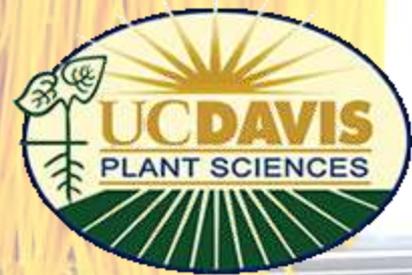


# **Increasing Resistant Starch in Wheat**

**Brittany Hazard  
Ph.D. Candidate  
Genetics Graduate Group  
University of California Davis**

**CWC Board Meeting  
September 4, 2014**



# Dubcovsky Laboratory



# Wheat in Our Diet

## WHEAT

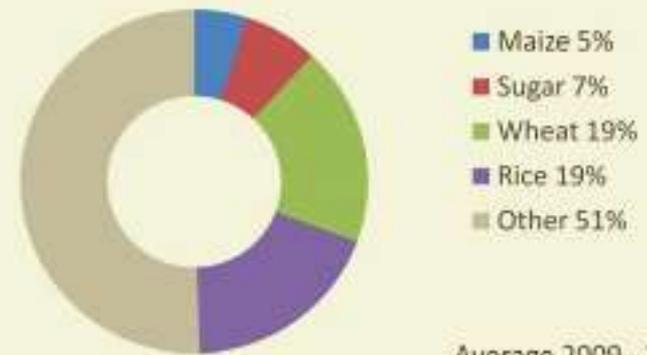
Today, wheat is grown on more land area than any other commercial crop and continues to be the most important food grain source for humans.

WHEAT IS THE LARGEST PRIMARY COMMODITY

GLOBAL PRODUCTION IS APROX.  
**700 million tonnes**



WHEAT PROVIDES **19%** OF OUR TOTAL AVAILABLE CALORIES



Global production from 1961 to 2013



# Wheat in Our Diet

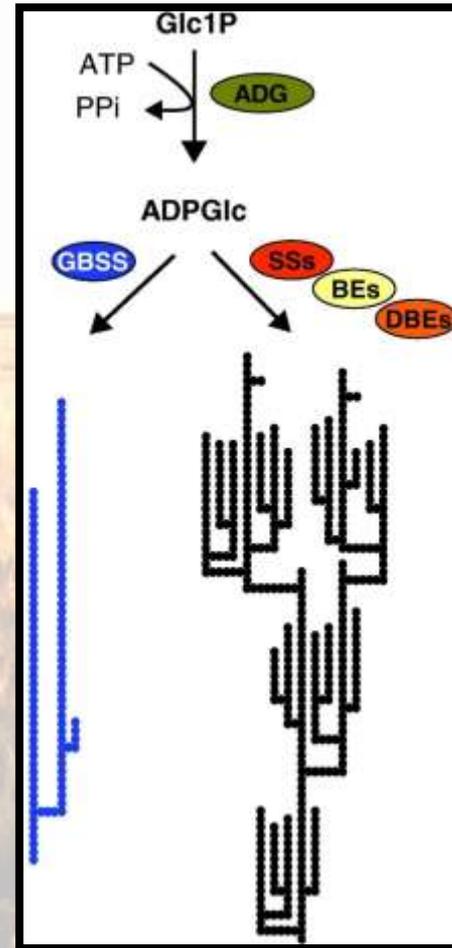
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- Starch is the major component of the wheat kernel (~50-70%).
- Improvements in starch composition have potential to deliver nutritional benefits.



# Starch: Amylose and Amylopectin

- **Amylose**  
~ 20-30%
- **Amylopectin**  
~ 70-80%



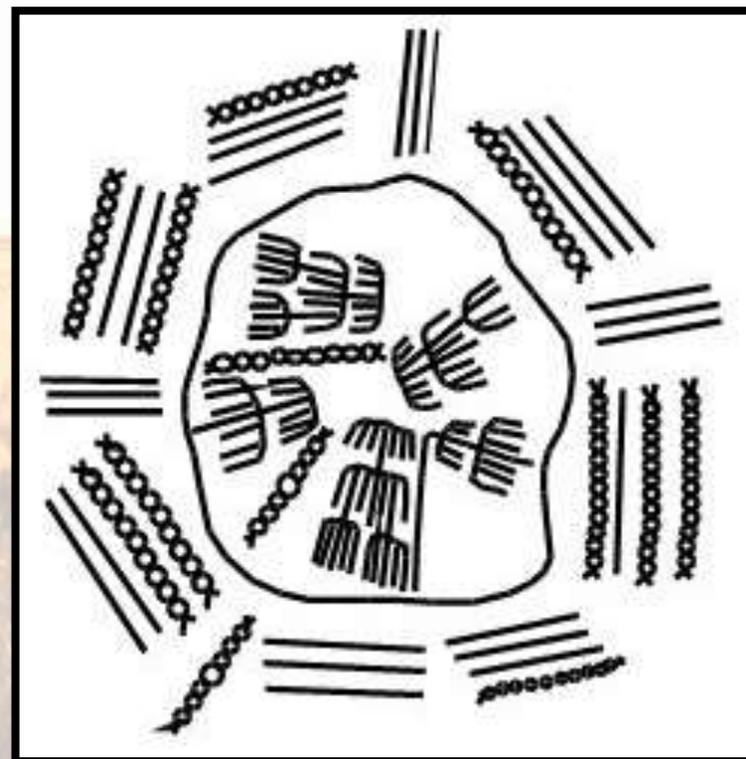
Amylose

Amylopectin

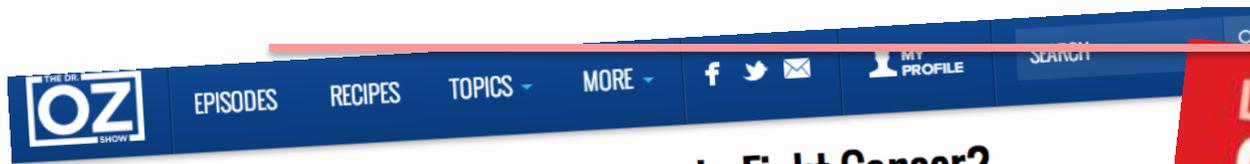
# Resistant Starch (RS)

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- High-amylose starch → **increased RS**
- Inaccessible to  $\alpha$ -amylase digestion
- Component of dietary **fiber**



# Health Benefits of Resistant Starch

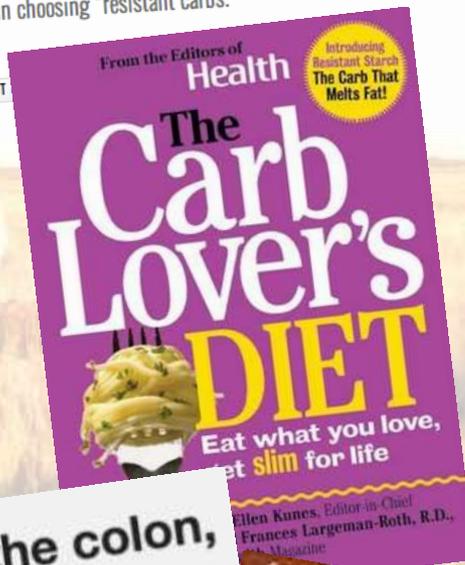
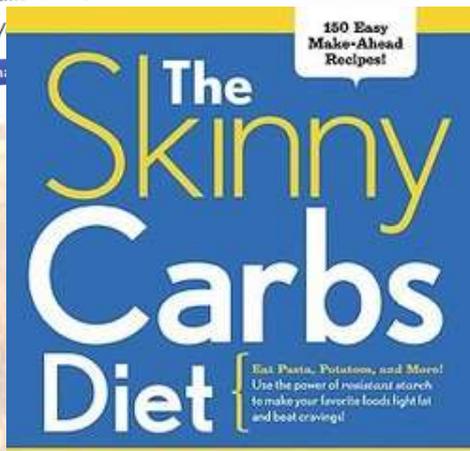


## Resistant Carbs: The Secret Weapon to Fight Cancer?

To many dieters, carbs are viewed as public enemy #1. But research shows that not all carbs cause weight gain. In fact, some may serve as weapon against deadly diseases from cancer to diabetes. The secret lies in choosing "resistant carbs."

Posted on 4/

f Like Sh



## 'Resistant' starches heal the colon, prevent cancer and diabetes

LIVE SCIENCE

By Christopher Wangk - Published February 28, 2013

f 119 t 68



From Around



Food Trick "The



### LOVE YOUR GUTS!

**HEALTH BITES**

SHOW YOUR GUTS SOME LOVE BY EATING A MIX OF DIETARY FIBRES, INCLUDING RESISTANT STARCH.

#### WHAT IS IT?

RESISTANT STARCH IS A TYPE OF DIETARY FIBRE THAT DOESN'T GET DIGESTED IN YOUR SMALL INTESTINE. INSTEAD, IT PASSES THROUGH TO YOUR BOWEL, WHERE IT HAS POSITIVE EFFECTS ON BOWEL HEALTH. IT'S SOMETIMES CALLED THE THIRD TYPE OF FIBRE, IN ADDITION TO SOLUBLE AND INSOLUBLE FIBRE.

#### WHAT DOES IT DO?

- \* HELPS KEEP YOU REGULAR
- \* ENCOURAGES HEALTHY BACTERIA IN YOUR BOWEL
- \* PROMOTES BET HEALTH
- \* HELPS KEEP YOUR BLOOD SUGAR AT A HEALTHY LEVEL

#### WHY SHOULD YOU CARE?

AUSTRALIA HAS ONE OF THE HIGHEST RATES OF BOWEL CANCER IN THE WORLD. IT'S ACTUALLY THE SECOND MOST COMMONLY DIAGNOSED CANCER IN AUSTRALIA. SO BE GOOD TO YOUR GUTS!

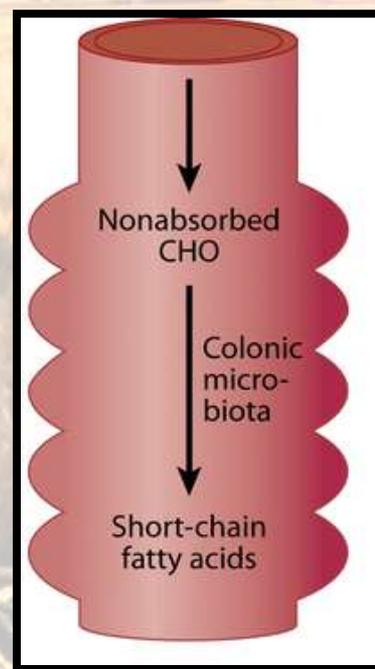
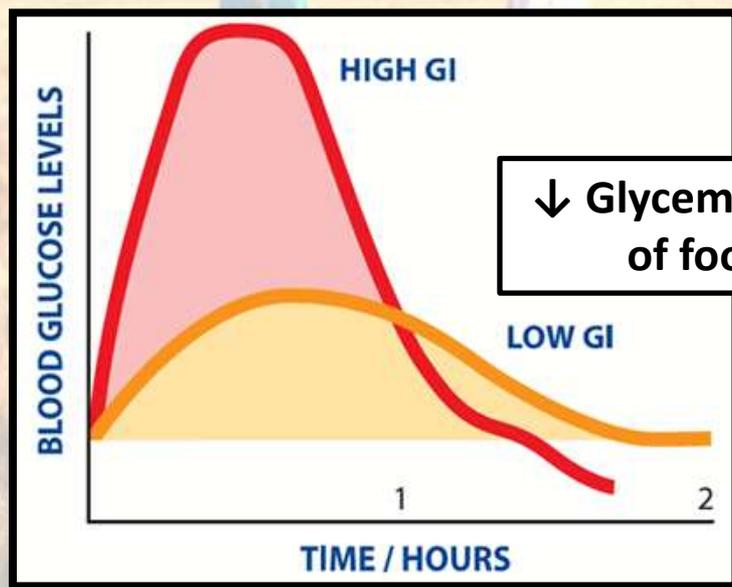
#### GET SOME IN YOUR GUTS:

- \* COOK SOME LENTLES FOR DINNER
- \* SERVE SOME COLD POTATO OR PASTA SALAD
- \* EAT FIRM BANANAS
- \* TRY SOME FOODS CONTAINING OUR BANLEYMAX™ SEAM

#### WE THINK YOU SHOULD AIM TO EAT ABOUT 20 GRAMS OF RESISTANT STARCH PER DAY, ALMOST FOUR TIMES MORE THAN WHAT PEOPLE TYPICALLY EAT.

# Health Benefits of Resistant Starch

- Reduced risk of diseases
  - Diabetes, obesity, heart disease and cancers of the colon and rectum
- Benefits in the large intestine and systemic health benefits



↑ Short Chain Fatty Acids

# Resistant Starch Content

Product	% Resistant Starch
Long grain rice (brown)	1.7
Rice pudding	0.2
Whole wheat breads	1.9 – 2.8
Refined grain products	0.2 – 1.1
Potato salad	1.0
Boiled potatoes	0.5
Legumes	3.4 – 3.5



# Consumption of Resistant Starch

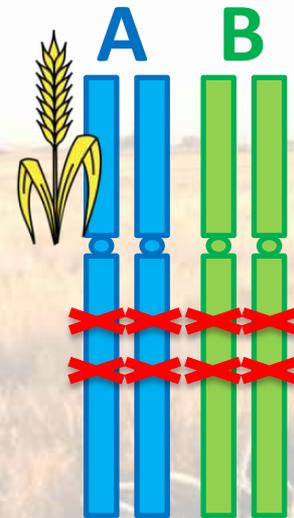
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- Recommended dietary fiber in the US
  - 19 to 38g per day
  - <5% of Americans consume this
- Recommended resistant starch
  - Australia's Division of Human Nutrition
    - 20g per day
  - Americans consume ~5g per day
- **Breads and cooked cereals/pastas contribute ~40% of RS intake**

# Starch Branching Enzyme II (SBEII)

---

- Catalyzes addition of branching points during amylopectin starch synthesis



- SBEIIa and SBEIIb

# Previous Studies: Transgenic Approach

- Amylose content in the wheat grain can be increased by down regulating *SBEII* transcript levels.
- Down regulation of *SBEII* genes by RNAi → increase amylose content (25-70%)
  - Bread wheat (Regina et al., 2006)
  - Durum wheat (Sestili et al., 2010)

## High-amylose wheat generated by RNA interference improves indices of large-bowel health in rats

Ahmed Regina<sup>\*1</sup>, Anthony Bird<sup>\*2</sup>, David Topping<sup>\*3</sup>, Sarah Bowden<sup>4</sup>, Judy Freeman<sup>5</sup>, Tina Barsby<sup>6</sup>, Behjat Kosar-Hashemi<sup>\*7</sup>, Zhongyi Li<sup>\*8</sup>, Sadequr Rahman<sup>\*9</sup>, and Matthew Morell<sup>\*10</sup>

<sup>\*</sup>Commonwealth Scientific and Industrial Research Organization, Food Futures National Research Flagship, P.O. Box 93, North Ryde 1670, NSW, Australia; <sup>1</sup>Commonwealth Scientific and Industrial Research Organization, Plant Industry, G.P.O. Box 1600, Canberra ACT 2601, Australia; <sup>2</sup>Commonwealth Scientific and Industrial Research Organization, Human Nutrition, P.O. Box 10041, Adelaide BC 5000, SA, Australia; and <sup>3</sup>Biogenma UK Limited, 200 Science Park, Milton Road, Cambridge CB4 0GZ, United Kingdom

Communicated by William Lonnie Ross, Commonwealth Scientific and Industrial Research Organization, Canberra, Australia, December 14, 2009

CSIR  
Plant Biology

RESEARCH ARTICLE

Open Access

## Increasing the amylose content of durum wheat through silencing of the *SBEIIa* genes

Francesco Sestili<sup>1</sup>, Michela Janni<sup>1</sup>, Angela Doherty<sup>2</sup>, Ermelinda Botticella<sup>1</sup>, Renato D'Ovidio<sup>1</sup>, Stefania Masci<sup>1</sup>, ... D. ... and Domenico ...

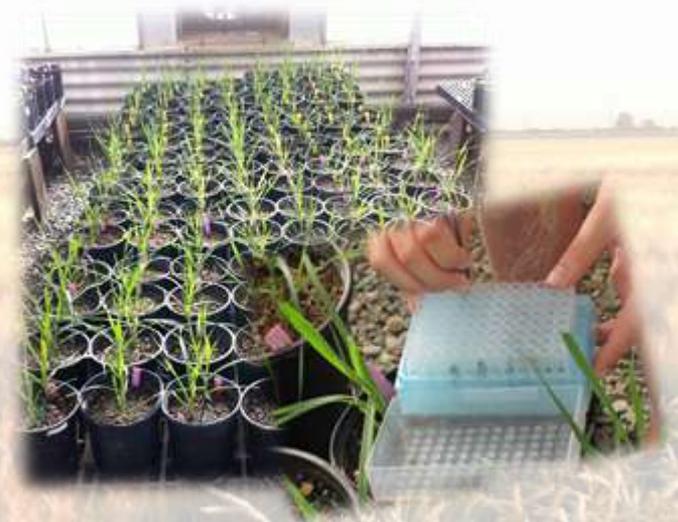
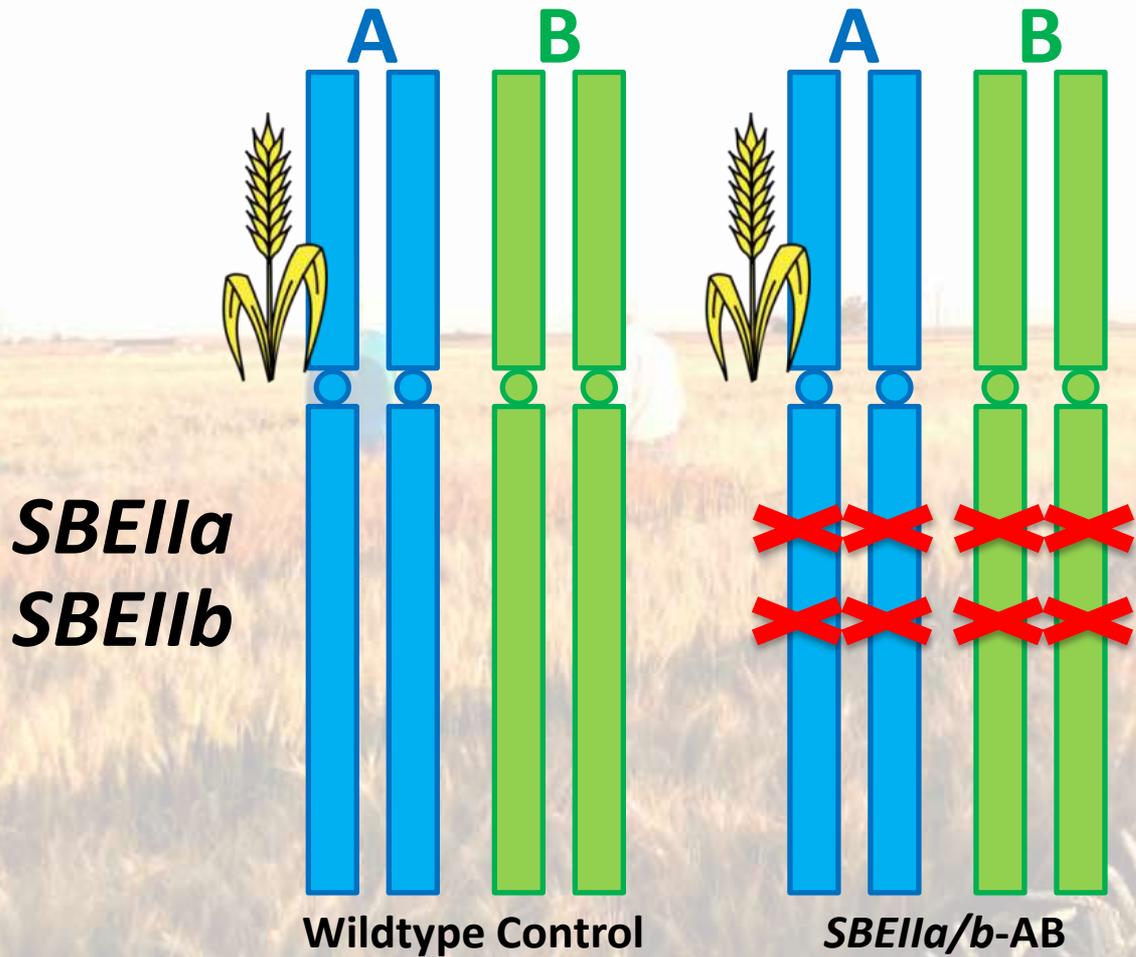
# Alternative approach: TILLING *SBEII* genes

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- Commercialization of transgenic wheat has not yet been implemented
- **TILLING**
  - Targeted Induced Local Lesions in Genomes
  - Induce mutations using a chemical mutagen
  - Screen for mutations in *SBEII* genes



# Combine *SBEIIa* + *SBEIIb* Mutations

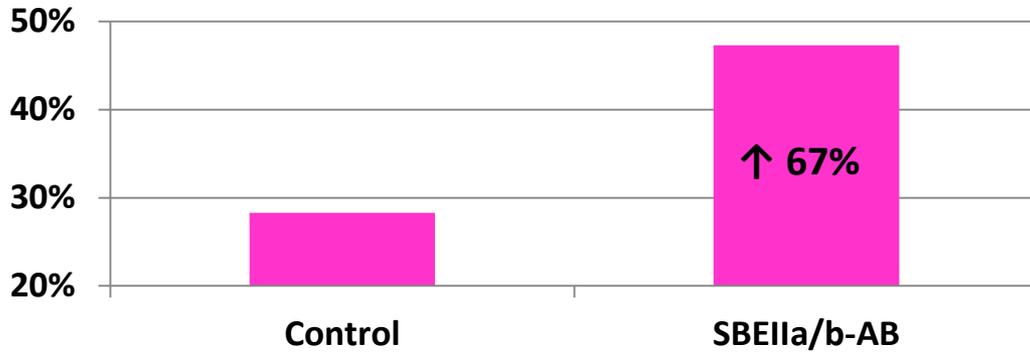


**A Genome** **B Genome**

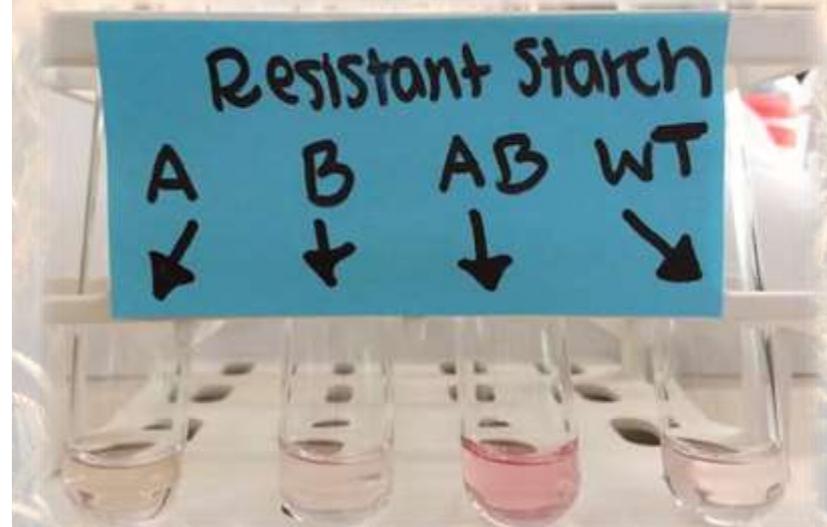
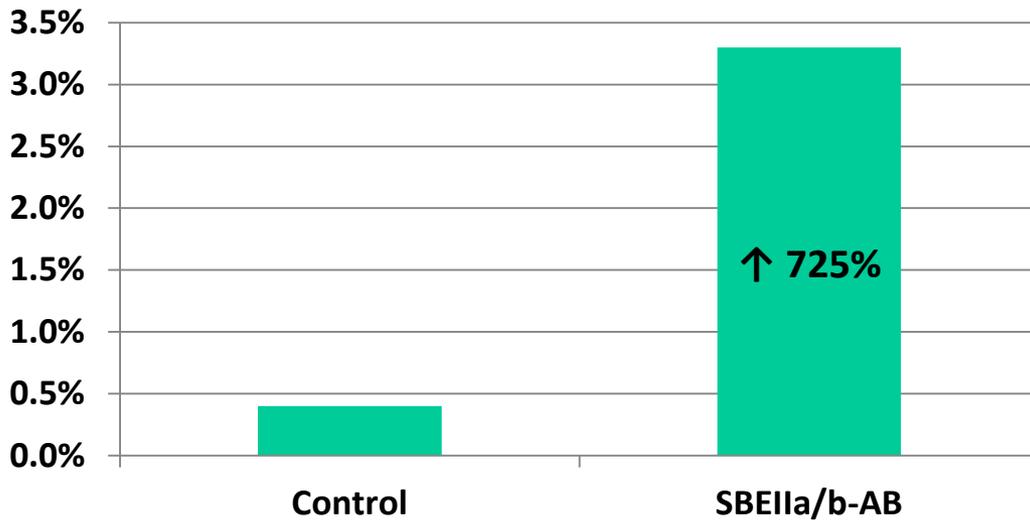
(Hazard et al., 2014)

# Results

## Amylose

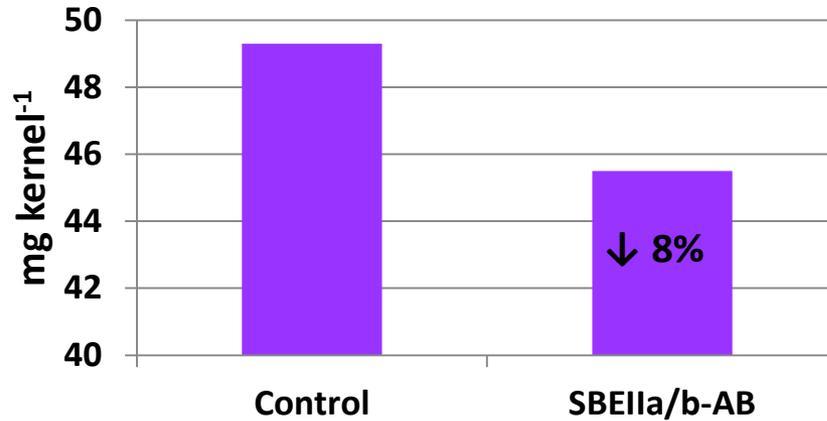


## Resistant Starch

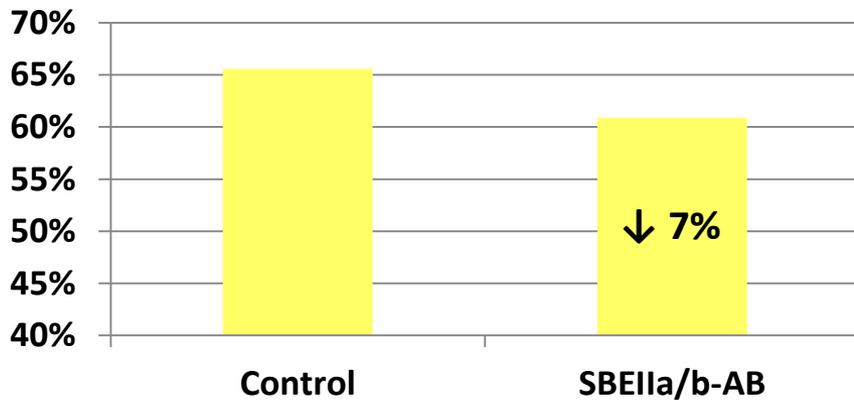


# Results

## Kernel Weight



## Total Starch



# Hazard et al. 2014

- **SBEIIa/b-AB line registered**
  - PI # 670160
- **Germplasm Resource**
  - National Center for Genetic Resources Preservation (NCGRP)
  - Germplasm Resources Information Network (GRIN)



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Registration of Durum Wheat Germplasm Lines with Combined Mutations in *SBEIIa* and *SBEIIb* Genes Conferring Increased Amylose and Resistant Starch

Brittany Hazard\*, Xiaoguo Zhang\*, Mahmoudreza Naemeh\* and Jorge Dobcovsky\*

## Search NPGS/GRIN Accessions

SBEIIa-SBEIIb AB

Search!

Sort by: Rank  Reverse Sort

Search only accession with status:

Active  Inactive

Results for SBEIIa-SBEIIb AB 1 to 3 of 3 results.

1 PI 670160 - *Triticum turgidum* subsp. *durum* - SBEIIa-SBEIIb AB - California, United States -- rank: 1000  
... *Triticum turgidum* subsp. *durum* (Desf.) Husn. POACEAE (durum wheat) SBEIIa-SBEIIb AB Dev  
... uniformity: Pureline. Form received: Seed. Accession names and identifiers SBEIIa-SBEIIb AB Id  
Transfer Agreements ... benefits. Ethyl methane sulphonate (EMS) mutations in the linked SBEIIa and  
http://www.ars-grin.gov/cgi-bin/npgs/html/acchtml.pl?1917347 - 4730 bytes -

# Goals for Current Experiments

---

## Evaluation of *SBEIIa/b*-AB Mutant Line

- Agronomic characteristics
  - Yield trials in 3 California locations
- Quality characteristics
  - Collaboration with California Wheat Commission Milling and Baking Lab
  - Grain, semolina and pasta quality

# Agronomic Characteristics

- 3 Locations

- Sacramento Valley in Davis, CA
- San Joaquin Valley in Five Points, CA
- Imperial Valley in Holtville, CA



- Yield Traits

- Total Yield
- Spike density
- Spikelet number
- Kernel number
- Kernel weight



# Preliminary Results

## Agronomic Characteristics

- Yield penalties are observed in *SBE11a/b*-AB lines
- ~5% decrease in kernel weight
- ~5-10% decrease in overall yield

# Grain and Semolina Quality Characteristics

- **Grain Quality**

- Test Weight
- Kernel Size Distribution
- Protein
- Ash
- Hard and Vitreous of Amber Color (HVAC)

- **Semolina Quality**

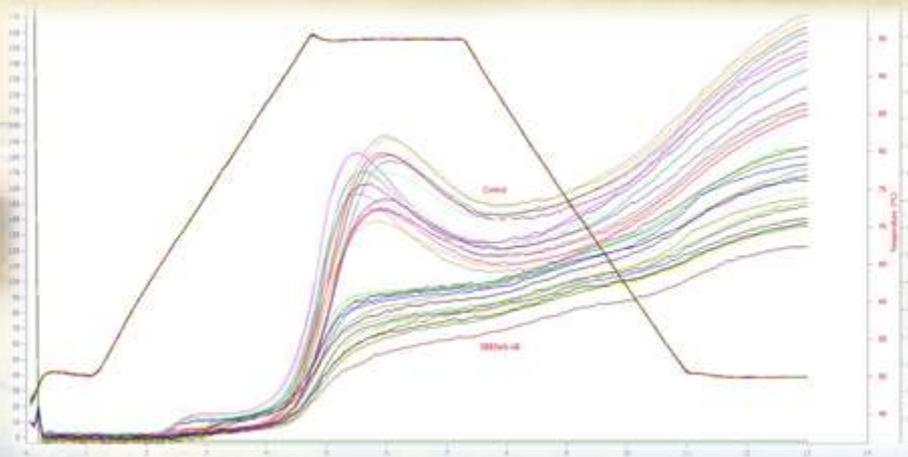
- Milling Tests
- Speck
- Color
- Alveograph
- Protein
- Ash
- RVA
- Falling Number
- Wet Gluten



# Preliminary Results

## Grain and Semolina Quality

- Decrease in test weight (~3%)
- Decrease in semolina extract (~4%)
- Increase in falling number (~40%)
- Changes in alveograph parameters
- Changes in RVA parameters



# Pasta Quality Characteristics

- **Pasta Quality**
  - Color
  - Cooked Weight
  - Cooking Loss
  - Firmness



# Preliminary Results

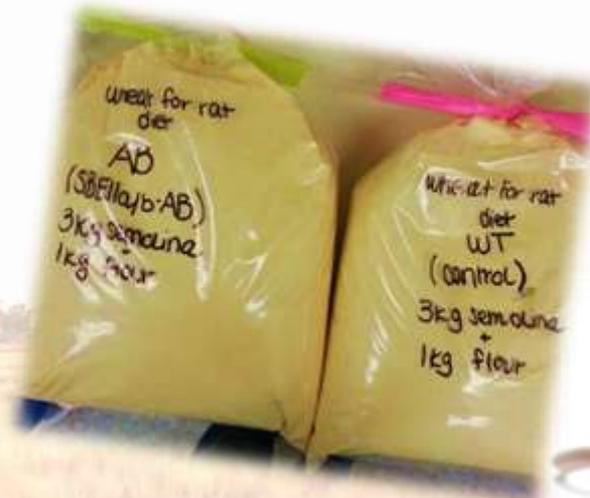
## Pasta Quality

- Increase in cooking loss (~20%)
- Increase in firmness (~12%)



# Future Directions

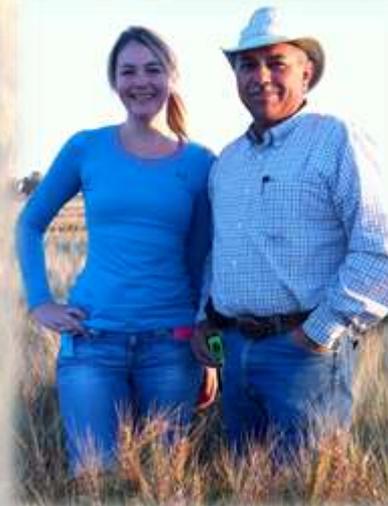
- Test RS in pasta
- Gut fermentation study in rodents
  - Cecal size, cecal pH, short chain fatty acids, gut peptides...
- Transfer and test mutations in common wheat



# Acknowledgments

## People

- **Dubcovsky Lab**
  - Jorge Dubcovsky
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  - Teng Vang
  - Janice Cooper
- **UC Research and Extension**
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  - Steve Wright
- **Regional Testing Program**
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  - Diane Prato-Mayo
  - Sam Fraser



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