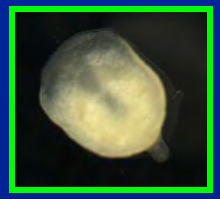
What has Genetic Engineering Taught Us about Wheat Quality?

Ann Blechl

10/20/10

USDA – Agricultural Research Service
Albany, CA

Genetic Engineering of Wheat



- Introduce DNA into embryo cells 10 daf
  - Sequence of DNA is known
- Occasionally (1/100) DNA integrates into a wheat chromosome
- Regenerate fertile plants from the embryo cells, selecting for those with new DNA
- New DNA is inherited
  - Becomes part of wheat's genome
  - The plant is said to be —trasgenic" or —trasformed"

Genetic Transformation vs. Traditional Breeding

- Allows introduction of genes from any source
- Changes one or a few known genes at a time
- One generation to recover original plant with change
- Allows tests of wheat gene & gene family function
  - Increase gene products by adding more gene copies
  - Decrease gene products by RNA interference

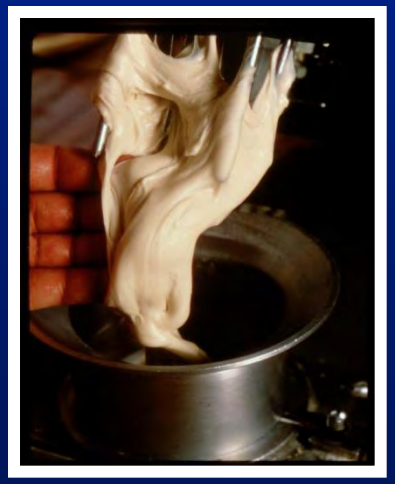
- Allows introduction of genes from sexually compatible relatives
- Half of genes in first generation hybrid are "new"
- 5-8 generations to recover original plant with change and nearby genes
- Mutations allow tests of gene function
  - If mutations in single genes have effects

Biotech (GE) (GM) Wheat and Baking Quality

- Genetic engineering allows us to change the levels of an individual wheat flour protein or of a family of wheat flour proteins
- Can increase levels by adding wheat gene copies
- Can decrease levels by RNA interference
- Changes in the levels of the appropriate proteins modulate mixing and baking quality
- The ultimate goal is predictable robust performance

Wheat Processing Quality

- Dough Handling Properties



Wheat End-use Properties Depend on Seed Proteins

- Total amount of protein
  - Nitrogen fertilizer, environment
  - Genes that affect grain maturation

Breeders introduced a chromosome segment from wild emmer wheat into domesticated durum wheat

Durum cultivar Langdon

Langdon + emmer wheat segment

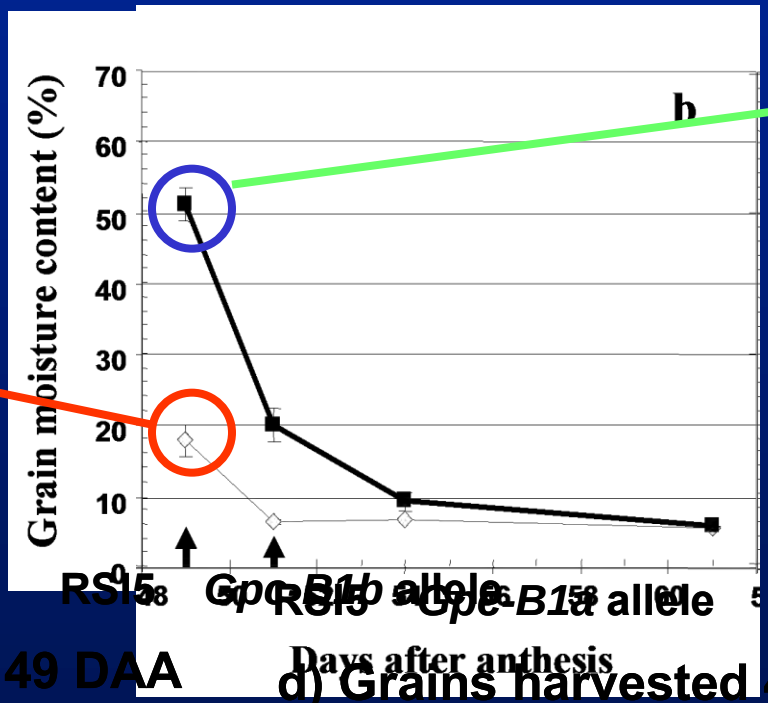
- Higher grain protein content
- Same time to anthesis, but shorter duration of grain fill due to earlier grain maturity

titution Lines

c) Water content 6 spikes

RSI5 *Gpc-B1a* allele

Langdon +
Wild emmer



RSI5 *Gpc-B1b* allele

LDN

c) Water content 6 spikes

RSI5 *Gpc-B1a* allele

DIC

RSI5 *Gpc-B1b* allele

Langdon

LDN

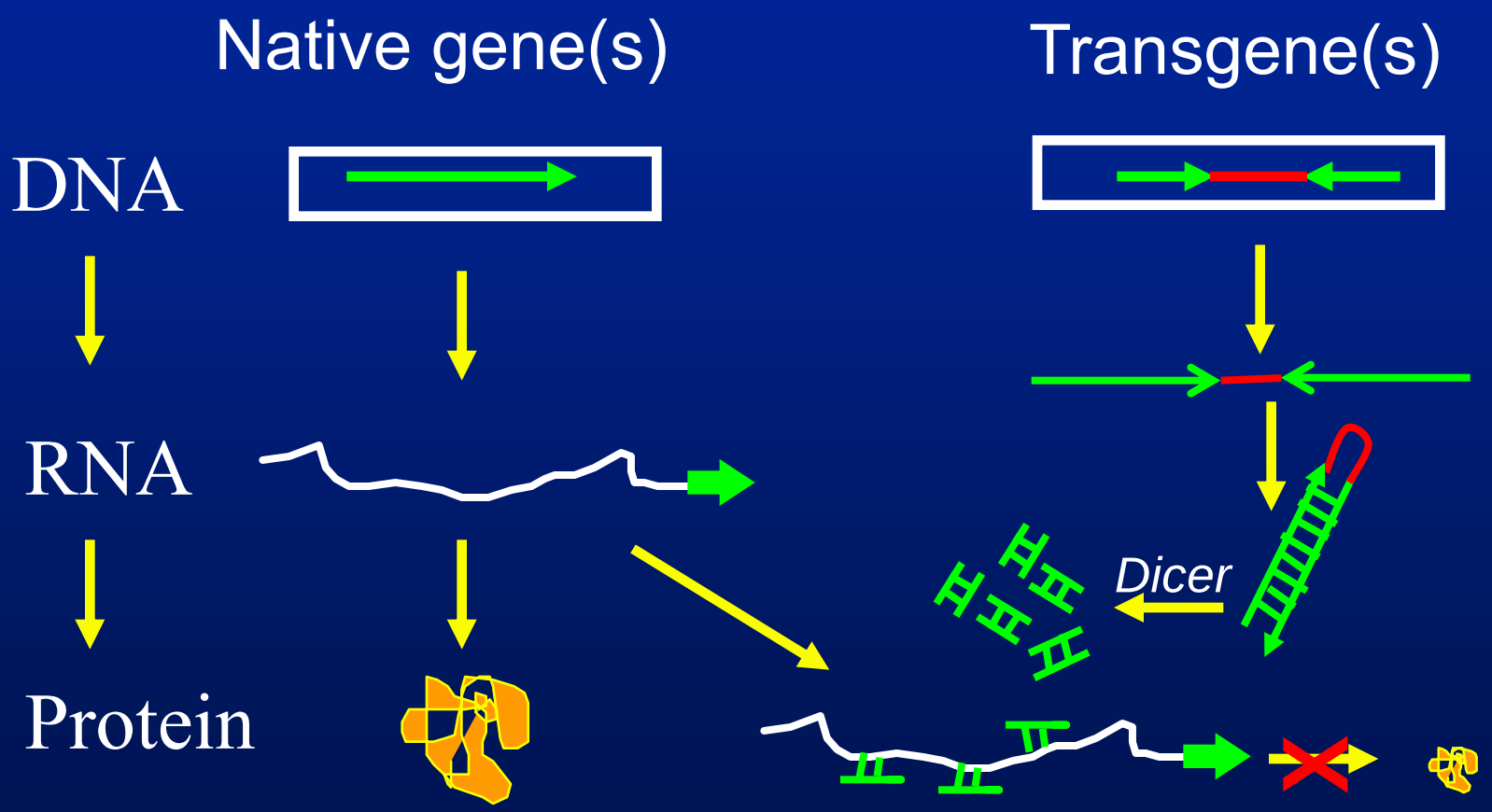
d) Grains harvested 49 DAA

d) Grains harvested 49 DAA

To isolate the individual gene and study how it works

- Cristobal Uauy, Daolin Fu and Jorge Dubcovsky (U. California – Davis) made a fine map of the emmer wheat DNA region
  - Linking DNA tags to the trait
- Isolated and sequenced DNA regions
  - Picked out candidate gene
    - Protein encoded predicted from sequence
    - Discover when and where the gene is expressed
- To prove their gene identification was correct, my lab transformed a version of the candidate gene designed to decrease its expression in bread wheat

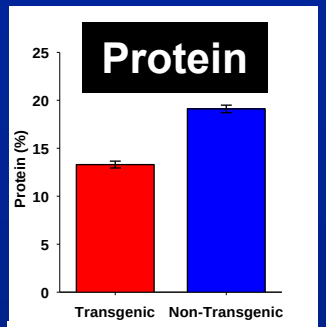
RNA Interference to Decreases Gene Expression

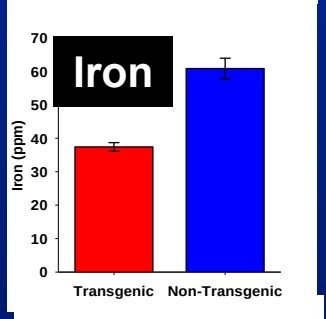


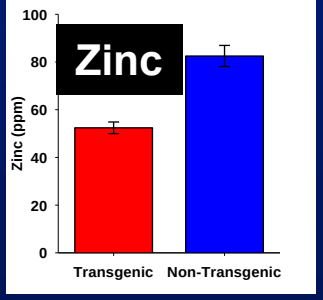
Grain Protein Content Gene:

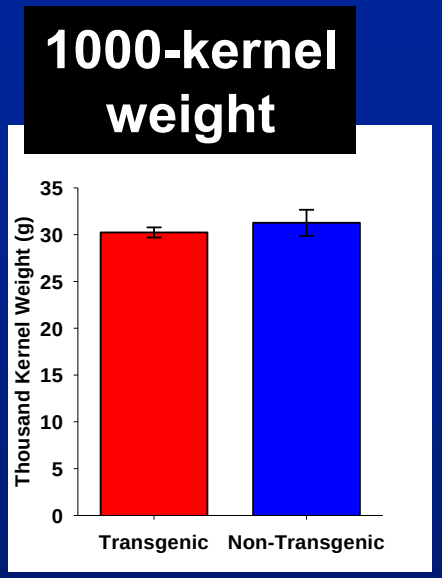
Effects of Decreasing Candidate Gene Expression

Extends time for grain maturation









No change in kernel weight

Lowers grain protein, iron and zinc contents

Summary

- A single gene from wild emmer wheat accelerates maturation and nutrient remobilization to the grain.
  - Increases grain protein, zinc and iron
- The DNA sequences of the wild emmer gene has been used to design molecular markers for use by breeders.
- Jorge Dubcovsky is releasing durum and bread wheat cultivars with these genes in California-adapted varieties

Protein Determinants of Wheat End-Use Properties

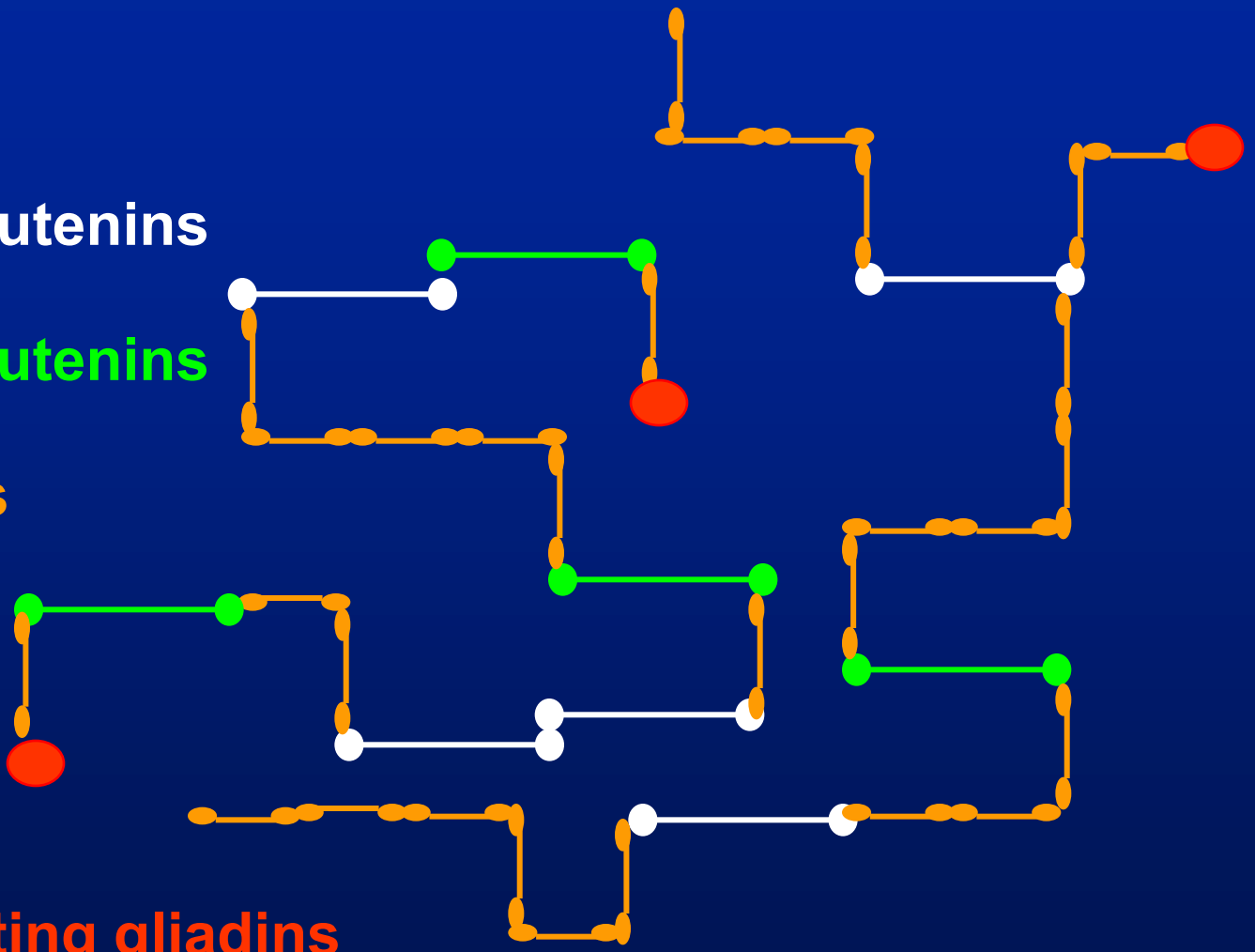
- Total amount of protein
  - Nitrogen fertilizer, environment
  - Genes that affect grain maturation
- Amount of protein found in polymeric network
  - ratio of glutenins to gliadins
- Size distribution of polymeric protein
  - Glutenin composition

The gluten polymer is the backbone of dough

x-type HMW-glutenins

y-type HMW-glutenins

LMW-glutenins

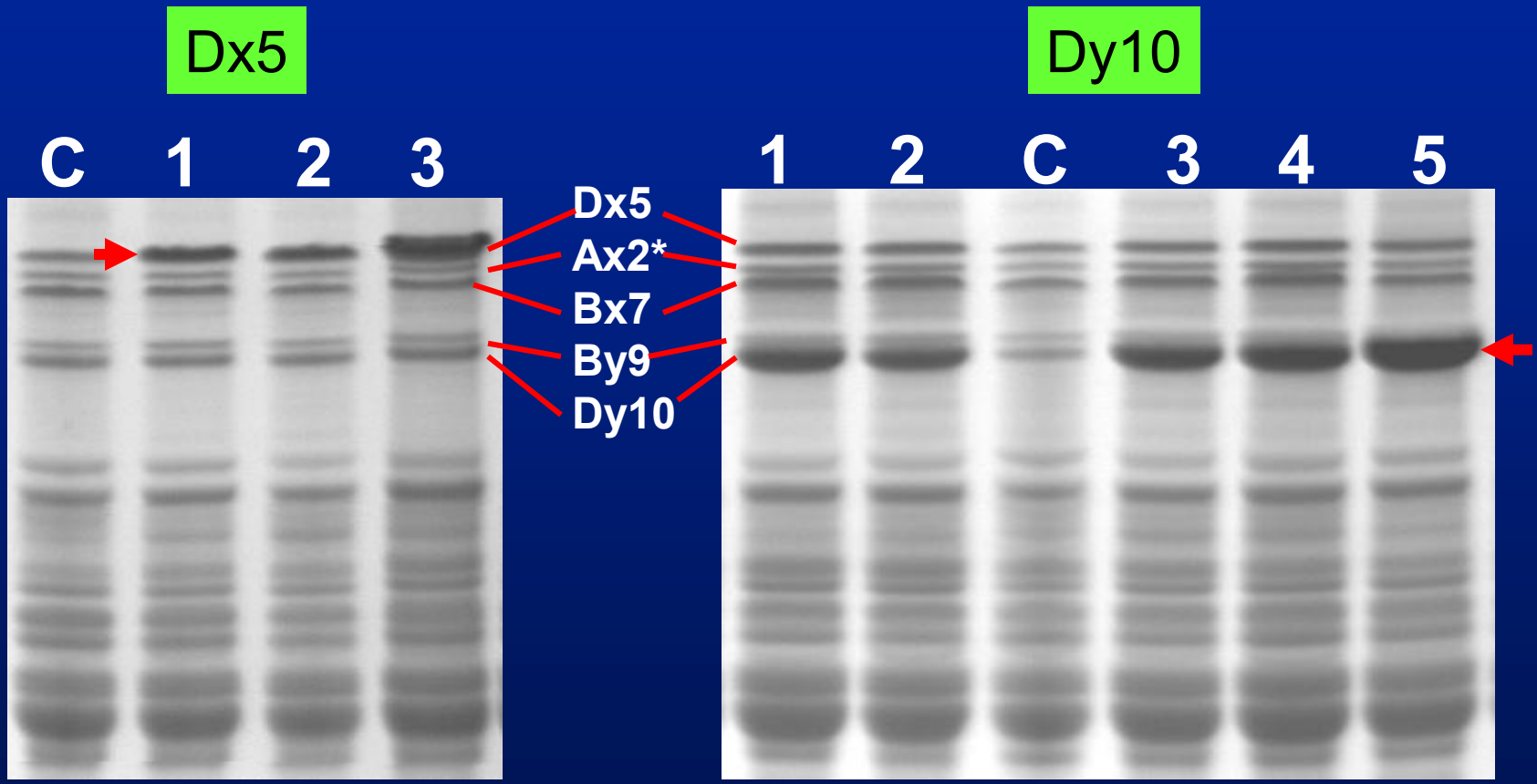


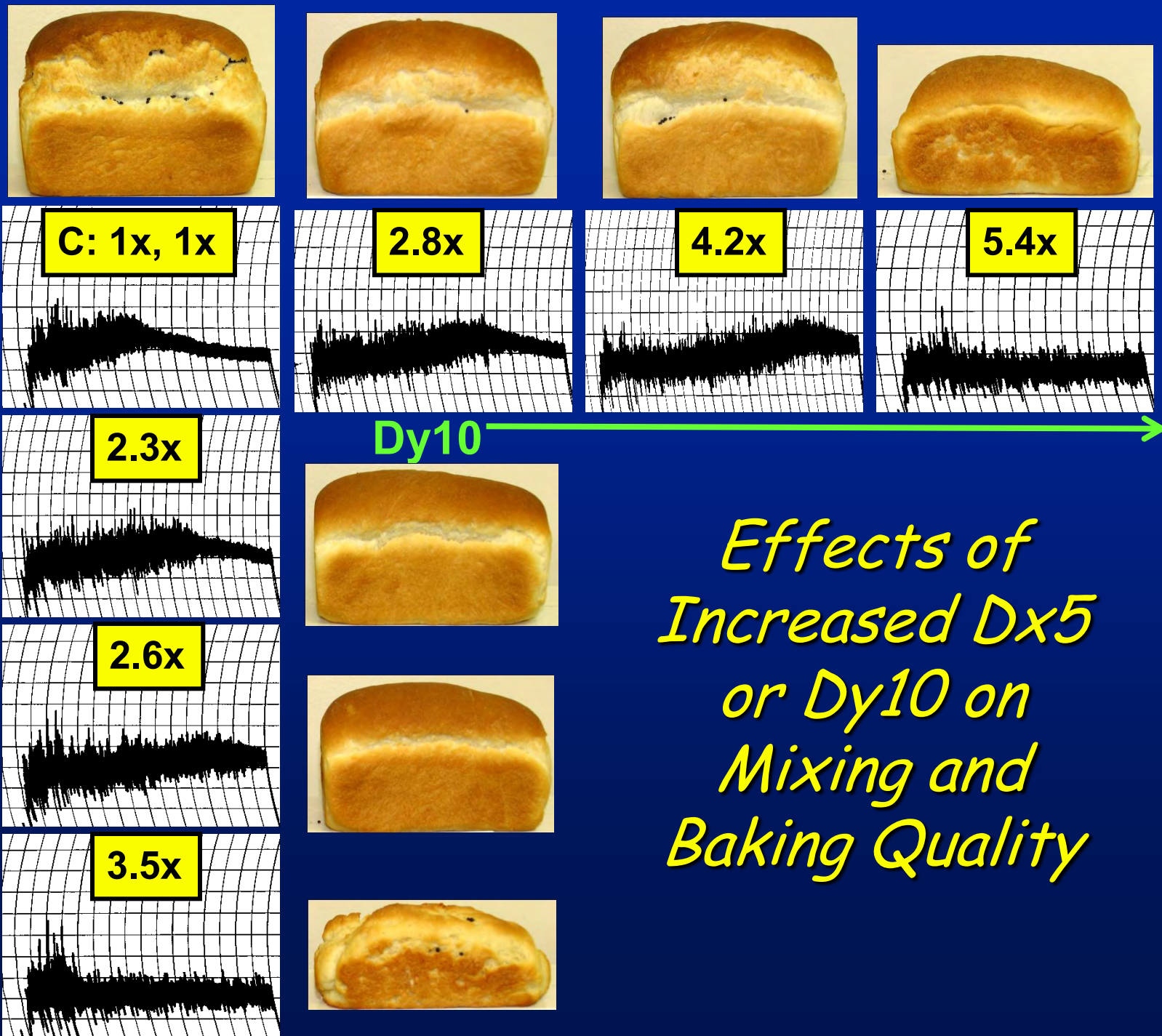
Chain-terminating gliadins

HMW-glutenin subunits Dx5 and Dy10

- Encoded by separate closely linked genes
  - Always inherited together
- The Dx5/Dy10 pair is associated with superior bread-making potential compared to other alleles
- Dx5 has potential for one more linkage than other x-type subunits
- Aim to understand their separate contributions

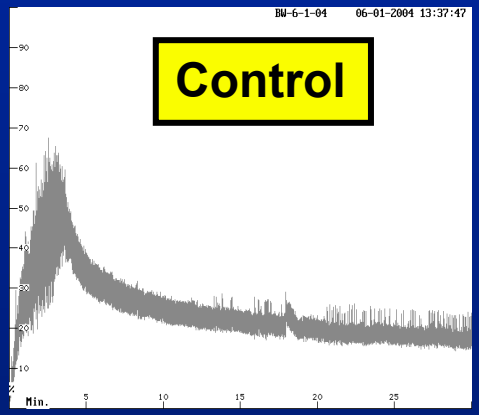
Seed Proteins of Transgenic Bread Wheats with Extra Copies of the Genes Encoding Dx5 or Dy10

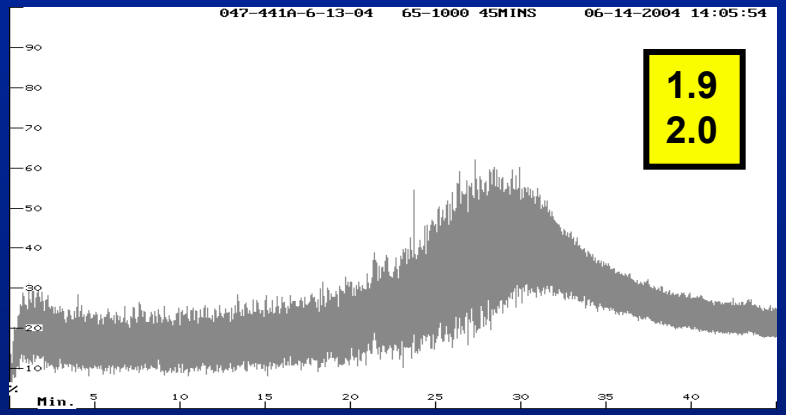




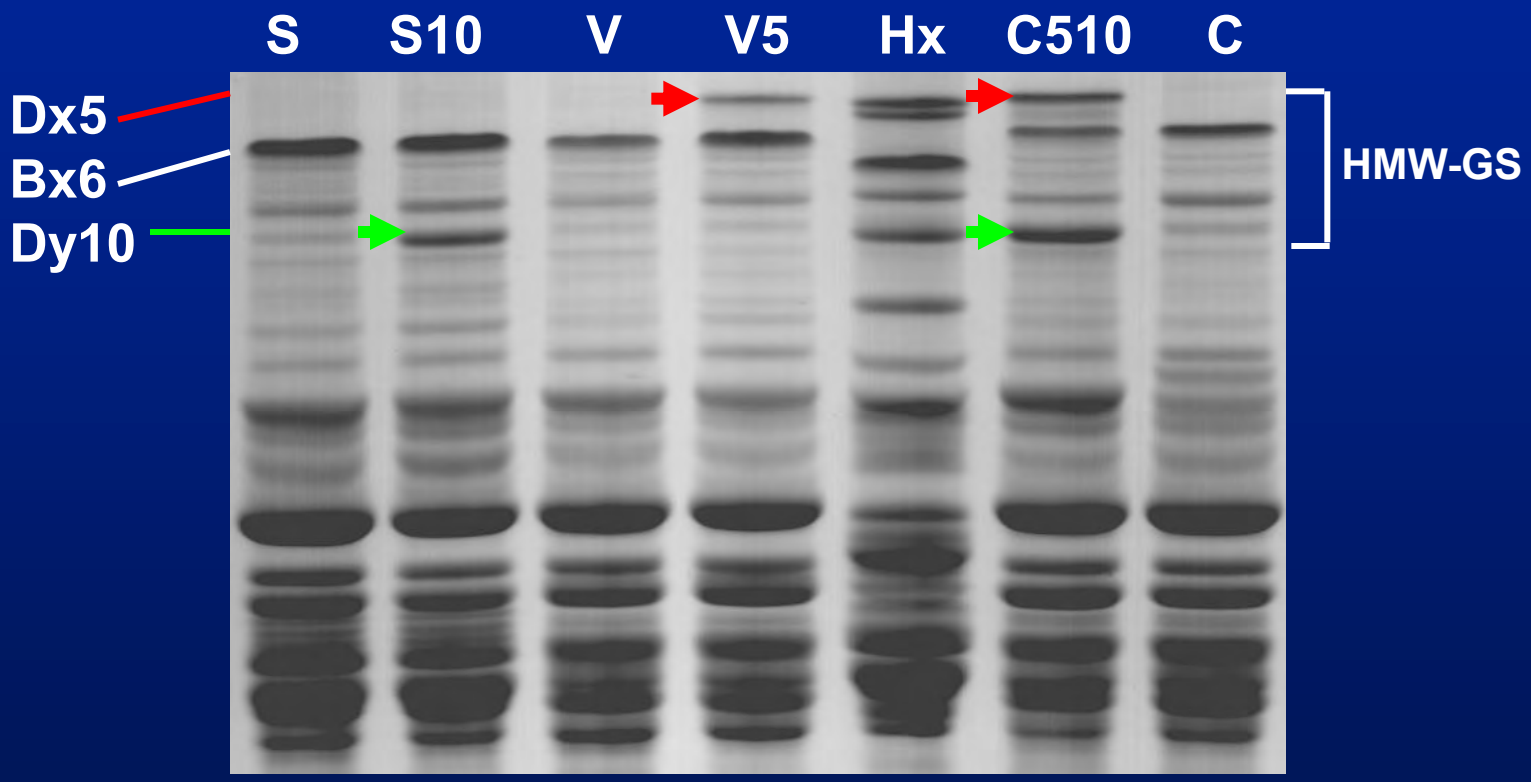
Effects of Increased Dx5 or Dy10 on Mixing and Baking Quality

2-gram mixograms of transgenic with balanced Dx5 and Dy10 levels





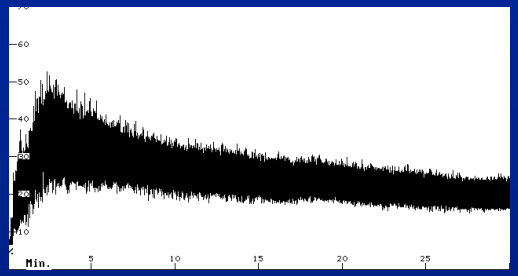
Seed Proteins of Transgenic Durum Wheats and Their Parents

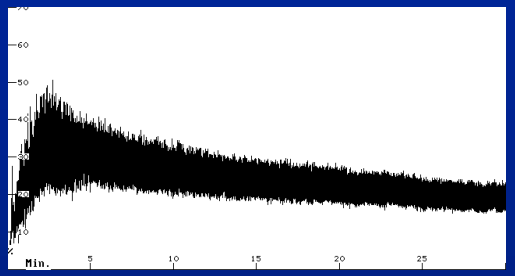


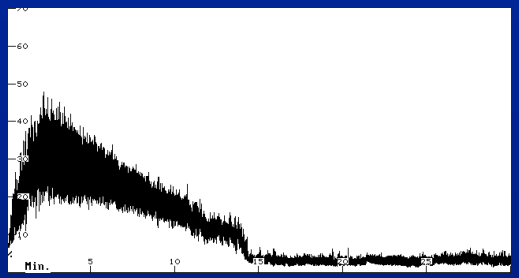
Agata Gadaleta – U. Bari, Italy

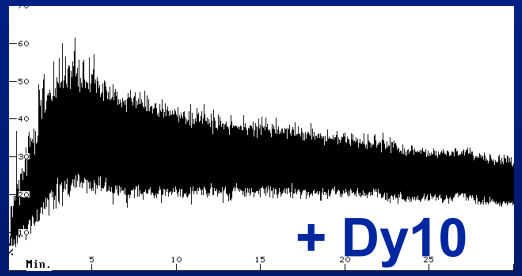
Representative 2-gram mixographs

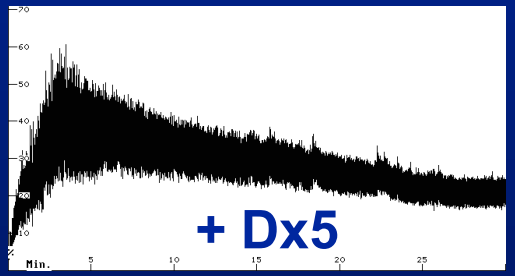
Svevo **Varano** **Creso**

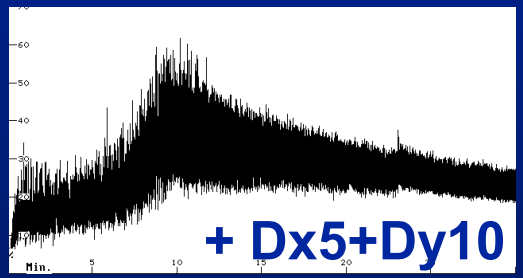












Gadaleta et al. (2008) Molecular Breeding 22:267-279.

Bread Loaves from Field-Grown Transgenic Durum Wheat

Rugby Lebsock Alzada Creso Svevo Varano

Creso Dx5+Dy10

Varano Dx5

Svevo none Dy10

What we've learned so far

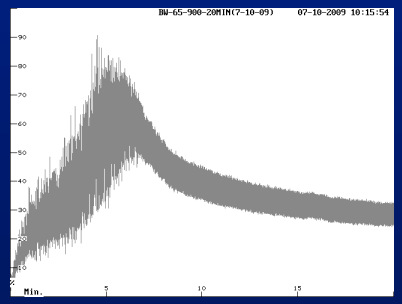
- Small increases in Dx5 and/or Dy10 increase mixing tolerance, especially in durum and bread wheat varieties with poor mixing tolerance
- Increases in Dx5 and/or Dy10 increase mixing strength
- Only small increases (<1.5x) in Dx5 can be made without affecting loaf volume
- Small to medium (3-4x) increases in Dy10 improve mixing properties without affecting loaf volume

Wheat Varieties Containing Rye Translocations

- Introduced by breeders to bring in multiple pest resistances
- Increased yield

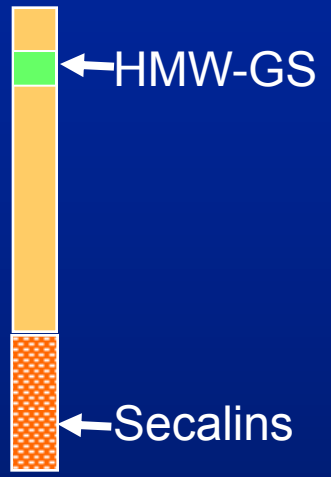
BUT often inferior quality

- Poor mixing tolerance
- Sticky doughs
- Low loaf volumes

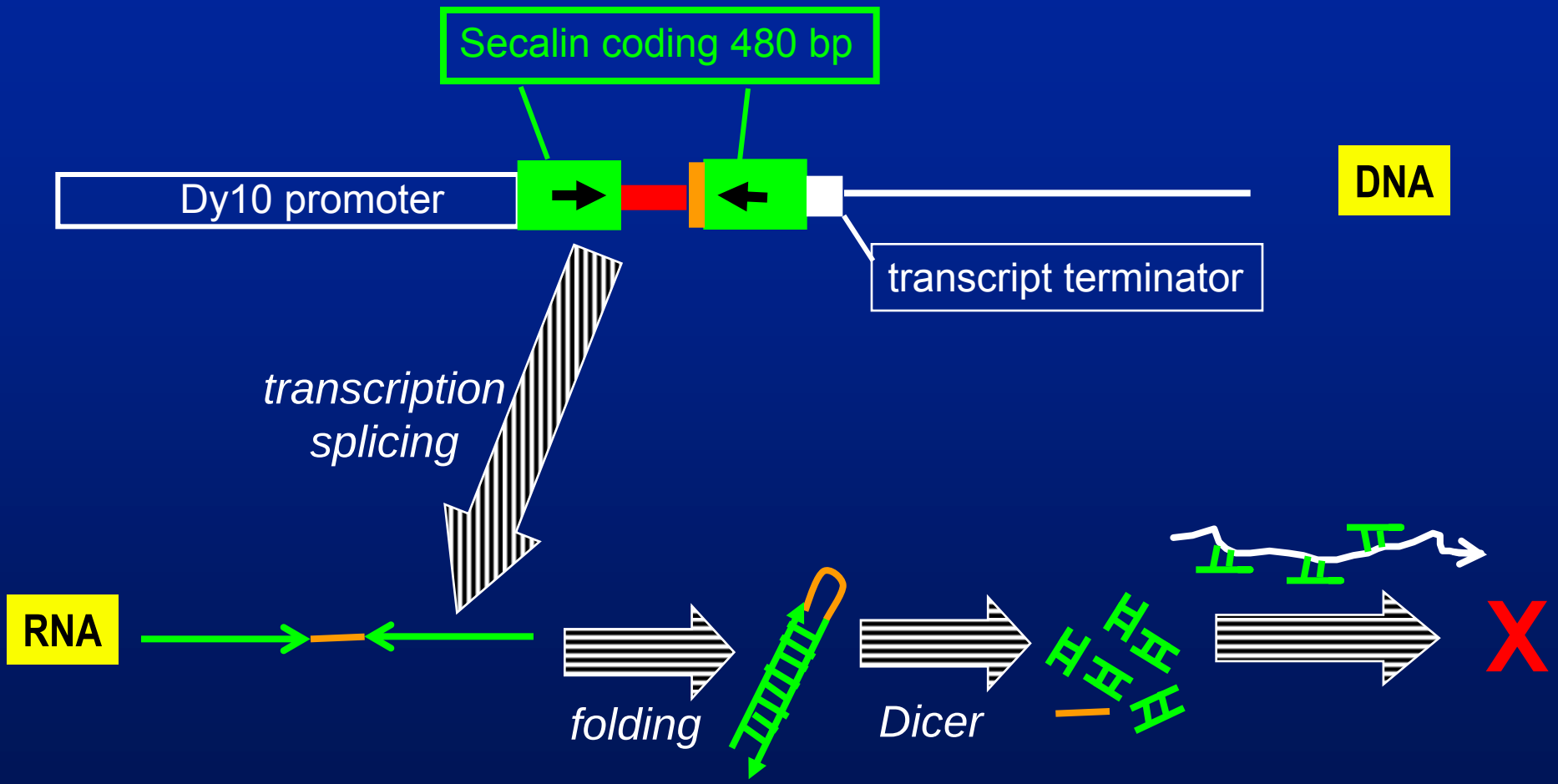


Rye translocations: stickiness factor(s)

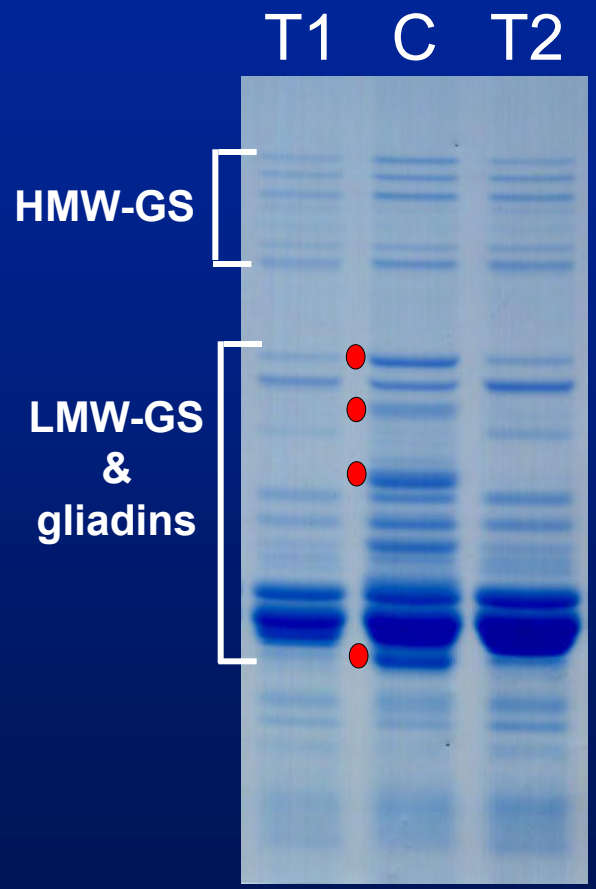
- Presence of rye seed storage proteins (omega secalins)
- Absence of wheat proteins encoded by *Glu-3* locus
  - LMW-GS, omega and gamma gliadins
- Reconstitution experiments suggest water-soluble
  - Omega secalins
  - Ferulic acid-linked carbohydrate
  - Pentosans



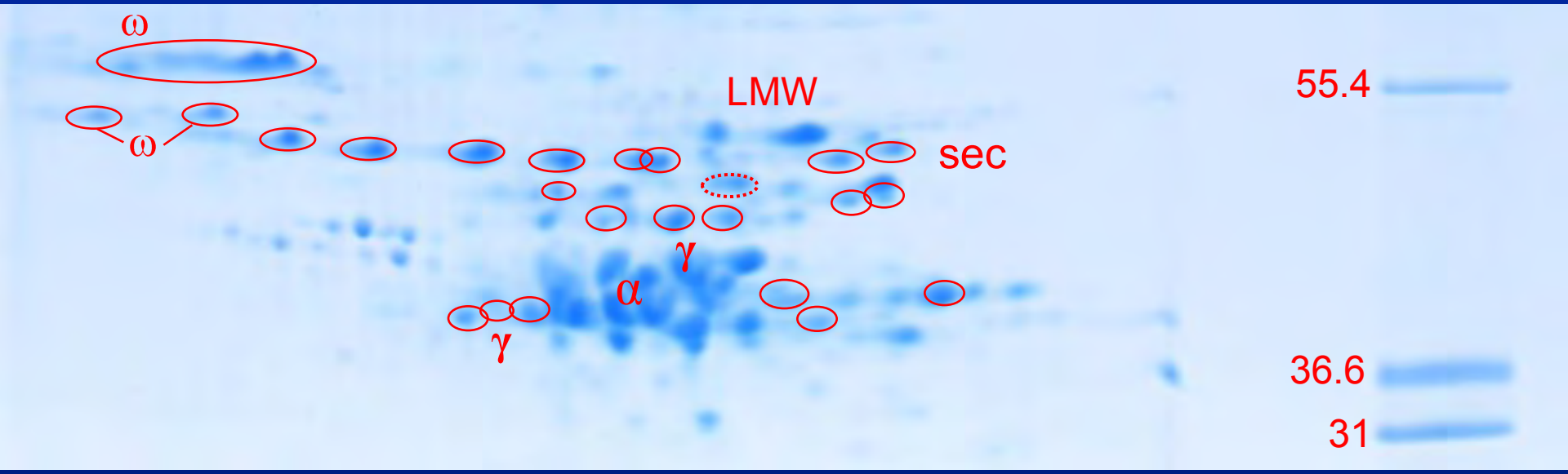
RNA interference (RNAi) construct

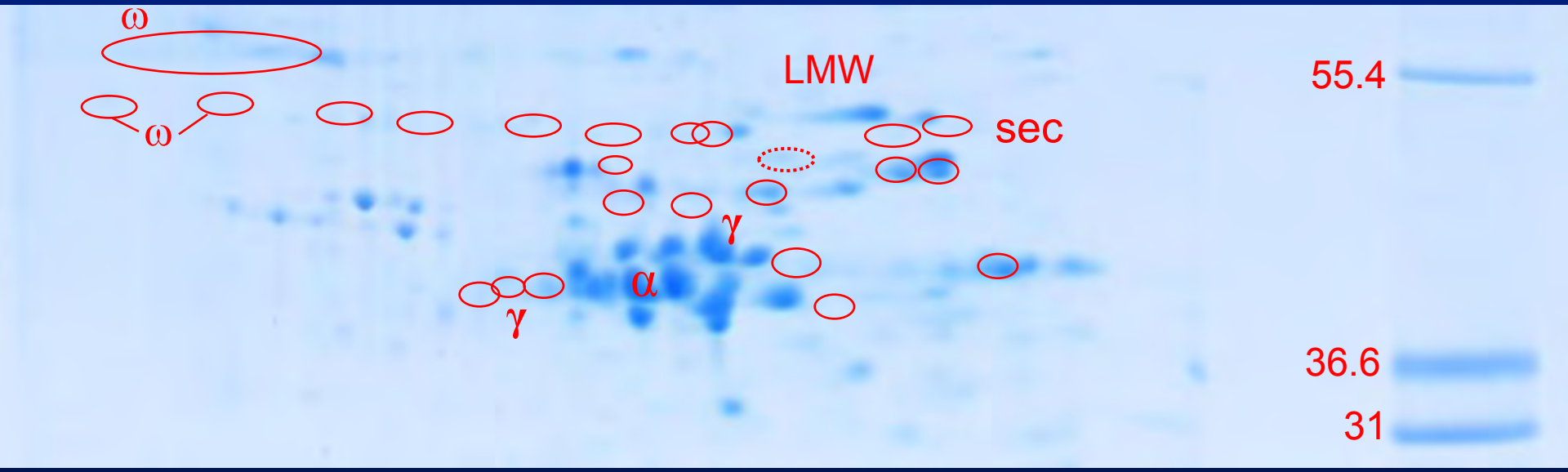


Effects of Secalin RNAi on Seed Proteins



Bobwhite



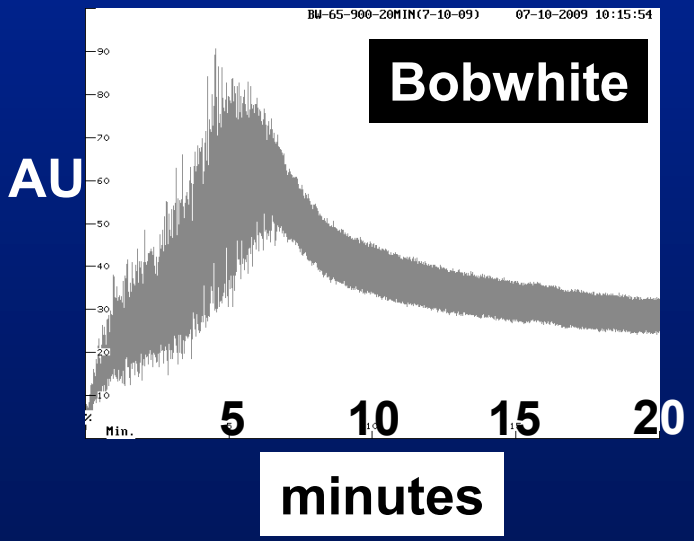


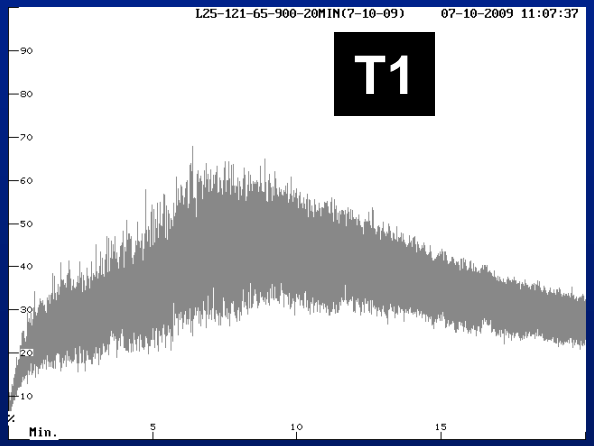
Transgenic

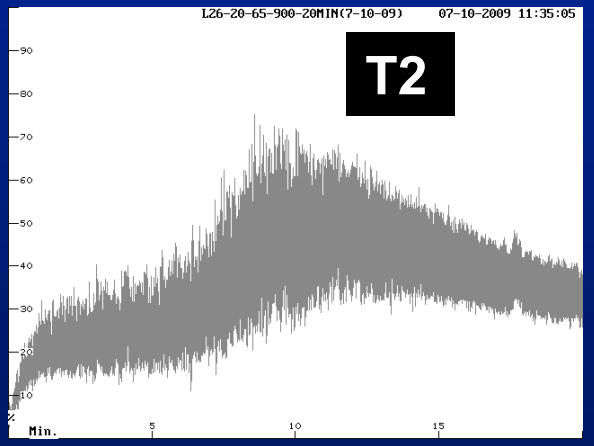
Quantitative Differences

- Secalins
  - Nine spots decrease 86-98%
- Omega gliadins
  - Four spots decrease 83-95%
- LMW-GS
  - Four spots decrease 8-47%
- Gamma gliadins
  - Three spots behave like omegas, nearly gone
  - Four spots behave like LMW-GS, decrease up to 50%
- Alpha gliadins
  - Five spots **INCREASE** 10-80%

2-gram Mixograms







Sticky doughs??

Field Trials: Goals

- Obtain sufficient seed quantities for measuring mixing and baking parameters in standard tests
- Evaluate agronomic characteristics of transformed wheat lines

2002: Three Locations

Phil Bregitzer and Doug Fiedler

ARS – USDA, Aberdeen ID

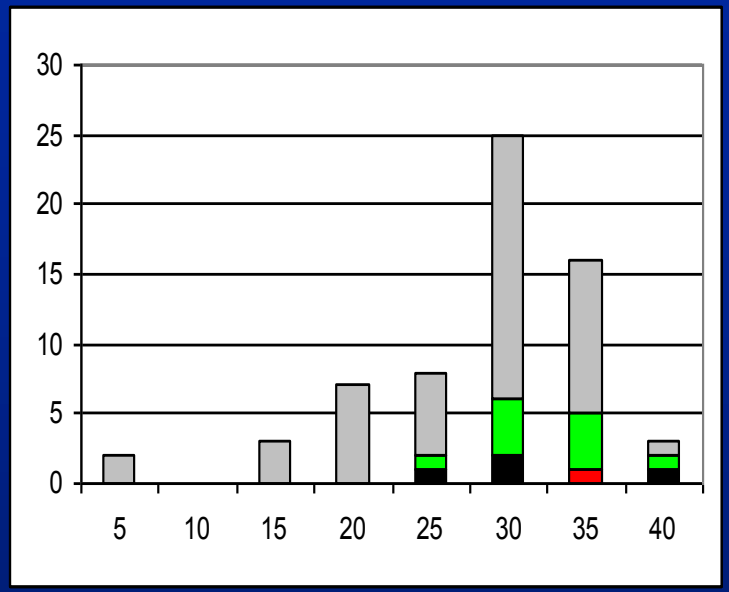
Jorge Dubcovsky and Oswaldo Chicaiza

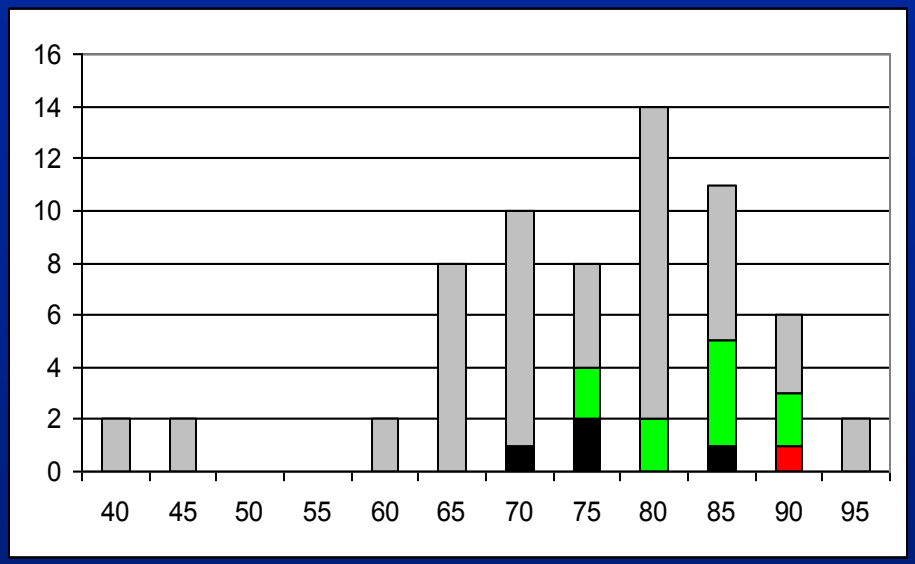
University of California - Davis

Paul Sebesta and Jose Fernandez de Soto

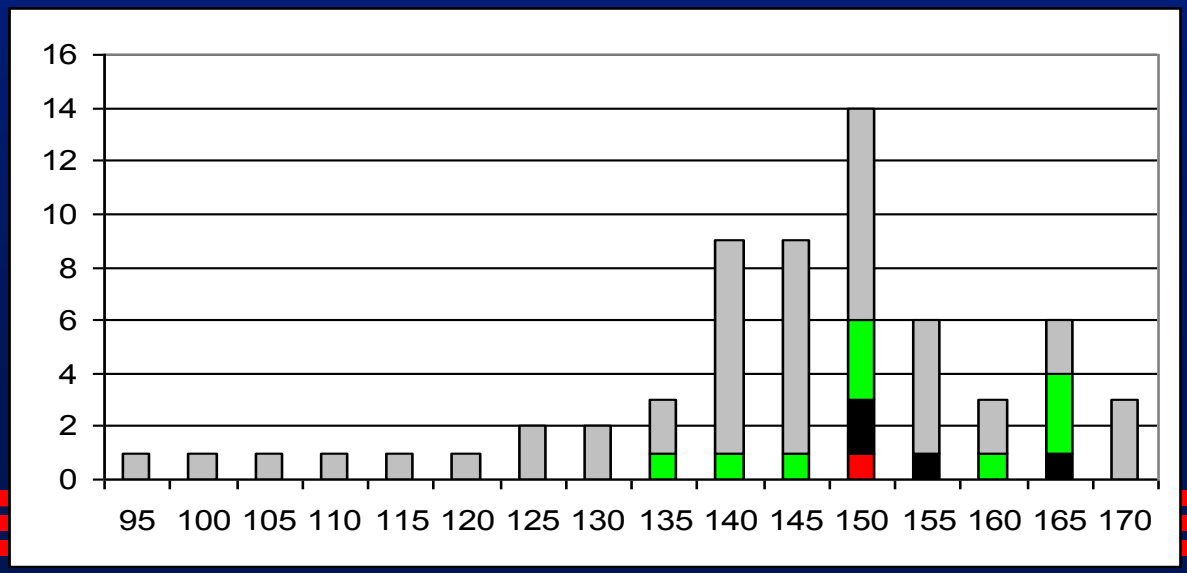
University of California – El Centro

Yields





Yield (bu/acre)



Summary of Field Trial Results

- We can make transgenic wheats with higher glutenin content without yield penalties or other detectable changes in growth and development.
- About 10% transgenic wheats have lower yields than their parent
  - Gene insertion is random
  - Presence of antibiotic- and herbicide-resistance marker genes used to identify transformed cells.

Active Wheat Transformation Programs

- U.S.
  - ARS, Albany, CA
  - Oklahoma State U. - Stillwater
  - U. Minnesota - St. Paul
  - Kansas State U. - Manhattan
  - Montana State U.- Bozeman
  - U. Florida - Gainesville
  - U. Nebraska - Lincoln
- Americas
  - Ag and Ag-Food Canada
  - CIMMYT, Mexico
  - Argentina
  - Chile
  - Brazil
- Europe
  - UK
  - Spain
  - Italy
  - Germany
  - Hungary
  - Czech Republic
  - Zurich
- Asia
  - China
  - India
  - Japan
- Australia
- Africa
  - Egypt

Problem: Genetic engineering is controversial

2007 Swiss authorities give GM wheat trials the green light
The Federal Environment Office has given Swiss scientists the go-ahead to carry out crop trials involving genetically modified (GM) wheat.

Foreign DNA

GMO's

2008 Jose Bové on hunger strike against GMOs
French activist and head of the farmers union Confederation Paysanne José Bové started a hunger strike demanding a m… GMO cultivation in France.

2007 Germany ends ban on Monsanto GMO maize type
A temporary sales ban on GMO-giant Monsanto's genetically modified MON810 maize was lifted after the company agreed to extra crop monitoring in Germany, German… announced.

2007 Paris, France — Greenpeace activists today hung a 12 by 3 metre banner reading —Ba GMO's NOW" on the Arc de Triomphe in Paris urging the French government to ban genetically modified organisms.

Frankenfood!

2007 EU wins WTO ex… n to end GMO bans
The European U… n extension
until… h the World
…ruling from last
…se which pitted the EU
…ne United States and other GM crop
…over its bans on GMO imports
…l bans as well as a general
approvals illegal… nder WTO rules.

2007 Three million people vote to make Italy GM-free
More than 3 million Italians have signed a petition … to ban all genetically modified foods.
producin…

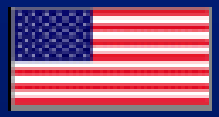
2007 West Australia considers GM cotton for the Ord
The Western Australian government says it will consider an exemption to its genetically modified crop ban, to allow GM cotton to be grown in the Ord Vall…

Unnatural!

Trilateral Statement on Wheat Biotechnology Commercialization

May 14, 2009

"In light of these resolutions, we will work toward the goal of synchronized commercialization of biotech traits in our wheat crops. While none of us hold a veto over the actions of others, we believe it is in all of our best interests to introduce biotech wheat varieties in a coordinated fashion to minimize market disruptions and shorten the period of adjustment. We are also committed to working with other stakeholders to address their needs and concerns as we travel the road to commercialization."



National Association of Wheat Growers
U.S. Wheat Associates
North American Millers' Association

Grain Growers of Canada
Western Canadian Wheat Growers Association
Alberta Winter Wheat Producers Commission

Grains Council of Australia
Grain Growers Association
Pastoralists and Graziers Association of Western Australia (Inc.)

"Cis-genics and Intra-genics": Strategies that address consumer and regulator concerns

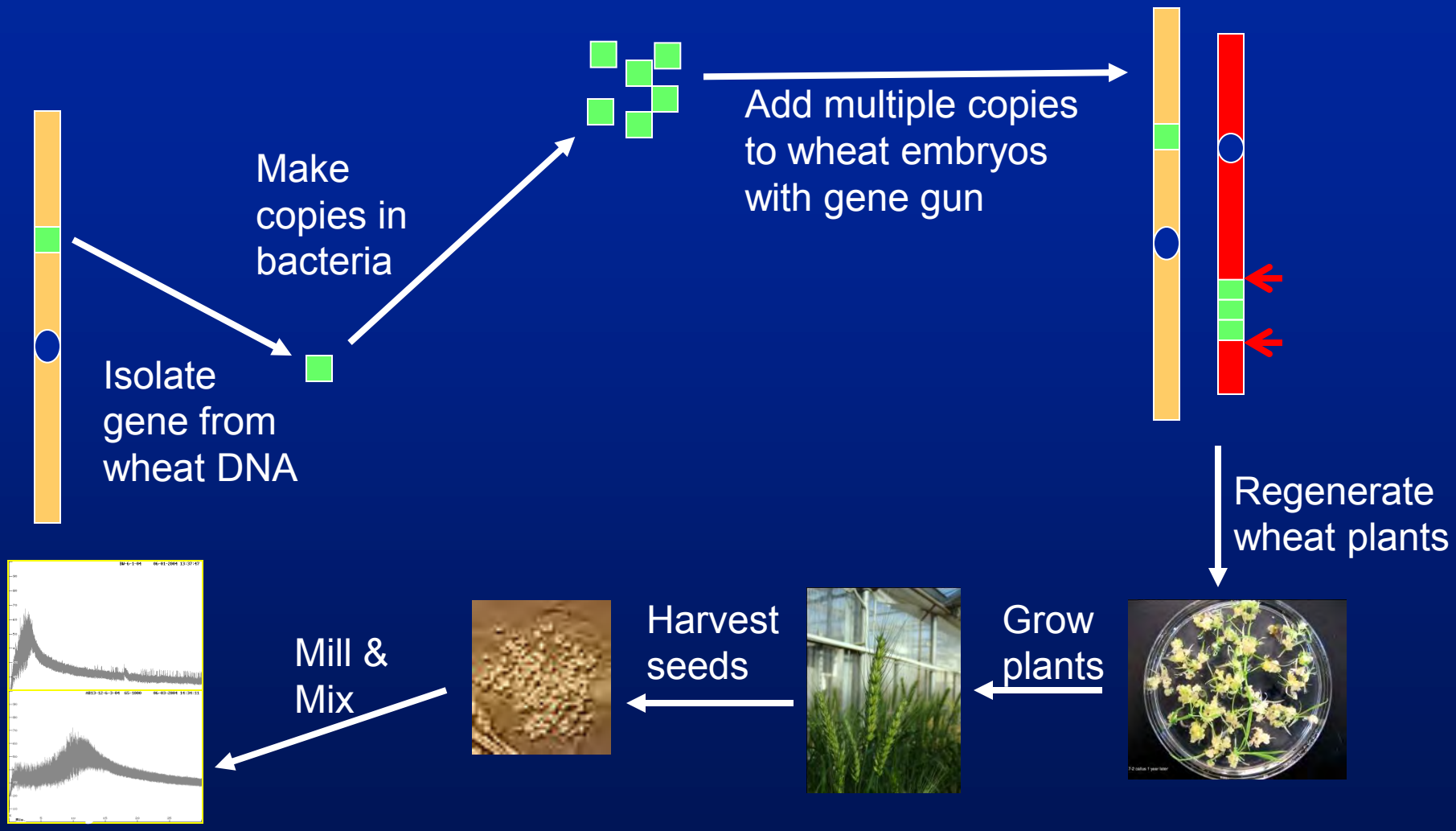
- Refinements in transformation technology
- Make new transformed wheat plants using "intragenic" or "cisgenic" methods
- Final plant will contain only wheat DNA and wheat proteins
- For gluten strength, introduce more copies of wheat Dy10 or Dx5+Dy10 genes
- Transformation technically more challenging, but investment of time up front could save time and trouble later

Intragenics: All native DNA

- Final plant only has wheat DNA

- In contrast to "transgenics"
- No antibiotic or herbicide resistance genes in final plant
- More precise than traditional breeding because only known DNA added
- Not yet clear how such plants will be regulated by U.S. regulators FDA and APHIS
- New DNA junctions yield DNA tags unique for each line
  - Inheritance of new genes can be followed by breeders
  - Plants, seeds, and flours can be identified in marketplace

Example: Using intragenic biotechnology to increase wheat gluten strength



Wheat genomics research is providing the research community with more and more useful wheat genes

- Gluten proteins (dough strength and elasticity)
- Grain Protein Content
- Starch properties
- Grain hardness
- Genes that control flowering timing in response to cold and/or day length
- Disease Resistance
- Yield components

All are candidates for making changes using Intragenics

Acknowledgements

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- Susan Altenbach
- Olin Anderson

ARS, Albany, CA

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- Intramural USDA Research

Acknowledgements: Collaborators I

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ARS, Aberdeen, ID

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- Doug Fielder

University of California - Davis

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- Cristobal Uauy
- Daolin Fu
- Assaf Distelfeld

Acknowledgements: Collaborators II

California Wheat Commission

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Colorado State University

- Jim Quick

University of Bari, Italy

- Agata Gadaleta
- Antonio Blanco

ARS, Pullman, WA

- Brian Beecher

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