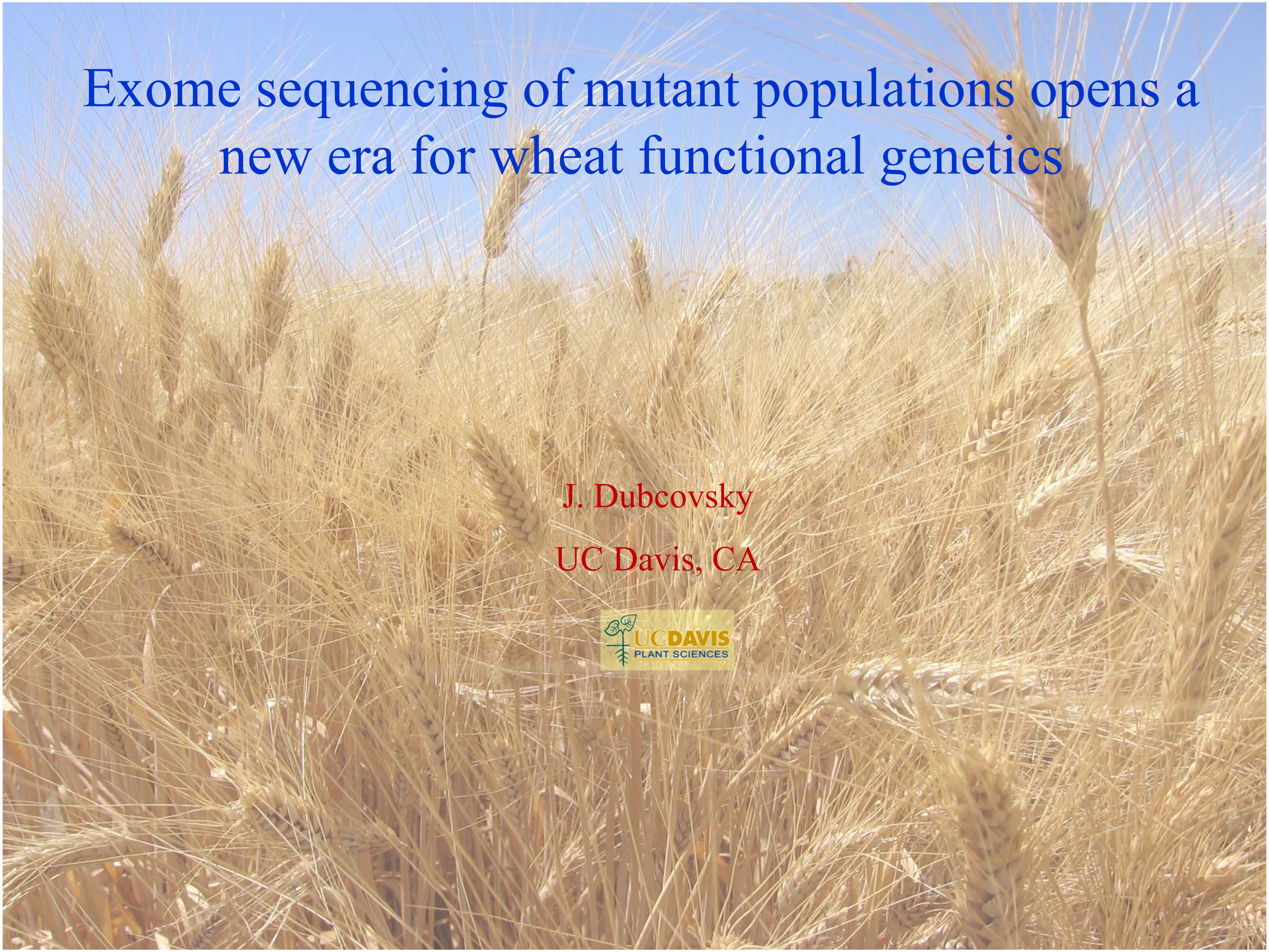

Exome sequencing of mutant populations opens a new era for wheat functional genetics

J. Dubcovsky

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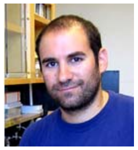
Acknowledgements

Tetraploid Team @ UCDavis

K.V. Krasileva, H. Vasquez-Gross, F. Paraiso, X. Wang, T. Howell, J. Dubcovsky

Hexaploid Team @ John Innes Centre & The Genome Analysis Centre (TGAC)

P. Bailey, S. Ayling, A. Phillips (Rothamsted). UK leader: C. Uauy



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- Howard Hughes Medical Institute
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hhmi
Howard Hughes
Medical Institute

GORDON AND BETTY
MOORE
FOUNDATION

USDA NIFA
United States Department of Agriculture
National Institute of Food and Agriculture

Funded sets of 100 Kronos mutant lines.

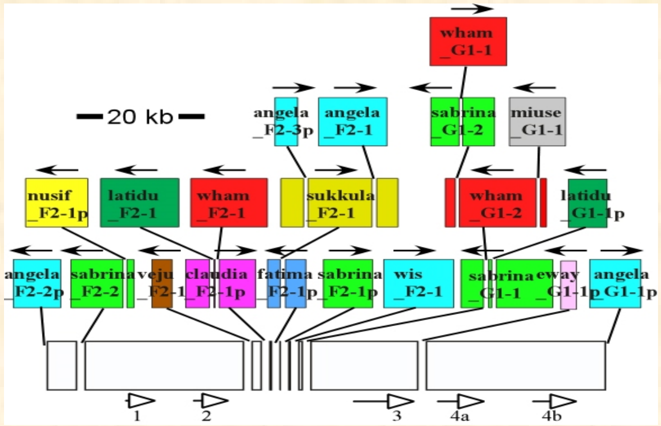
- USDA-ARS Cereal Disease Laboratory, St. Paul, MN
- Washington State University, Pullman WA
- Crop Development Center, University of Saskatchewan, Saskatoon, Canada
- Shandong Agricultural University, Tai’an, Shandong, China.

Wheat exome capture

- **Wheat genome had a large expansion** due to transposable elements (TE): high-copy no., ~sequence→ difficult assembly

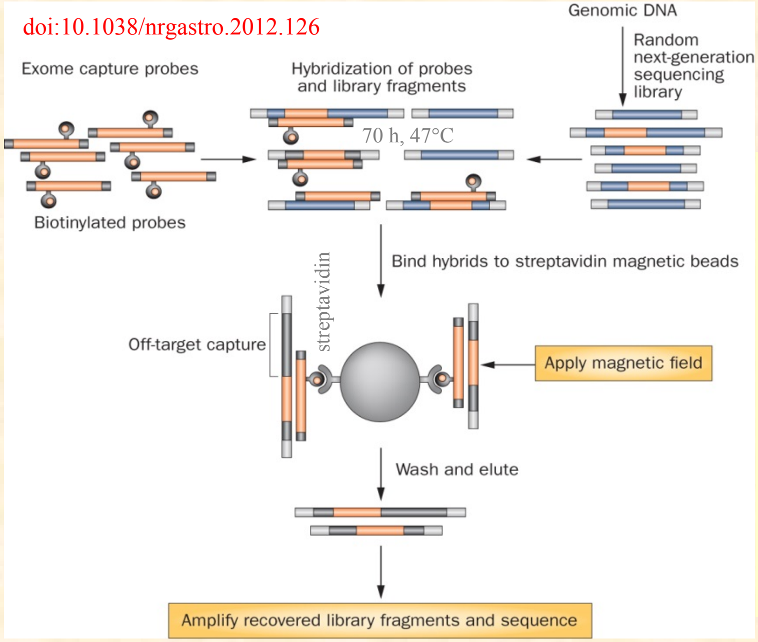
A. thaliana
- 135 Mb
- 55% repetitive

T. t. ssp. durum
- Genomes AABB
- 13,000 Mb
- **98% repetitive**



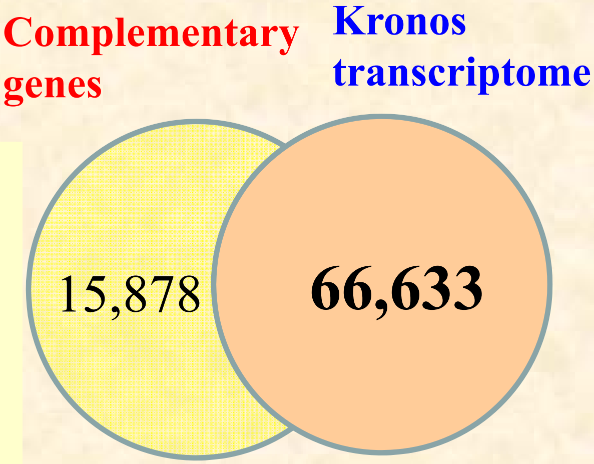
- **Exome Capture**

Collaboration with NimbleGen


doi:10.1038/nrgastro.2012.126

- 1.1 million wheat ESTs
- NCBI nr wheat genes
- *T. aestivum* full length cDNAs
- 4 *T. aestivum* transcriptomes
- Homologs to barley genes
- Annotated genes from our labs

Merged initial CD-HIT
Add non-redundant by CD-HIT2D t
Final CD-HIT EST 99% cutoff

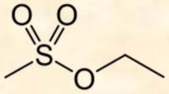


Total 82,511 genes

Genome Biology 2013, 14:R66

Exon capture re-sequencing of wheat TILLING populations

EMS treatment

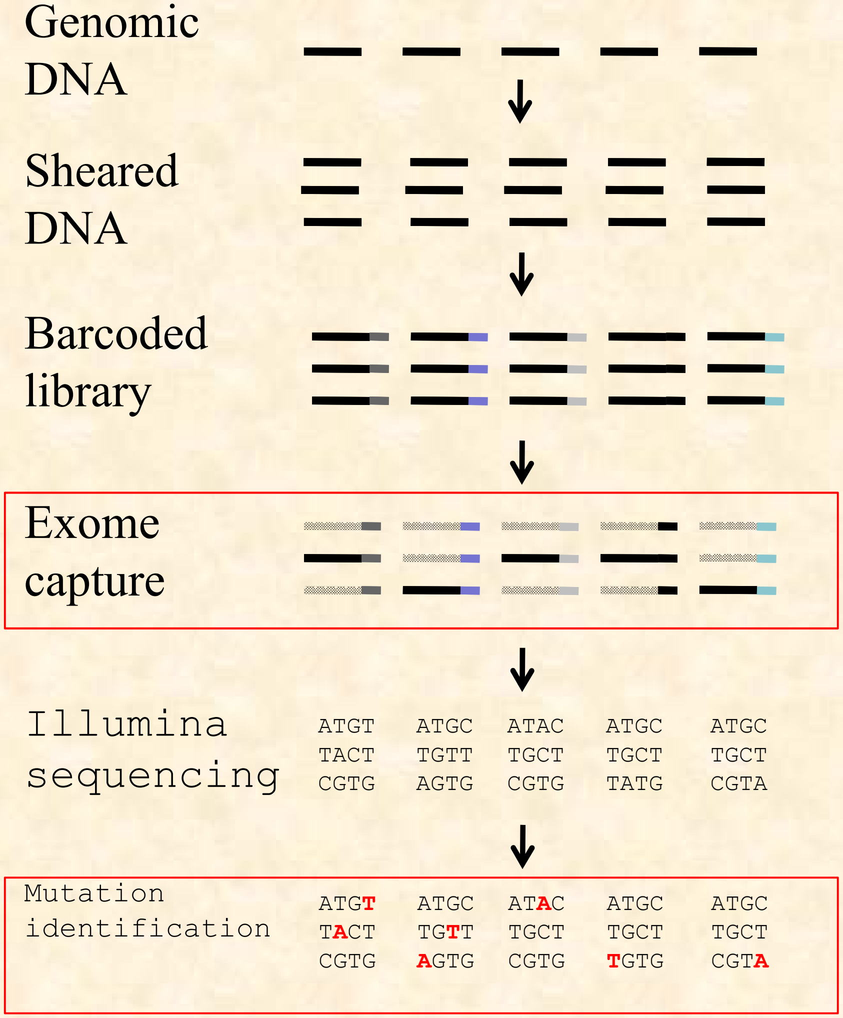


M1

M2

Genomic DNA

~ 2,000 lines (4x)
~ 1,800 lines (6x)



Genomic DNA

Sheared DNA

Barcoded library

Exome capture

Illumina sequencing

ATGT	ATGC	ATAC	ATGC	ATGC
TACT	TGTT	TGCT	TGCT	TGCT
CGTG	AGTG	CGTG	TATG	CGTA

Mutation identification

ATG**T**	ATGC	AT**A**C	ATGC	ATGC
T**A**CT	TG**T**T	TGCT	TGCT	TGCT
CGTG	**A**GTG	CGTG	**T**GTG	CGT**A**

MAPS pipeline to identify mutations

- Mapping by BWA with Illumina PE 200. Ensembl Reference + unmapped assembled contigs
- Polymorphism detection MAPS pipeline from Luca Comai Lab (we adapted it to polyploid wheat)
  - http://comailab.genomecenter.ucdavis.edu/index.php/MAPS
  - Published in ***Plant Cell*** 2014, 26:1382–1397

Noise:

Varietal SNP

Paralog/Homeolog SNP

Sequencing/PCR error

Off target sequences

Signal:

EMS Mutation

Reference
CAGTGTGCCCACCTGTGGCTTTTGACTAATGTGTACAGCAACGTA

Mutant 1
CAGGGTGCCGACCTGTGACTTTTGACAAATGTGTACAGCAAGGTA
CAGTGTGCCGACCTGTGACTTTTGACAAATGTGTACAGCAACGTA
CAGTCTGCCGACTTGTGGCTTTTGACAAATGTGTATAGCAACGTA
CAGTCTGCCGACTTGTGGCTTTTGACAAATGTGTACAGCAACGTA

Mutant 2
CAGTGTGCCGACCTGTGACTTTTGACAAATGTGTACAGCAACGTA
CAGTGTGCCGACCTGTGACTTTTGACAAAAGTGTACAGCAACGTA
CAGTCTGCCGACCTGTGGCTTTTGACAAATGTATACAGTAACGTA
CAGTCTGCCGACCTGTGGCTTTTGACAAATGTGTACAGCAACGTA

Mutant 3
CAGTGTGCCGACCTGTGACTTTTGACAAATGTGTACAGCAAGGTA
CAGTCTGCCGACCTGTGGCTTTTAACAAATGTGTACAGCAACGTA
CAGTCTGCCGACCTATGGCTTTTAACAAATGTGTACAGCAACGTA
CAGTCTGCCGACCTGTGGCTTTTAACAAATGTGTACAGCAACGTA

Characterization of mutations

Tetraploid wheat TILLING status

614 Online with Mutations

942
Mapped

1250
Sequenced

1547
Captured

- Target **1,547** lines
- **Target 3 M** mutations

Hexaploid Wheat TILLING status

342
Mapped

573
Sequenced

1245
Captured

291 Captures
Pending

Target **1536** lines
Target 3 M mutations

Wheat TILLING populations

Tetraploid wheat cultivar KRONOS: USA, UC Davis. *Contact Jorge Dubcovsky*

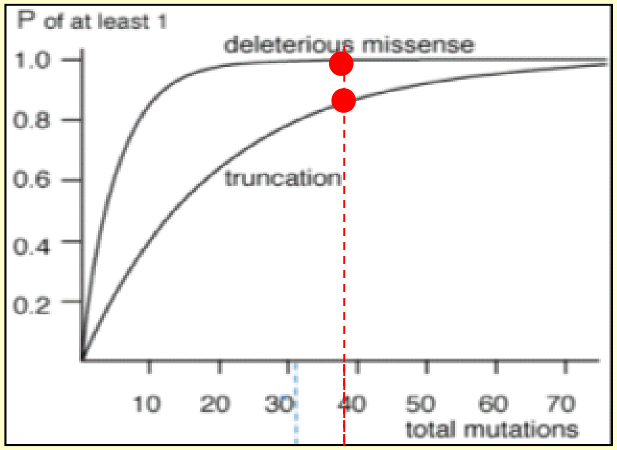
Hexaploid wheat cultivar CADENZA: UK, John Innes Centre/TGAC. *Contact Cristobal Uauy.*

Seeds from the mutants will be available at cost

Kronos mutagenized TILLING lines

Generation of new allelic variants

- Probability of truncations and deleterious missense mutations



Tetraploid wheat

- 1547 lines

~37 mutations/kb in all lines

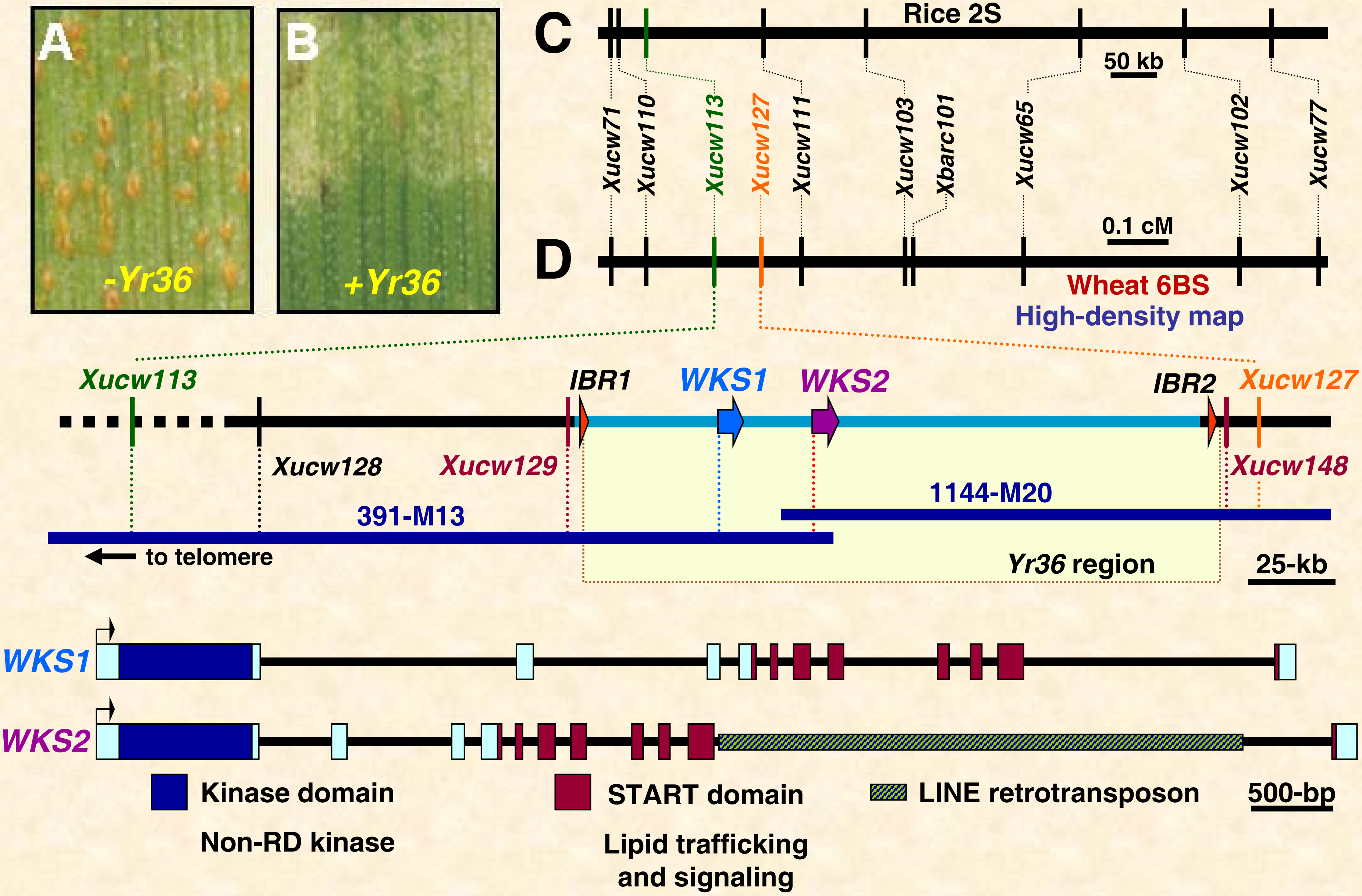
Hexaploid wheat

- 1,536 hexaploid lines

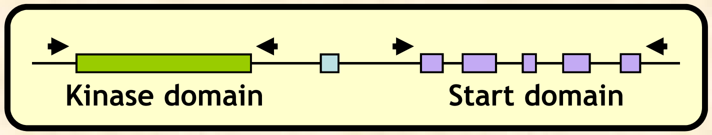
~42 mutations/kb in all lines

- The current mutation density is expected to produce amino acid mutations in almost every gene and truncations in ~80% of the targeted gene. Truncations are preferable in functional studies.
- TILLING has changed the paradigm of what can be done in functional genomics in wheat!
  1. **Validation candidate genes**
  2. **Pathway engineering**
  3. **Pathway dissection**

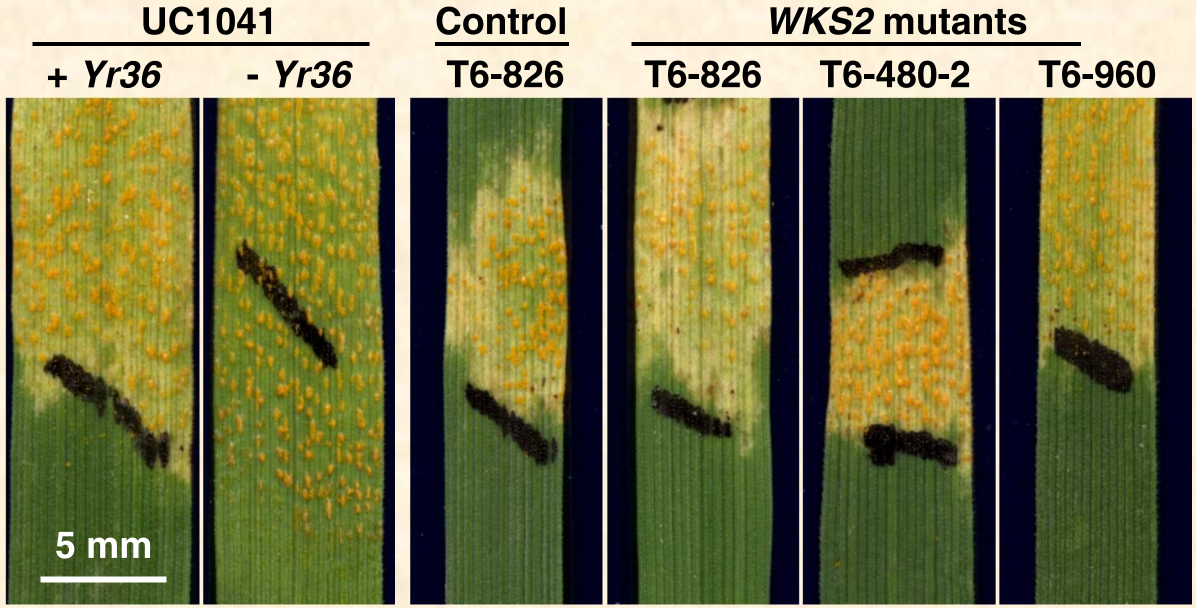
1. Use of TILLING mutants to validate candidate genes



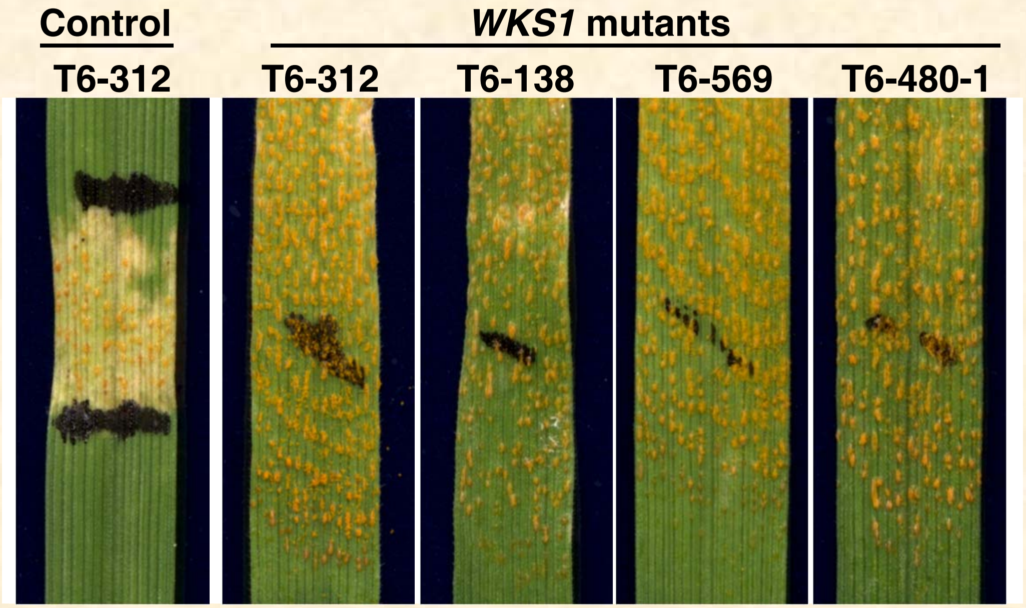
WKS1 validation by TILLING



- 2 genes and 2 domains
- 117 mutants detected,
- 9 mutants selected



WKS2 is not essential to confer resistance

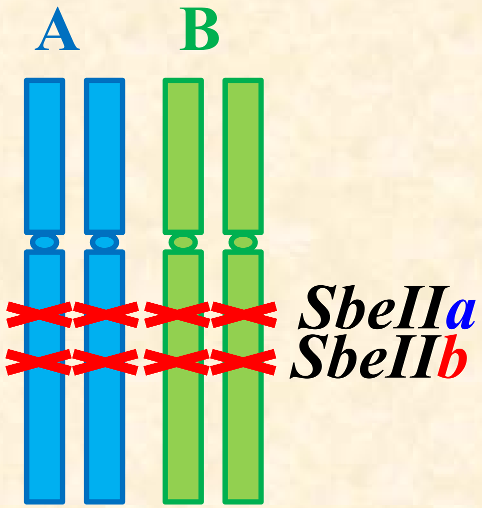


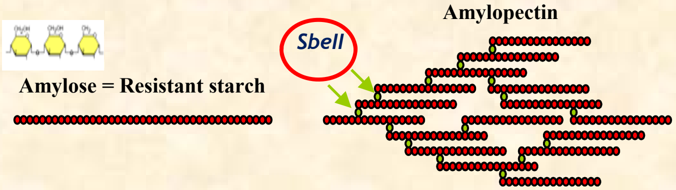
Mutations in WKS1 kinase or START domains result in susceptible plants

***WKS1* is required for resistance**

Science. 2009. 323:1357-1360

2. Engineering the starch biosynthetic pathway





Knock out **all** ***SbeII***

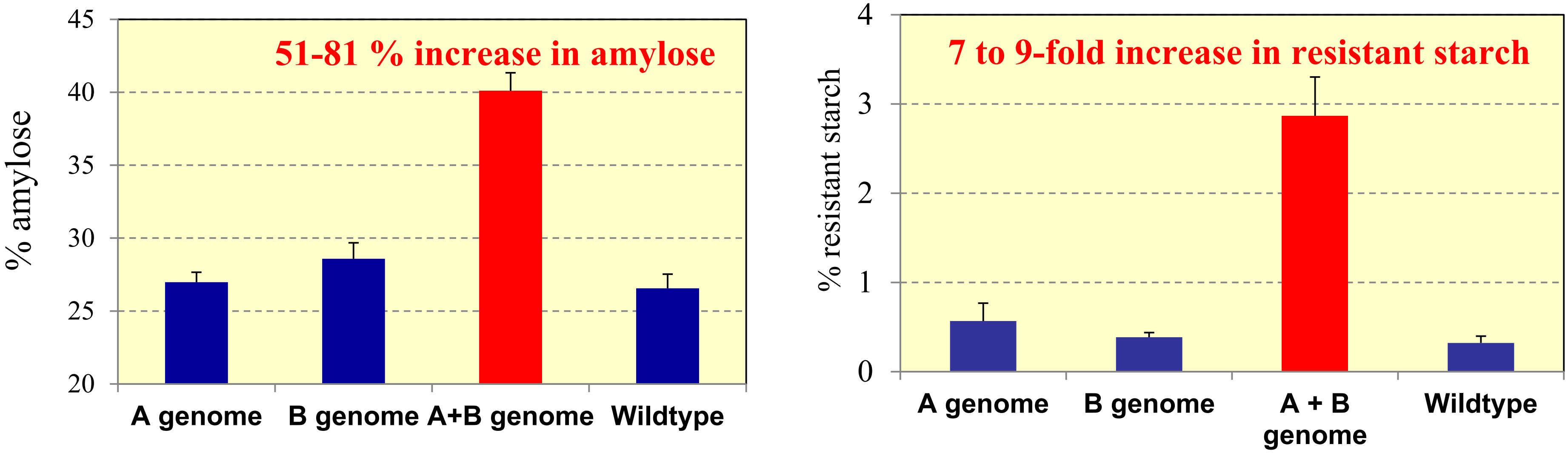
- Found mutations in *SbeIIa* & *SbeIIb* genes
- Backcrossed 2 generations
- Combined the 4 mutations
- Introgressed into commercial varieties (6 BC)

Crop Science.

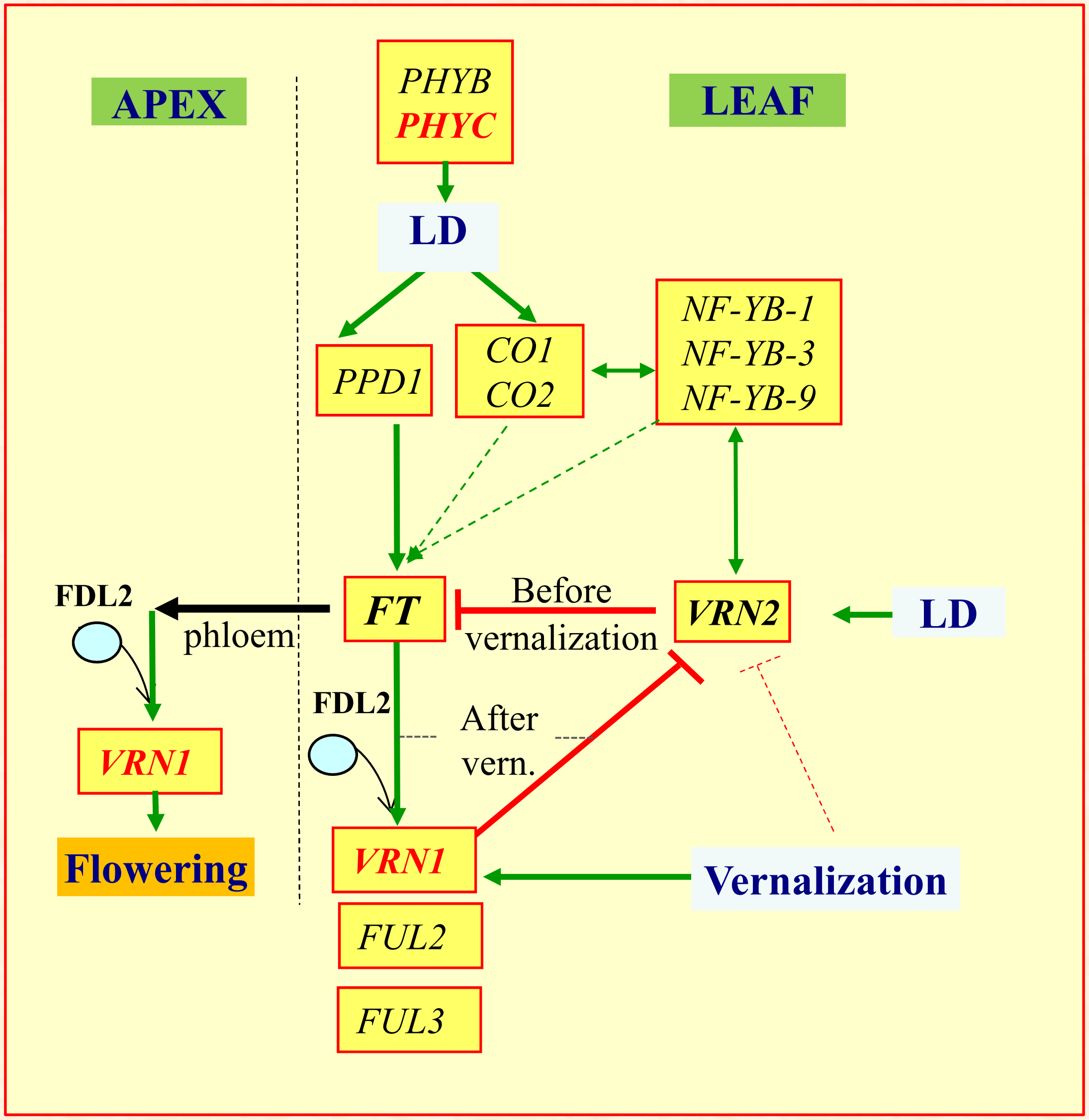
2012 52:1754

J. Plant Reg.
2014 in press

Effect of *sbeIIa* + *sbeIIb* quadruple mutant on amylose and RS



3.- Use of TILLING mutants to dissect complex pathways



- All mutants are in the same genetic background “Kronos”.
    - Winter-spring
    - Photoperiod sensitive-insensitive
- Combination of different mutants to study epistatic interactions.
- RNASeq studies of mutant lines to identify downstream genes (*phyC* and *phyB null* completed)

We have an exciting future of wheat research ahead of us!

Thank you