Meristematic Barley Transformation

A collaboration between the USDA and the Wisconsin Crop Innovation Center

Marcus Vinje
Research Geneticist
USDA-ARS, Cereal Crops Research Unit

Who, What, Where, When, Why, and How
Who & Where?

USDA Agricultural Research Service
U.S. DEPARTMENT OF AGRICULTURE

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Research Leader
Cereal Crops
Research Unit
(Madison, WI)

Phil Bregitzer
(Lead)
Research Geneticist
Small Grains and
Potato Research
Unit (Aberdeen, ID)

Marcus Vinje
Research Geneticist
Cereal Crops
Research Unit
(Madison, WI)

ASBC

MASTER BREWERS ASSOCIATION OF THE AMERICAS ESTD 1887

Who, What, Where, When, Why, and How

Who & Where?

Shawn Kaeppler, WCIC
Director
Agronomy Professor at
UW-Madison

Wisconsin Crop Innovation Center

COLLEGE OF AGRICULTURAL & LIFE SCIENCES

UNIVERSITY OF WISCONSIN–MADISON

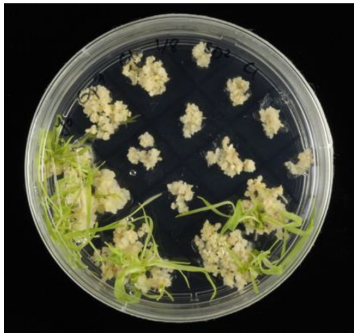
Michael Peterson, WCIC
Associate Director
(Retired)

Alvar Carlson –
Research
Program Director

Why was ARS interested in Barley Transformation?

Accessible genetic tool for barley researchers

- U.S. based transformation service
- Circumvent somaclonal variation caused by callus regeneration



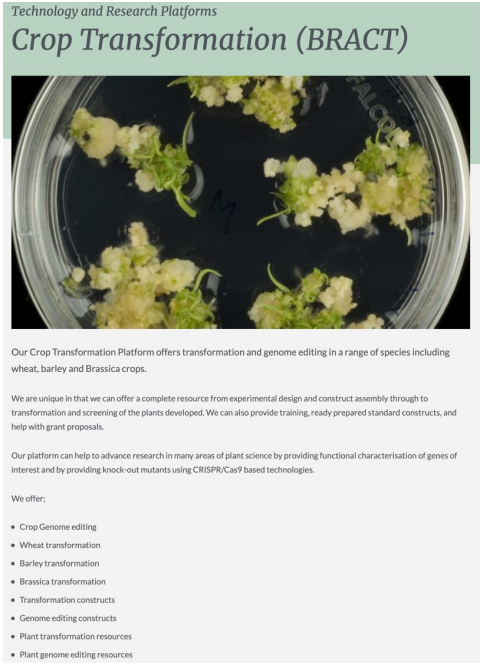
https://www.jic.ac.uk/app/uploads/2018/12/Transformation-chapter-figures-final.pdf

Previous options for barley transformation

- Do it yourself
- Collaborate

Contract Service

John Innes Centre
Norwich, England



Technology and Research Platforms

Crop Transformation (BRACT)

Our Crop Transformation Platform offers transformation and genome editing in a range of species including wheat, barley and Brassica crops.

We are unique in that we can offer a complete resource from experimental design and construct assembly through to transformation and screening of the plants developed. We can also provide training, ready prepared standard constructs, and help with grant proposals.

Our platform can help to advance research in many areas of plant science by providing functional characterisation of genes of interest and by providing knock-out mutants using CRISPR/Cas9 based technologies.

We offer;

- Crop Genome editing
- Wheat transformation
- Barley transformation
- Brassica transformation
- Transformation constructs
- Genome editing constructs
- Plant transformation resources
- Plant genome editing resources

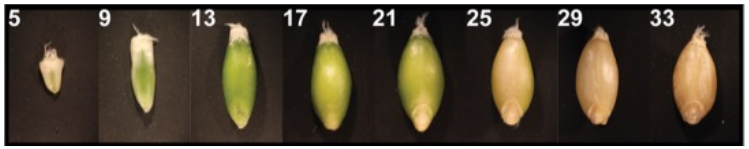
How? Established a Non-Assisted Cooperative Agreement with WCIC

- Non-Assisted Cooperative Agreement (NACA) established in 2018 with WCIC
  - Renewed in 2019, 2020
- **MAIN GOAL**: Develop an efficient system for barley transformation using meristems with direct regeneration of plants from barley meristems.
- **Hypothesis**: Using this system will enable the transformation of elite barley cultivars as opposed to the traditional barley transformation system.

What is considered “traditional” Barley Transformation?

- Immature barley embryos harvested from developing barley grains
- Golden Promise
  - Salt-tolerant mutant (gamma-ray treated seeds)
  - Mutant origins may explain why its amenable to callus regeneration and transformation
  - Other cultivars recalcitrant to callus regeneration
- Method of gene delivery
  - Biolistic/*Agrobacterium*
- Plant regeneration via callus

- Developing Grains



Vinje et al., 2019

- Isolate Embryos

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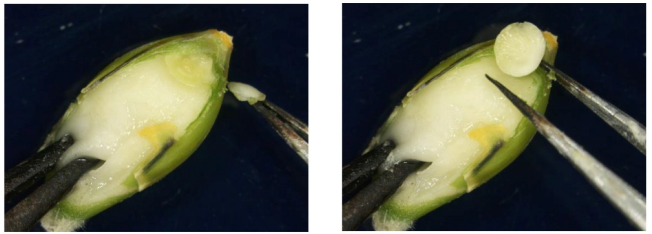

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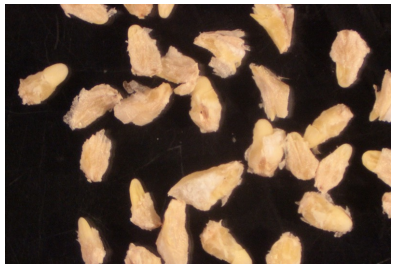
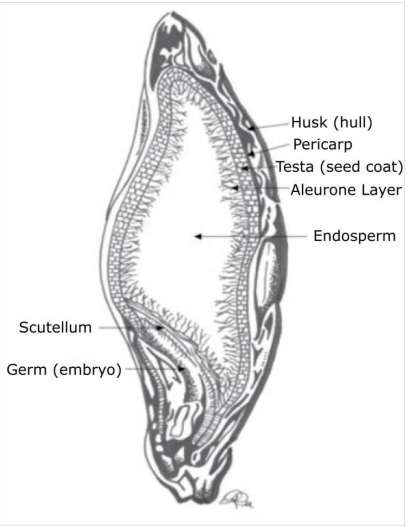
What is Direct Meristematic Transformation?

- Meristem : tissue found in plants consisting of undifferentiated cells capable of cell division.
  - Embryo
    - Shoot and Root apical meristems
- Plant regeneration via the mature embryo.
- Patented (Bayer and WCIC)
  - Patent Application Publication US 2020/0396918 Al

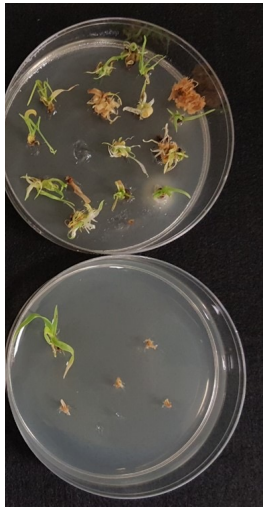
- Excised mature barley embryos



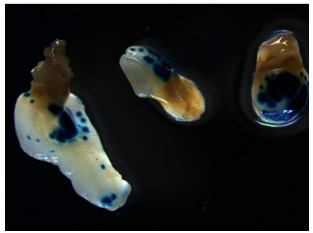
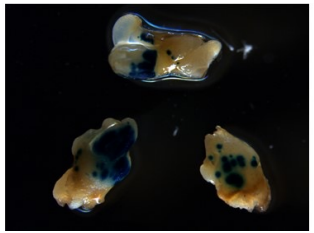
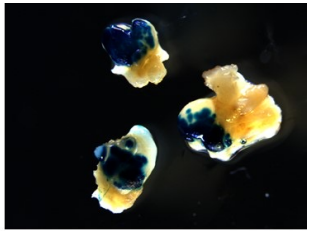


- Ed Williams at WCIC was instrumental in the barley transformation protocol

Success!!!



Differences in selective agents

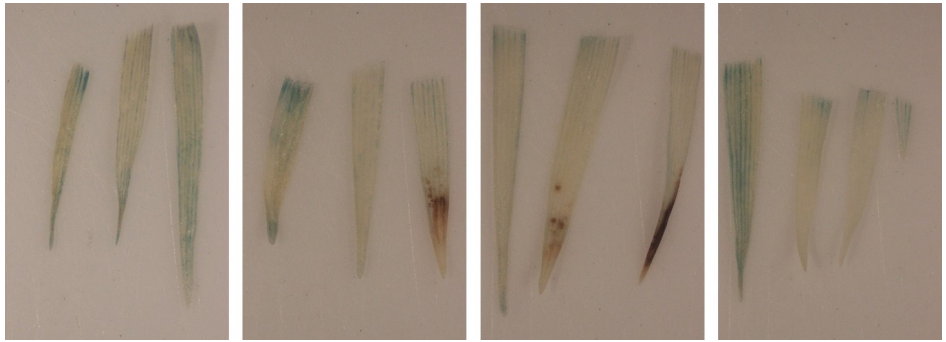




Transient expression of GUS in meristems

Meristems were able to successful root and shoot to produce viable plants in the greenhouse

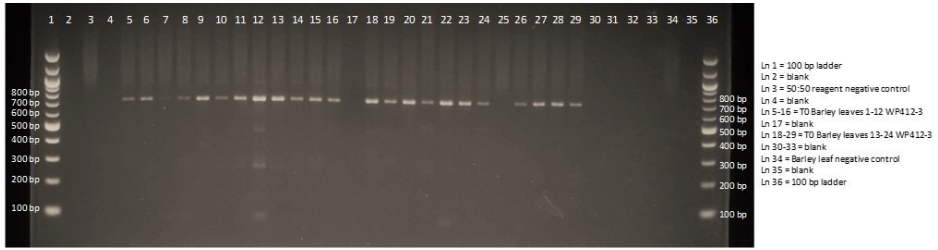
Success!!!

T0 barley event
WP412-1



Stable expression of GUS in T0 leaves from WP 412-1 (13/13 leaves)

T0 barley event
WP412-3



tdTomato gene expression in T0 leaves from WP 412-3
PCR positive (23/24 leaves)
GUS expression was positive in 20/24 leaves

Germline Transmission

Barley apical meristems have 2 cell layers: L1 and L2 with only L2 giving rise to gametes

Event	Spike	# T1 Seeds Planted	# T1 Plants Germinated	% Germination	# T1 Plants GUS positive	% GUS positive T1 Plants
WP412-1	1	10	10	100%	7	70%
WP412-1	2	10	7	70%	3	43%
WP412-1	3	10	8	80%	7	88%
WP412-1	4	10	5	50%	5	100%
WP412-1	5	10	5	50%	4	80%
WP412-1	6	10	7	70%	5	71%
WP412-1	7	10	9	90%	7	78%
WP412-1	8	10	9	90%	8	89%
WP412-1	9	10	10	100%	6	60%
WP412-1	10	10	10	100%	7	70%
WP412-1	11	10	10	100%	8	80%
WP412-1	12	10	8	80%	6	75%
WP412-1	1-12	120	98	82%	73	74%

Barley Transformation Service at WCIC

Pocatello brewery makes special beer showcasing new craft malt variety

Pocatello's Portneuf Valley Brewing made the beer with a new malt variety, called Gem Craft, developed by USDA's Agricultural Research Service in Aberdeen.

Gemcraft

Gongshe Hu

https://www.brewingwithbriess.com/blog/another-bighorn-barley-tour-in-the-books/

Wisconsin Crop Innovation Center

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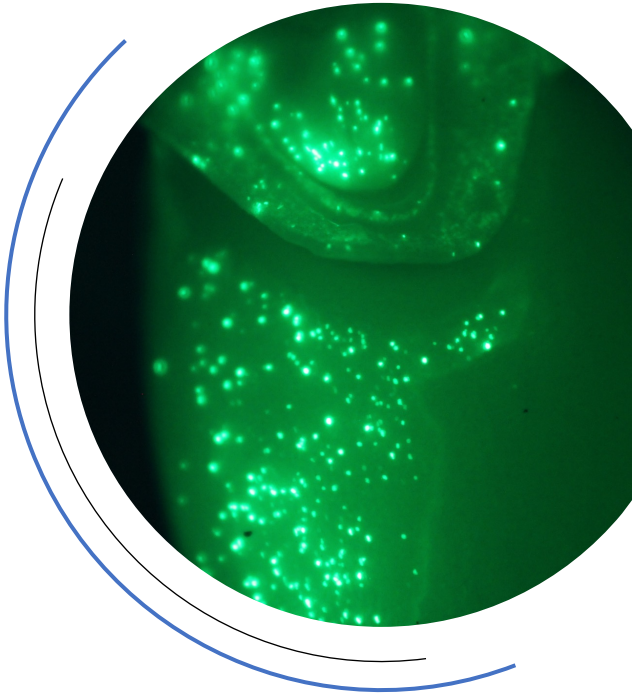
- Established transformation services for corn, soybean, sorghum, brachypodium.
- Barley - Malting cultivar Gemcraft
  - Internal UW rate = $4,021
  - External public rate = $5,066
  - Delivers T1 seeds up to 6 events/construct.

Deliverables from NACA with WCIC

- Each USDA PI received 2 constructs and 6 events/construct
- Phil Bregitzer
  - *Fusarium* head blight resistance.
  - Transgenic.
- Mali Mahalingam
  - Heat and drought stress
  - Transgenic
- Marcus Vinje
  - Triple β-amylase CRISPR knockout

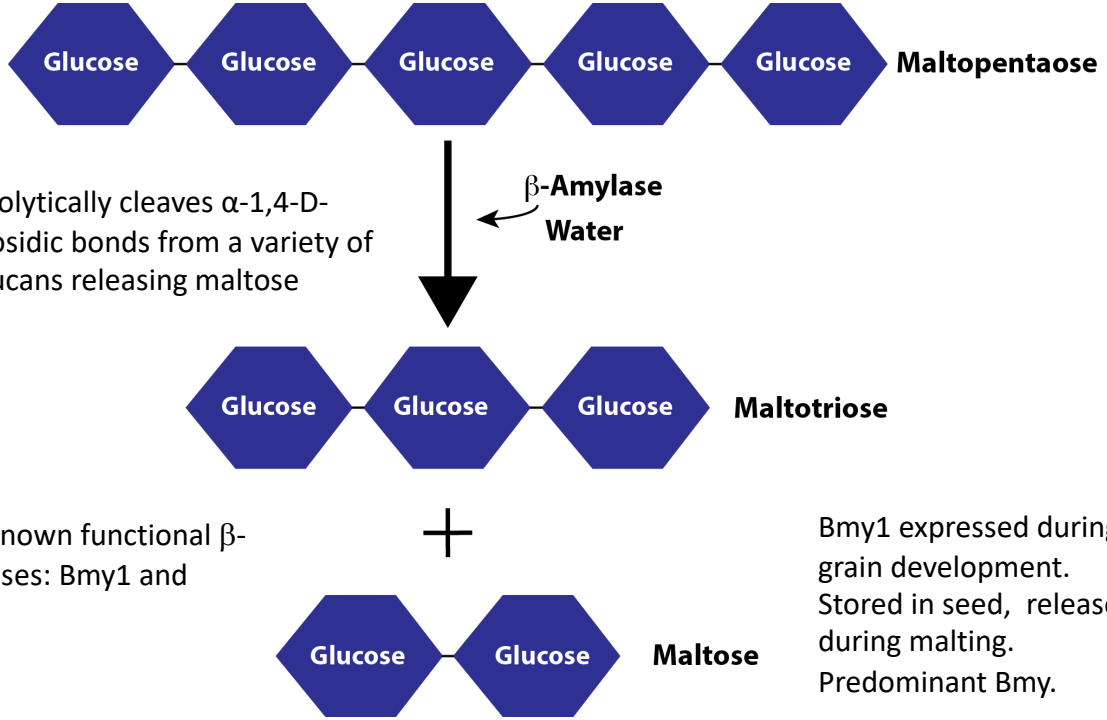


Barley β-amylase is the main driver of Diastatic Power

Correlation coefficients (*r*) between diastatic power and amylase activity		
β-Amylase Activity	α-Amylase Activity	Reference
0.77	0.64	Arends et al. (1995) J Cereal Sci 21:63
0.79	0.61	Gibson and Solah (1995) J Inst Brew 101:277
0.9	0.74	Evans et al. (2005) J Am Soc Brew Chem 63:185
0.67	0.39	Evans et al. (2008) J Am Soc Brew Chem 66:223
0.91	0.78	Duke and Henson (2009) J Am Soc Brew Chem 67:206
0.87	0.24	Vinje et al. (2010) Crop Sci 50: 826
1	0.26	Cu et al. (2016) Mol Breeding 36:129
0.88	0.11	Huerta-Zurita et al. (2020) J Am Soc Brew Chem 78:50

Diastatic Power – total activity of malt enzymes that hydrolyze starch to fermentable sugars

β-Amylase



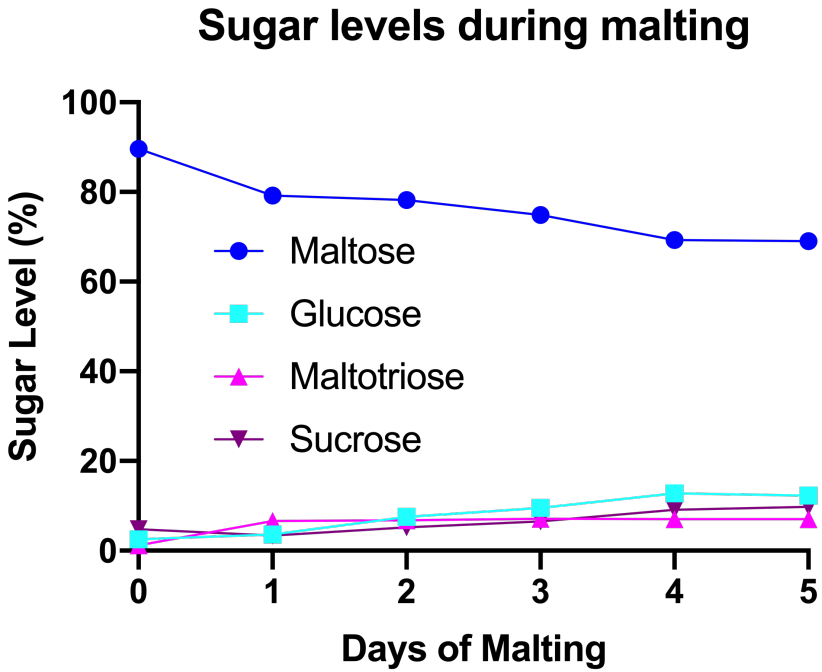
Hydrolytically cleaves α-1,4-D-glucosidic bonds from a variety of α-glucans releasing maltose

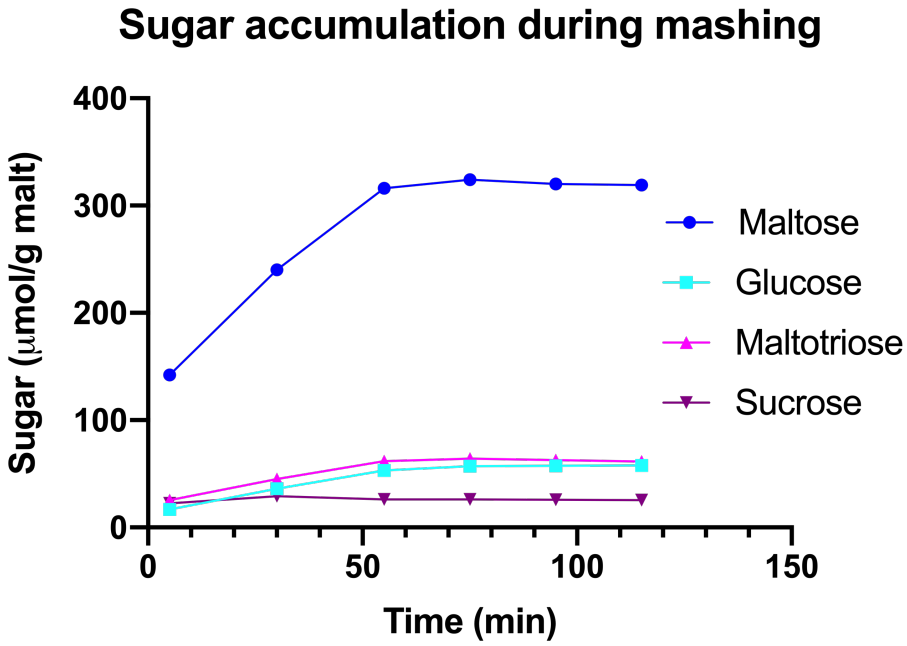
Two known functional β-amylases: Bmy1 and Bmy2

Bmy1 expressed during grain development. Stored in seed, released during malting. Predominant Bmy.

Bmy2 expressed in early grain development and malting.

Maltose is the most abundant sugar in malt and wort





Vinje MA et al. (2015) J Am Soc Brew Chem **73**: 195

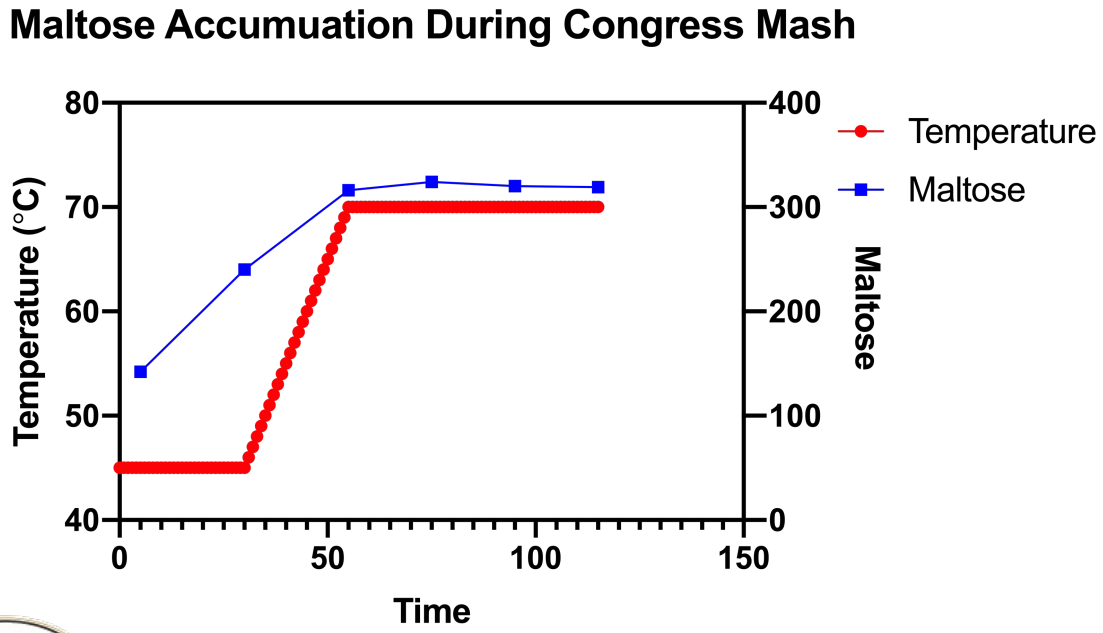
Duke and Henson (2011) J Am Soc Brew Chem **69**: 200 213

Barley β-Amylase is Thermolabile

Common mashing temperatures kill the enzyme.

T_{50} of β-amylase1 is between 55 and 59 °C

T_{50}: Temperature where 50% of enzyme activity remains



Congress Mash (EBC 4.5.1, ~ASBC Malt-4)

45°C for 30 min, Ramp 1°C/min for 25 min, 70°C for 60 min

Duke and Henson (2011) J Am Soc Brew Chem 69: 200 213

β-Amylase mutants and questions

- Thermostable *Bmy2* gene overexpressed during grain development
  - Better able to survive mashing temperatures?
  - More and/or quicker maltose production during mashing?
- *Bmy2* gene knockout (CRISPR)
  - What physiological role does the *Bmy2* gene have in developing and malting barley?
  - John Innes Center
    - Contract began summer 2019. Seeds arrived 8/2/22
- *Bmy1*, *Bmy2*, and *Bmy3* triple knockout (CRISPR)
  - What effect does a triple β-amylase knockout have on germination, development, and the malt and wort sugar profile?
  - Wisconsin Crop Innovation Center

Acknowledgements

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    - Mali Mahalingam
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  - Small Grains and Potato Germplasm Research
    - Phil Bregitzer
    - Kathy Klos
    - Dongying Gao
- Wisconsin Crop Innovation Center
  - Professor Shawn Kaeppler, Director
  - Michael Peterson, Assoc Director (Retired)
  - Alvar Carlson, Assoc Director
  - Heidi Kaeppler, Transformation Director
  - Ed Williams, Transformation R&D associate
  - Ray Collier, Molecular Technologies Lead
- John Innes Centre
  - Professor Wendy Harwood, Senior Scientist
  - Mark Smedley, Research Assistant

Questions?