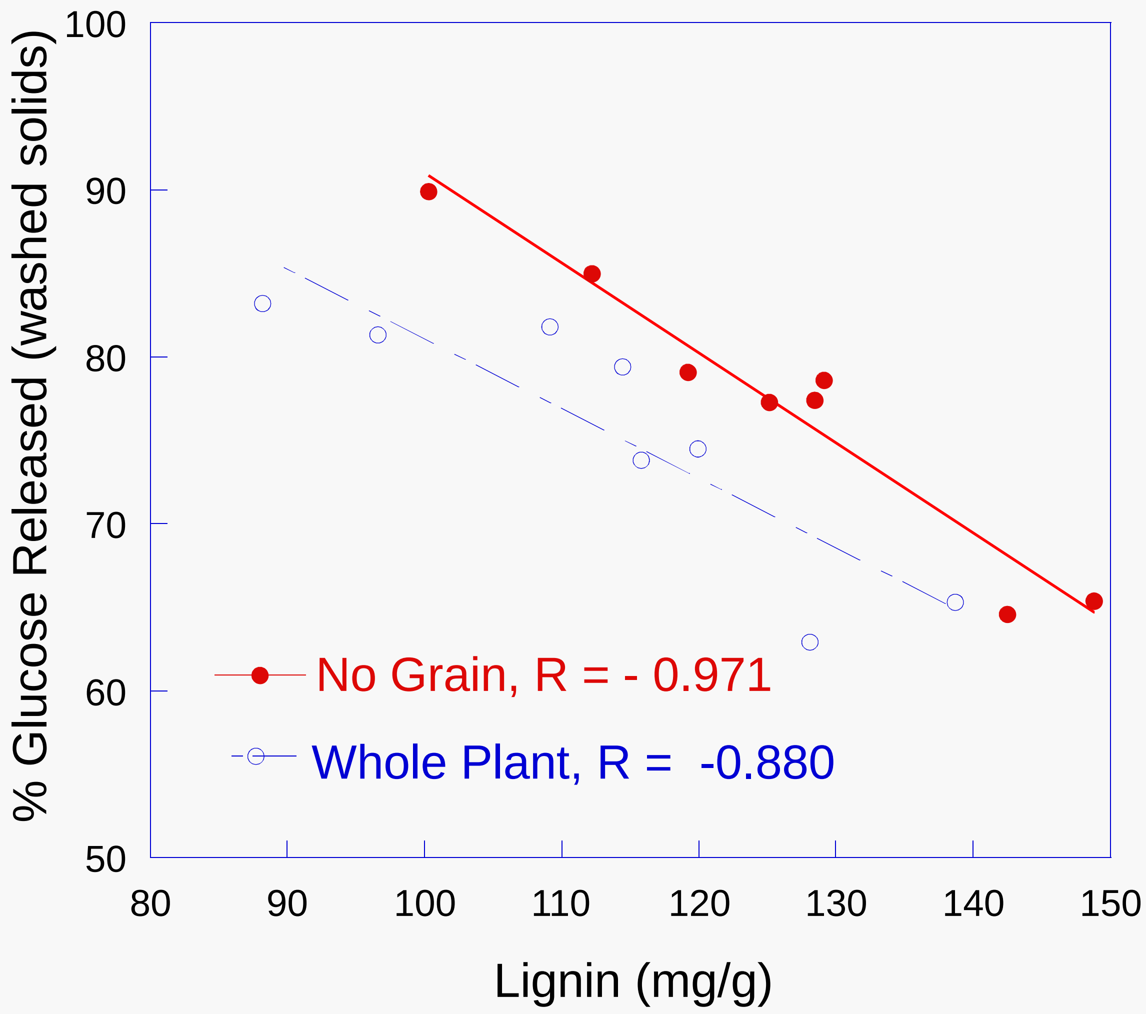
Analysis

Glucose
Other sugars (e.g. Arabinose, galactose, fructose, and xylose)

Lignin does not effect acid hydrolysis of xylan



Negative effect of lignin on glucose yield from cellulose



▪ Ethanol Fermentation Assay

Pretreatment conditions

pH = 1
Temp = 121°C
Time = 60 min
Neutralization: Calcium hydroxide until pH 5.0

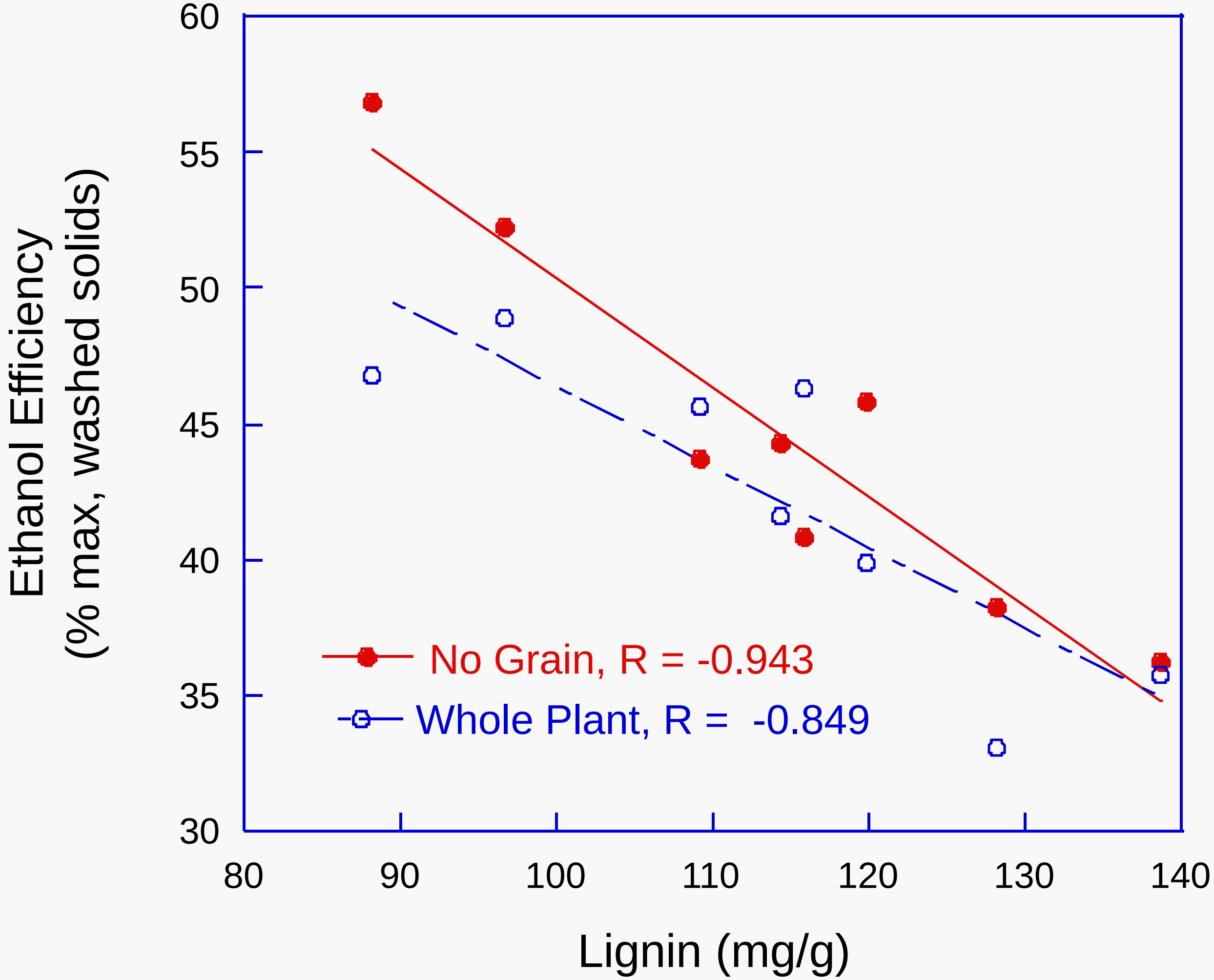
Simultaneous Saccharification & Fermentation

Biocatalyst = *Saccharomyces cerevisiae*
Cellulase: 5 FPU/g (db) + 12 U/g of beta-glucosidase
Temperature = 35°C & pH = 4.5
Time = 72 hr

Analysis

Ethanol
Nonfermentable Sugars (e.g. arabinose and xylose)

Lignin reduces ethanol yield from cellulose



Ammonium hydroxide pretreatment

Pretreatment conditions

Ammonium hydroxide: 4%

Temp = 170°C

Time = 20 min

Ammonia Removal: evaporated 48 hr at ambient temp

Simultaneous Saccharification & Fermentation

Biocatalyst = *Saccharomyces cerevisiae* D5A

Cellulase: 5 FPU/g (db) + 12 U/g of beta-glucosidase

Temperature = 35°C & pH = 4.5

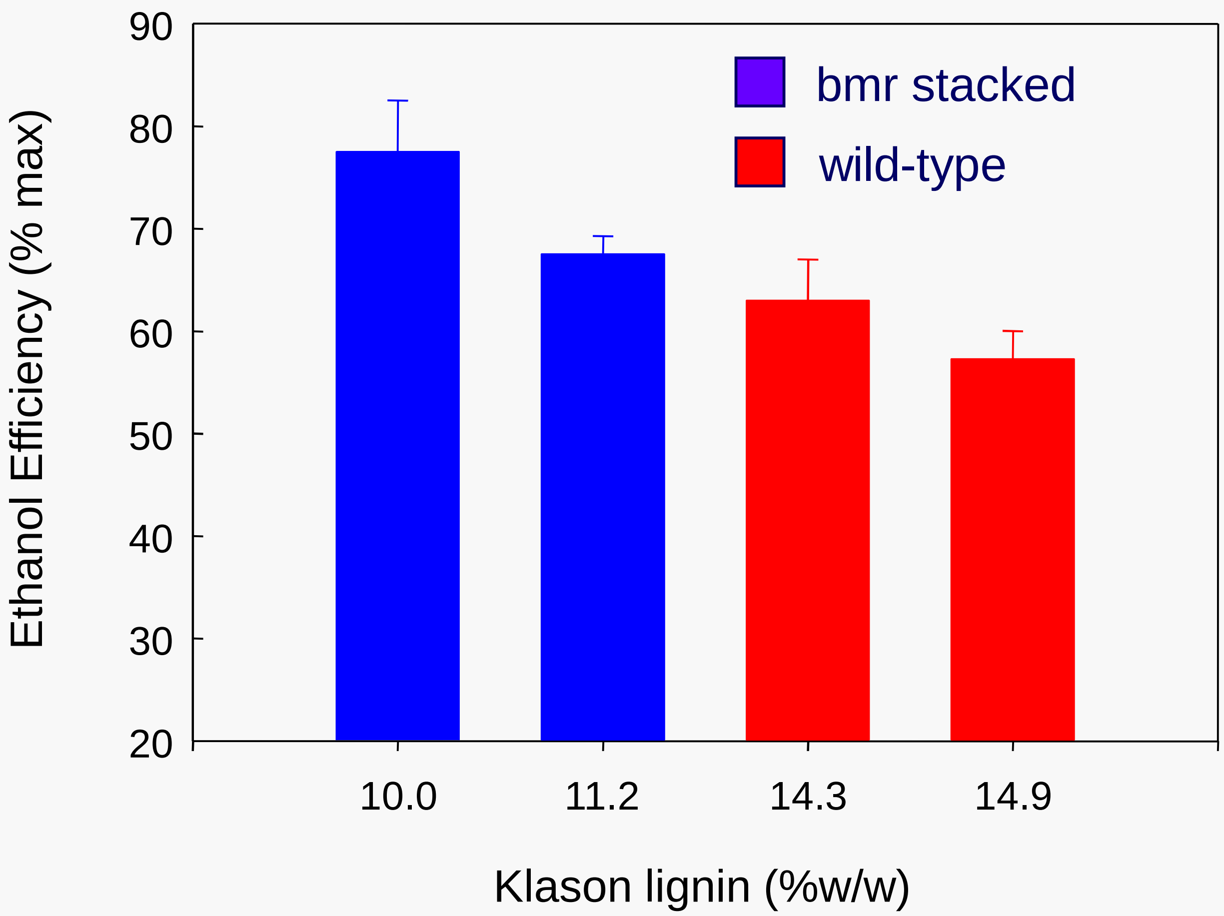
Time = 72 hr

Analysis

Ethanol

Nonfermentable Sugars (e.g. arabinose and xylose)

SSF of ammonium hydroxide pretreated stacked & wild-type sorghum samples



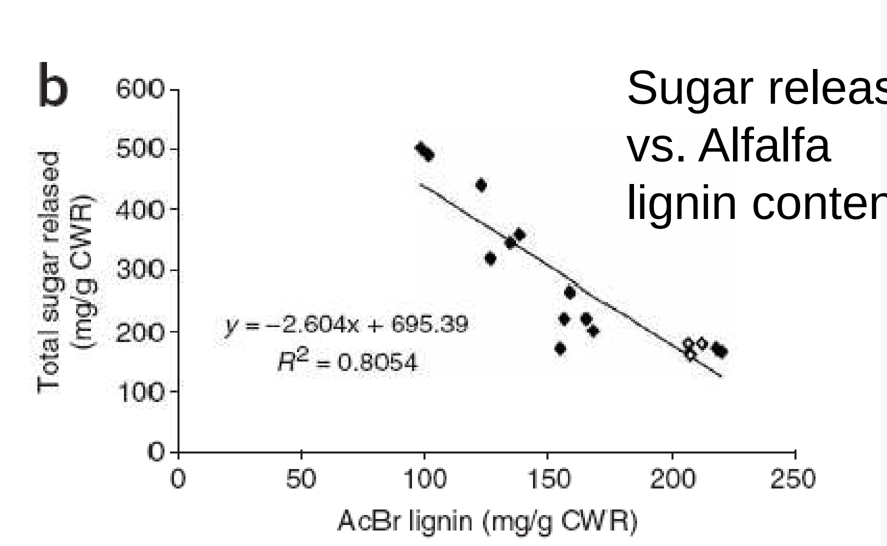
Comparison of ethanol and feed yields

Genotype	Lignin (%wt/)	Ethanol Eff[1] (% of max)	IVDMD[2] (% of max)
Stacked	10.6	54	86
bmr-6	12.4	44	78
bmr-12	12.7	43	82
Wild-type	14.6	37	75

[1]B.S. Dien, G.S. Sarath, J.F. Pedersen, D.L. Funnell, S. Sattler, J.J. Toy, & N.N. Nichols
[2]H. M. Dann, A. M. DiCerbo, J. F. Pedersen, and R. J. Grant (estimated from graph)

Prior reported results

- Determined that two bmr mutants in sweet sorghum improved enzymatic saccharificaiton by 30-60%. AI Saballos, W Vermerris, and G Ejeta. (2007). Development of Brown Midrib Sweet Sorghum as a Dual-Source Feedstock for Ethanol Production. Abstract
- Strong correlation found for enzymatic sugar yield vs. lignin in alfalfa engineered for reduced lignin. F Chen & RA Dixon. (2007) Lignin modification improves fermentable sugar yields for biofuel production.



Sugar release vs. Alfalfa lignin content

- Numerous studies detail advantages of *bmr* mutations for increasing forage digestibility & bmr sorghum seed is produced commercially. One example: AL Oliver, JF Pedersen, RJ Grant, & TJ Klopfenstein. (2005) Comparative Effects of the Sorghum bmr-6 and bmr-12 Genes: I. Forage Sorghum Yield and Quality.

Summary

- Chemical plant composition for near-isogenic sorghum lines carrying *bmr* were similar, except for lignin content.

- Glucose and ethanol yields for sorghum biomass samples pretreated with low severity dilute-acid were negatively correlated with Klason lignin content and differences in ethanol conversion efficiencies ranged over 20%.

- Lower lignin mutants also showed improved ethanol conversion efficiencies when pretreated using a higher temperature ammonium hydroxide pretreatment – the maximum efficiency for glucan conversion was 77%.

Acknowledgments

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