Reactivity of Forage Sorghums:

- **Wild vs. *bmr* mutant lines**
- **Dilute Acid Pretreatment vs. Deacetylation-Dilute Acid Pretreatment**

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Aims of the Study

- Use a laboratory-scale Pretreatment/Enzymatic Hydrolysis assay to compare the ***reactivity*** of near-isogenic ***bmr*** sorghum mutants to their wild type
- Identify Dilute Acid (DA) Pretreatment conditions that maximize reactivity
- Compare the effect on reactivity of Deacetylation before Dilute Acid Pretreatment (DDA)

What is Reactivity?

- The combined yield of glucose and xylose after PT/EH
- A proxy for suitability for cellulosic biofuel productions

Sorghum: a versatile bioenergy crop

forage/energy

biomass

sweet

sugars & biomass

grain

starch

- **C4 photosynthesis/Water Use Efficiency**
- **Wide Adaptation/High Yield Potential**
- **Drought Tolerance/Pest Resistance**
- **Existing Agricultural Infrastructure**

brown midrib to increase cell wall digestibility

Cell wall lignification is a major factor reducing digestibility

- ***brown midrib* (*bmr)* mutants have reduced lignin levels**
- ***bmr* mutants have been used for improving forage digestibility**
- ***bmr6* gene encodes the major cinnamyl alcohol dehydrogenase (CAD) of sorghum involved in monolignol synthesis.**
- ***bmr12* gene encodes the major caffeic *O*-methyltransferase (COMT) of sorghum involved in monolignol synthesis.**

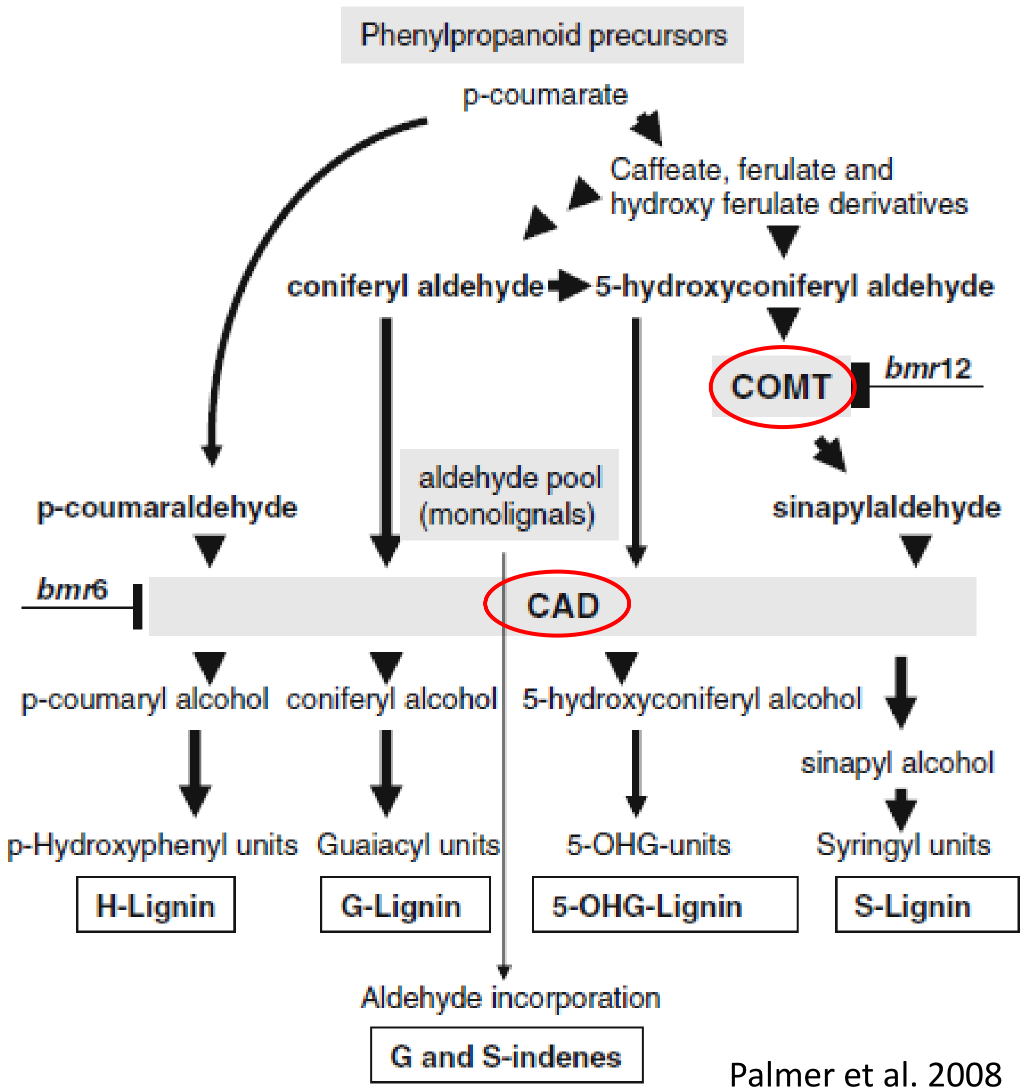
Pedersen JF, Vogel KP, Funnell DL (2005) Crop Science 45: 812-819

What are *bmr* mutants?

Brown midrib *(bmr)*

- Named after the phenotype
- Mutations in genes controlling lignin synthesis
- Feedstocks tend to have reduced lignin content and increased reactivity
- We studied three mutants and wild type in this study
  - *bmr12*- COMT
  - *bmr6* - CAD
  - stacked *bmr6+bmr12* mutant

Generalized scheme of lignin biosynthesis indicating points of sorghum *bmr* mutations

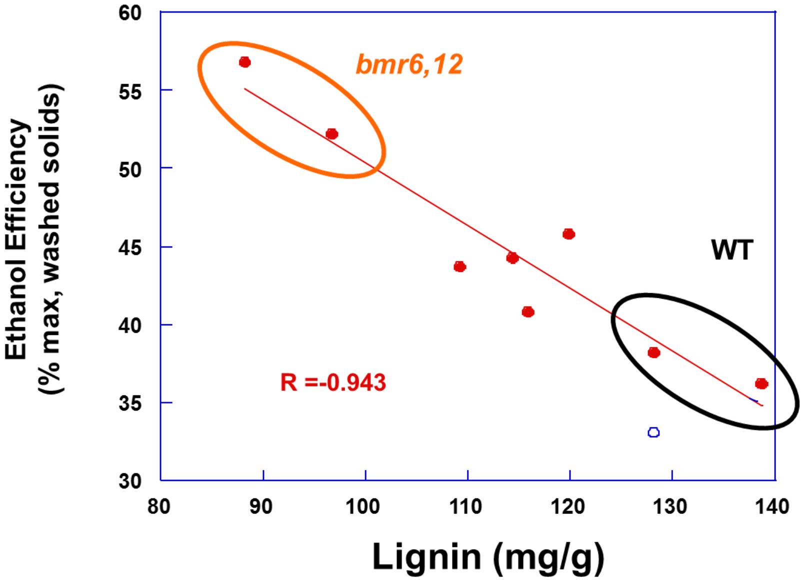


Palmer et al. 2008

Previous Work/Context

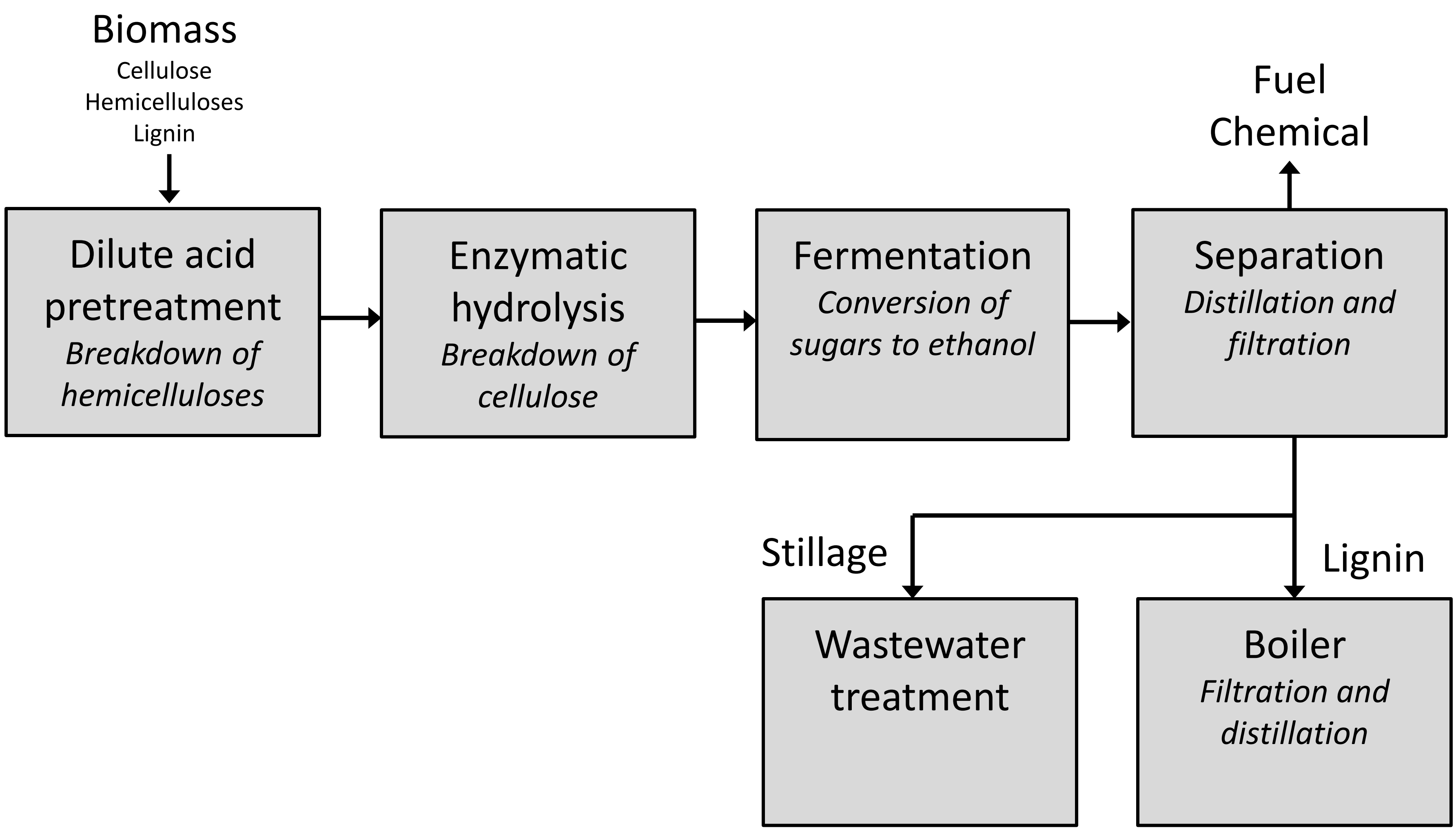
- Lots of data that supporting ***bmr*** mutations in forages reduce lignin content and improve digestibility

- Some data suggests ***bmr*** mutations reduce lignin content and biomass recalcitrance for dilute acid pretreatment & enzymatic hydrolysis

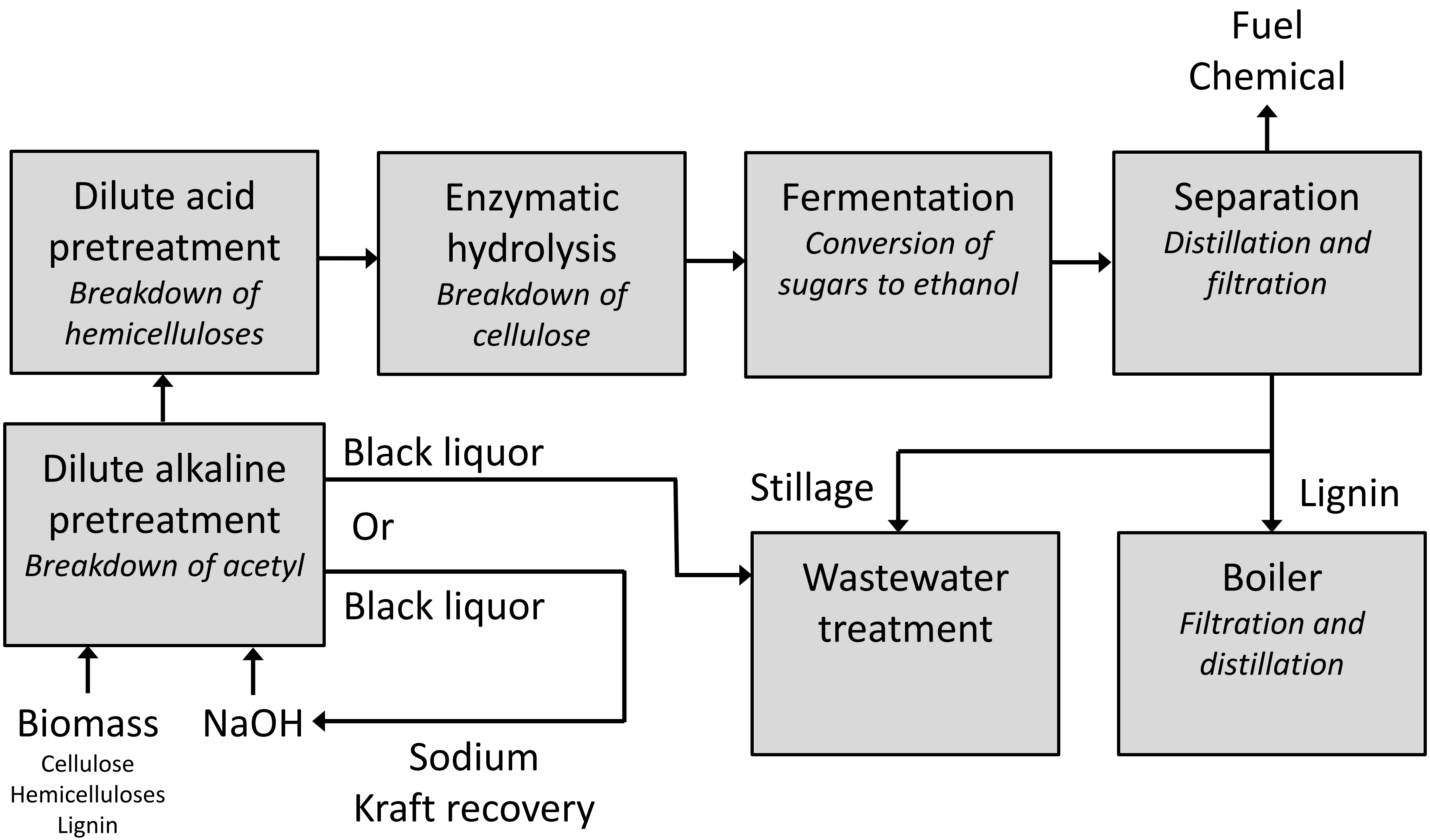


Dien, B, G. Sarath, J. Pedersen, S. Sattler, H. Chen, D. Funnell-Harris, N. Nichols and M. Cotta. 2009. BioEnergy Research 2: 153–164.

Dilute Acid Pretreatment (DA)



Deacetylation/Dilute Acid Pretreatment (DDA)



Materials and Methods

Four Forage Sorghum feedstocks (RTx430)

- Wild Type (no mutation)
- *bmr6* mutant
- *bmr12* mutant
- Stacked (*bmr6 & bmr12*) mutant

All materials milled to 2mm

Laboratory scale PT/EH Assay

Wild-type: outside
bmr12: inside
juicy stalk: left
dry stalk: right

Wild-type

bmr6

bmr12

bmr6 + bmr12

Sorghum Compositional Analysis

SAMPLE	EXTRACTIVES				STRUCTURAL				MB
	SUCROSE	WATER (other)	EtOH	TOTAL EXTRACT	LIGNIN	GLUCAN	XYLAN	ACETYL	
Wild Type	15.3	7.6	3.3	31.8	11.3	24.3	14.4	3.1	100.4
bmr-6	16.9	9.9	3.3	37.1	9.9	22.6	13.5	1.8	99.1
bmr-12	12.4	6.9	3.4	29.4	10.6	25.3	16.3	2.0	98.2
stacked	15.8	7.7	3.2	32.2	9.4	25.2	15.0	2.3	99.4

n=3

KEY OBSERVATIONS

- Mutants show lower lignin & acetyl content
- No trends in structural carbohydrates
- All samples have high total extractives & sucrose
- Excellent mass balance

Detail – Laboratory-Scale PT/EH Assay

Dilute Acid Pretreatment (DA)

- 10% solids loading
- 1.0% H_2SO_4 - 6 min static time @ multiple temps (x-y)
- Rinse with DI water (133 mL) @ 100°C

Deacetylation/Dilute Acid Pretreatment (DDA)

1. *Deacetylation*

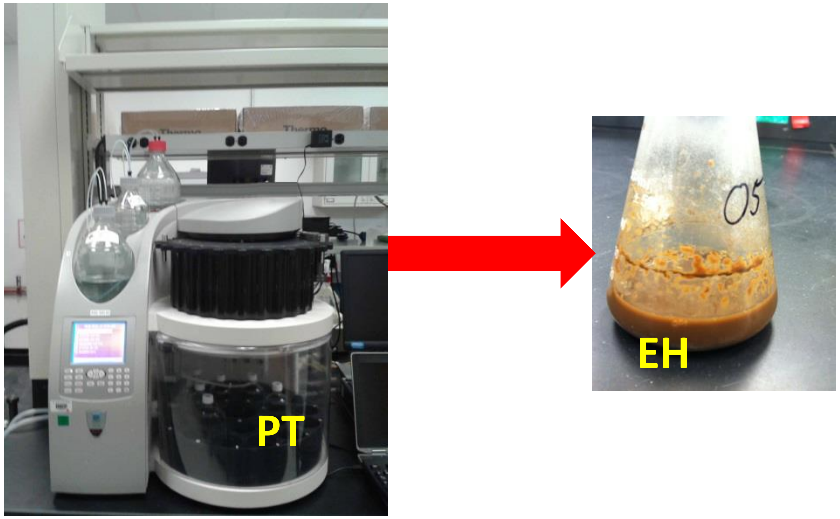
- 10% solids loading
- 0.2% (0.05 M) NaOH at 80°C - 30 min static time
- Rinse with 0.1% H_2SO_4 (67 mL) @ 100°C
- Rinse with DI water (133 mL) @ 100°C

2. Dilute Acid

- Solids NOT dried between deacetylation and dilute acid pretreatment
- 1.25% H_2SO_4 - 6 min static time @ multiple temps (x-y)
- Rinse with DI water (133 mL) @ 100°C

Enzymatic hydrolysis

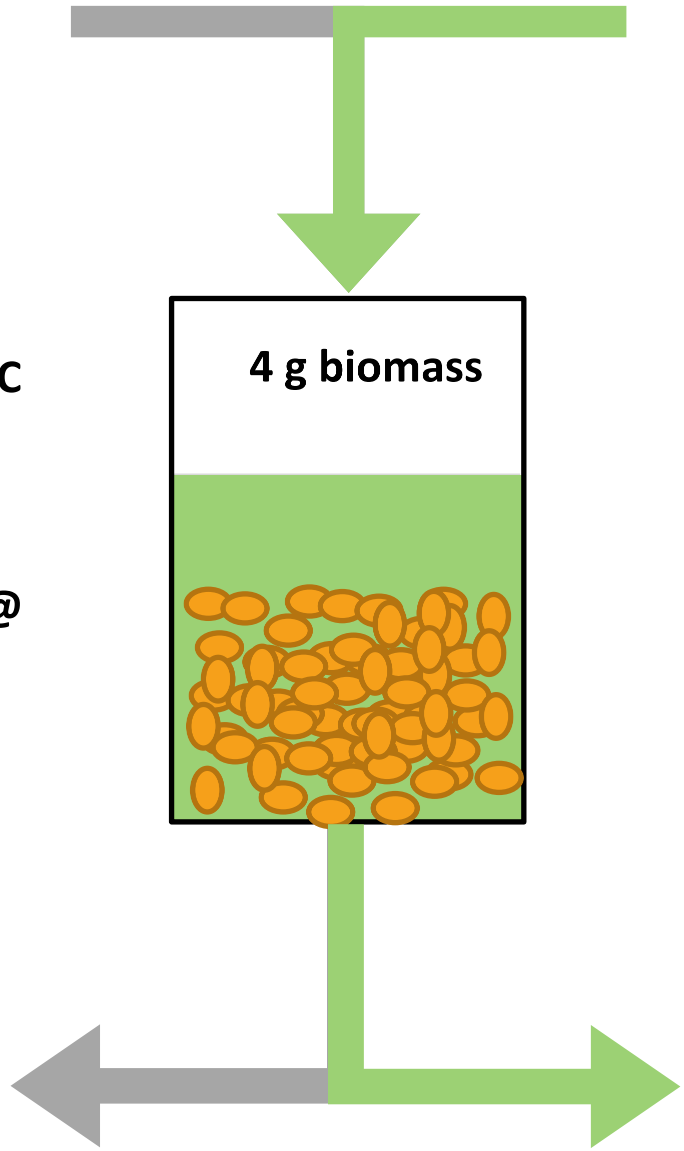
- 10mL total volume
- 10% solids loading
- CTec2 cellulase (20 mg/g TS)
- pH 4.8 (citrate buffer)
- Incubate @ 48°C for 5 days



DDA Pretreatment Schematic

0.2% NaOH (40mL)

40mL 1.25% H_2SO_4 (40mL)



Hold for 30min @ 80°C
Rinse with
- 0.1% H_2SO_4 (67mL) @ 100°C
- DI water (133mL) @ 100°C

Hold for 6min @ 130-180°C
40mL 1.25% H_2SO_4

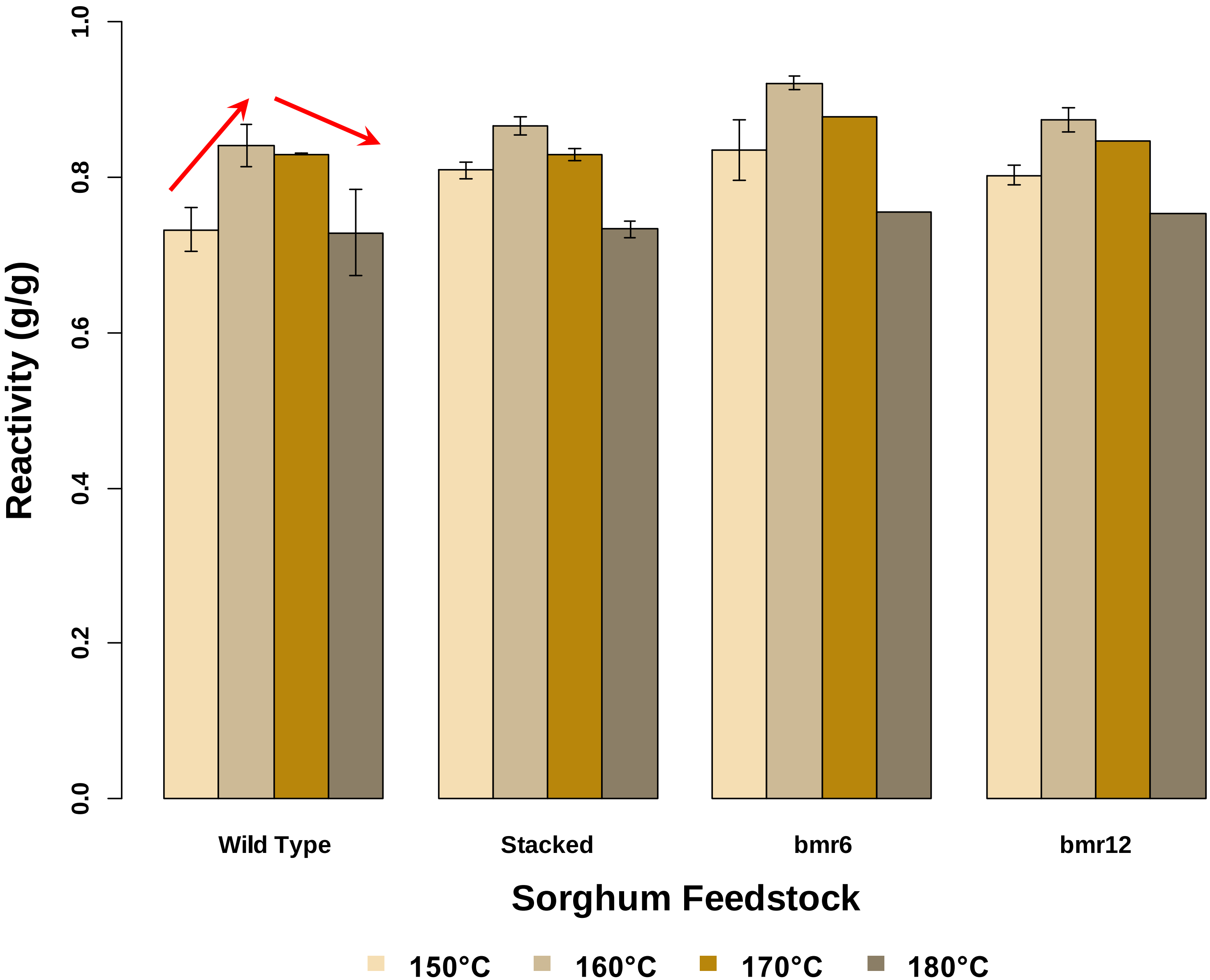
Rinse with DI water (133 mL) @ 100°C

Solids to EH Assay

to sugar analysis

to sugar analysis

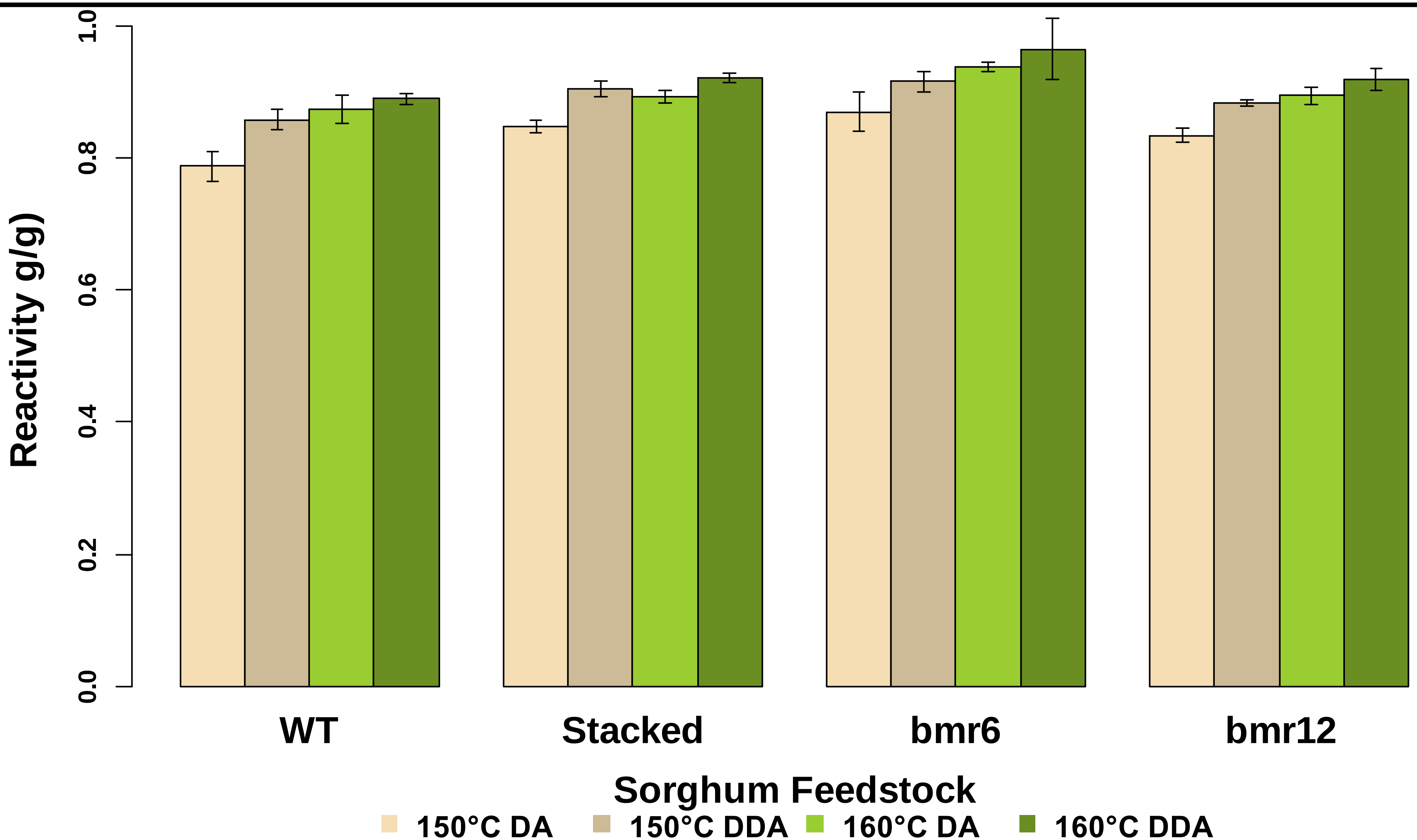
DA Pretreatment Results



Improved yield for mutants vs WT

Temperature affects yield

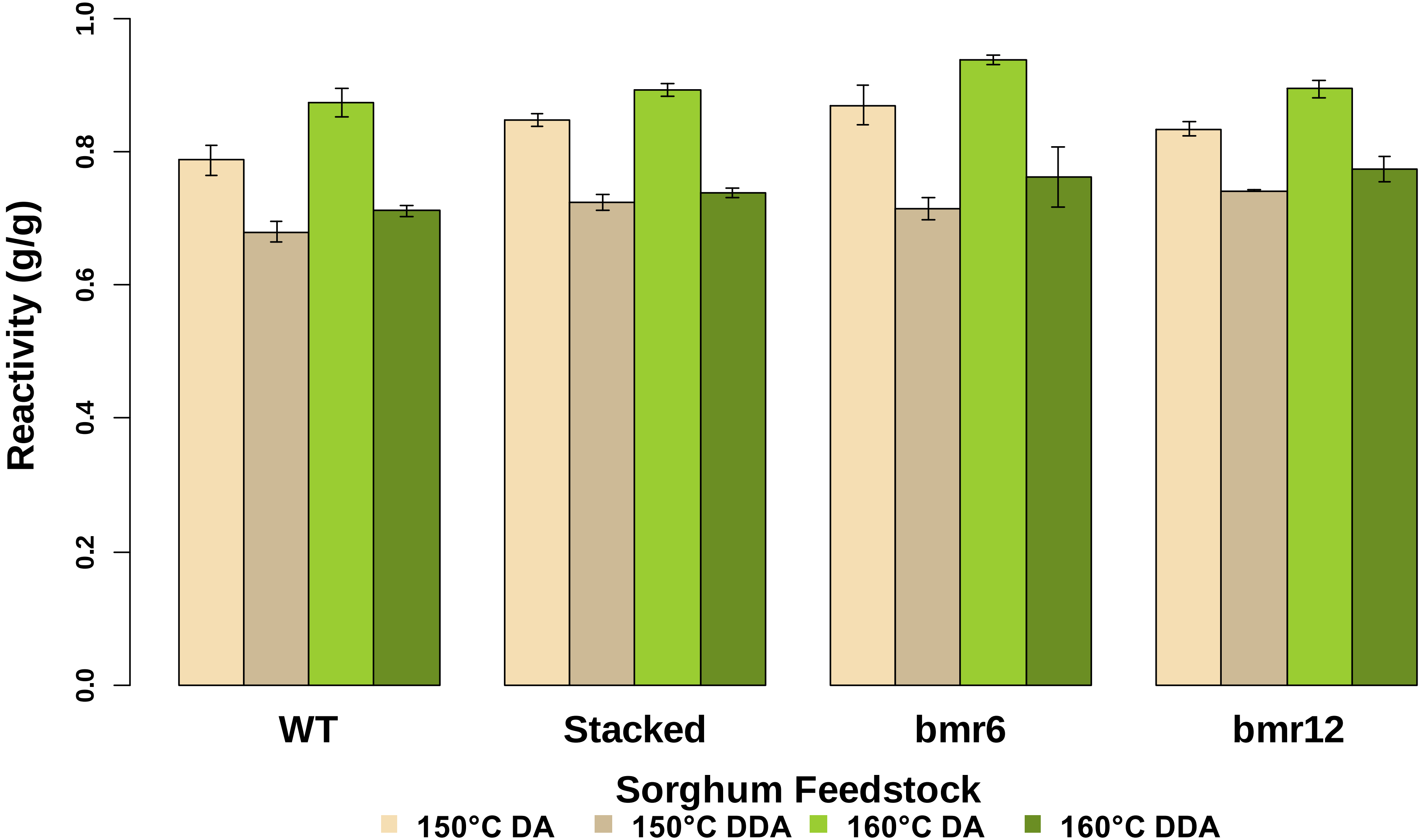
DDA Pretreatment Results



Recovery of the carbohydrates solubilized by deacetylation increases reactivity

Are these solubilized carbohydrates available?

DDA Pretreatment Results



Loss of the carbohydrates solubilized by deacetylation lowers reactivity

Are these solubilized carbohydrates available?

Deacetylation Solubilizes Acetyl & Sucrose

The Deacetylation Liquor contains large amounts of glucose and acetate; 20-30% of apparent glucan, 20-40% of acetyl originally in biomass

	Wild type	Stacked	*bmr6*	*bmr12*
Glucose*	0.25	0.25	0.28	0.20
Xylose	0.02	0.02	0.03	0.03
Acetyl	0.23	0.33	0.41	0.41

*from sucrose

Conclusions

- **Statistically significant decrease in lignin & acetyl content of stacked *bmr* mutant compared to wild type**
- **Statistically significant increase of reactivity for**
  - ***bmr*** mutants compared to wild type
  - Deacetylation prior dilute acid pretreatment
  - Higher DA pretreatment temperatures
- **Increasing Pretreatment severity decreases the differences in recalcitrance between *bmr* mutant & wild type**
- **Feedstocks with high non-structural carbohydrates will lose much of them to deacetylation; need a strategy to recover them or limit DDA to low-extractives feedstocks**

Acknowledgements

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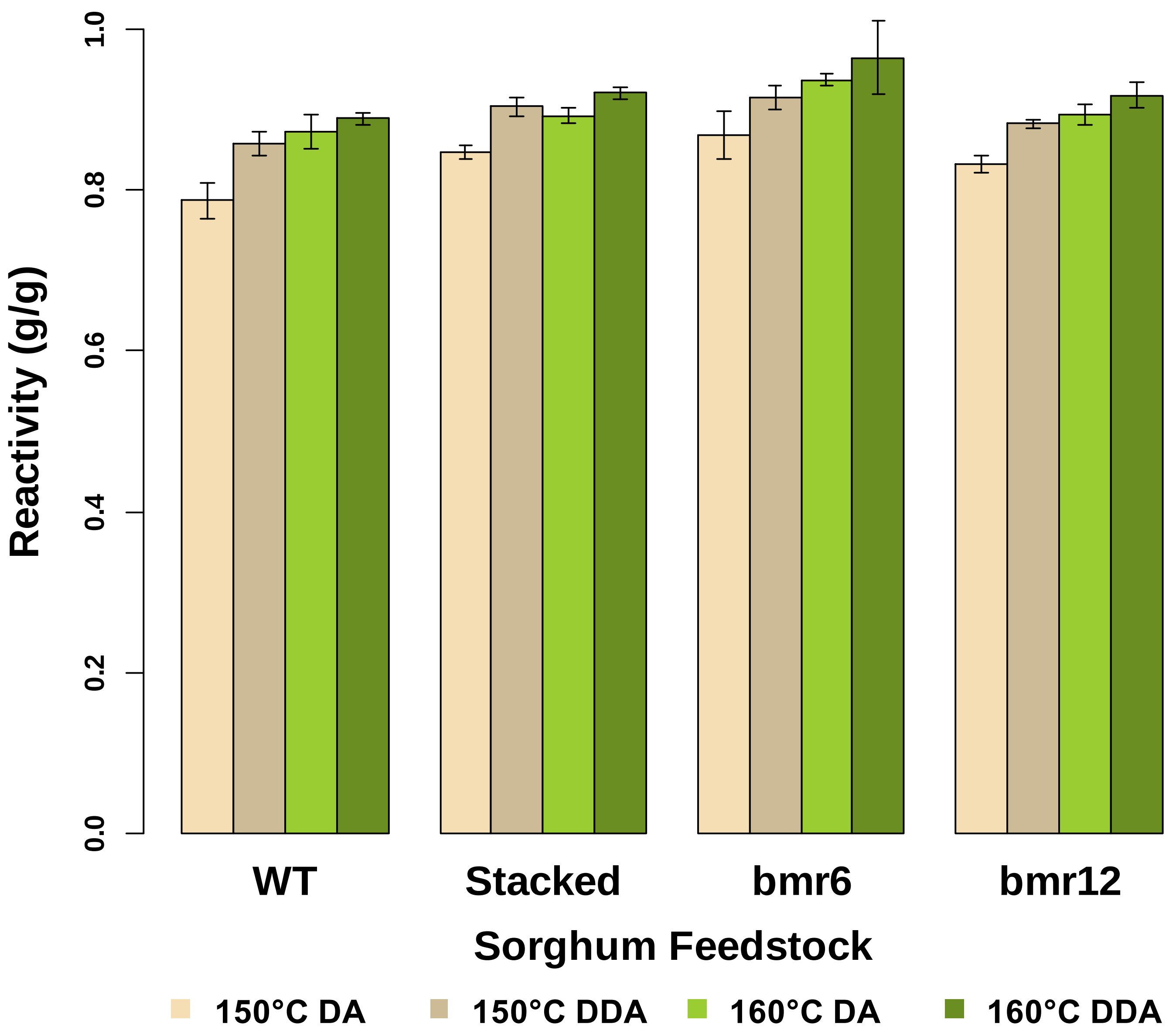
cra-w Wallonie

USDA ars

Questions

DDA vs DA (with recovery of solubles)



Mutants more reactive

Deacetylation improves reactivity

Higher temperatures improve reactivity

DDA has smaller temperature effect than DA