



**Reduced gluten and increased lysine
levels by single gene disruption in wheat**

IGW – March 15, 2018

Reduced Gluten (RG) Wheat: Rationale



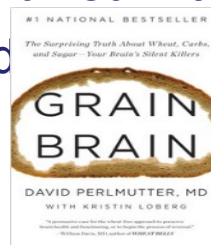
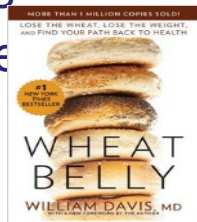
Opportunity:

- Reduced gluten wheat offers consumers a new food option
- Reduced gluten wheat can increase demand for wheat-based products

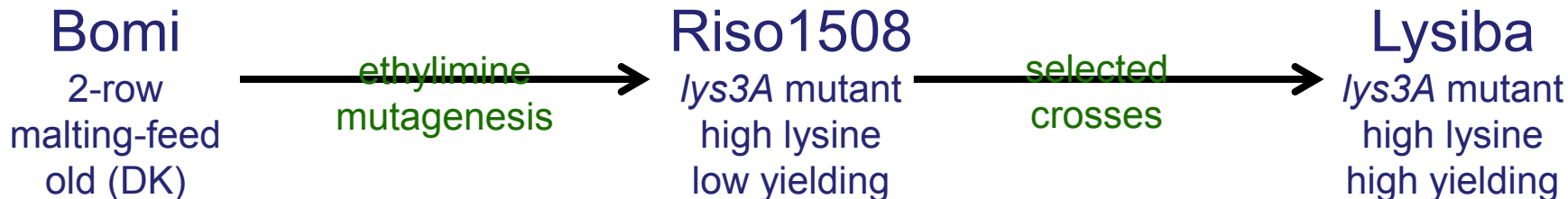


Solution:

- Non-GMO, patented, identity preserved specialty wheat
- Alternative to gluten-free diet, combined with an oral glutenase
- Improved protein content / increased amino acids



Barley high-lysine mutants



Developed for high-nutritional value feed

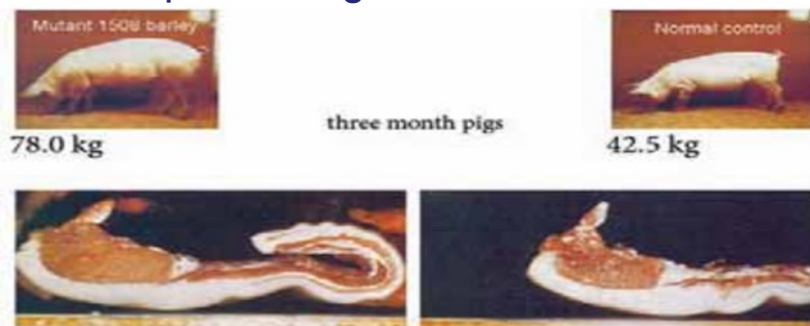
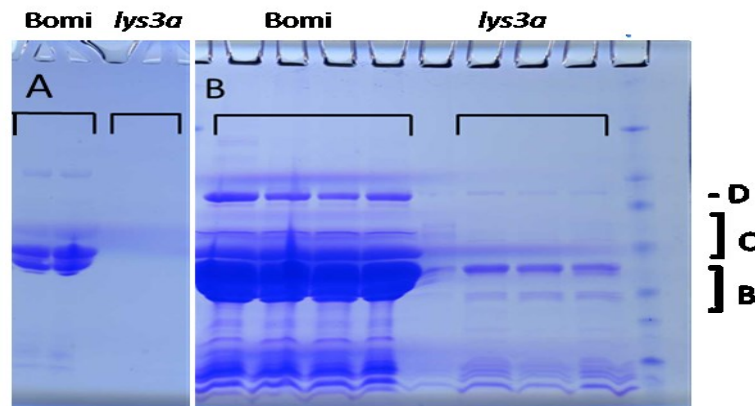


Figure 3 Functionality of improved protein by essential amino acids (lysine) demonstrated in a pig feeding trial with the Riso M-1508 (*lys 3.a*) mutant and a normal control without protein supplement. Mortensen and Madsen, 1988. See **Table 3**.

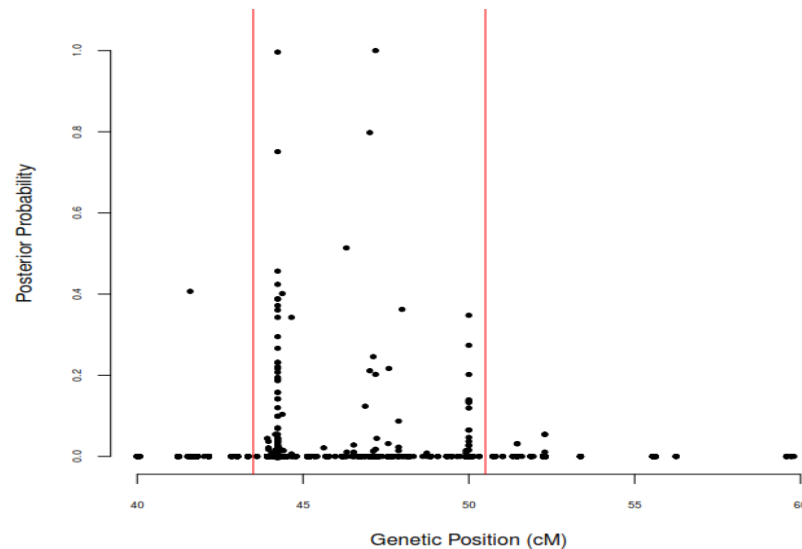
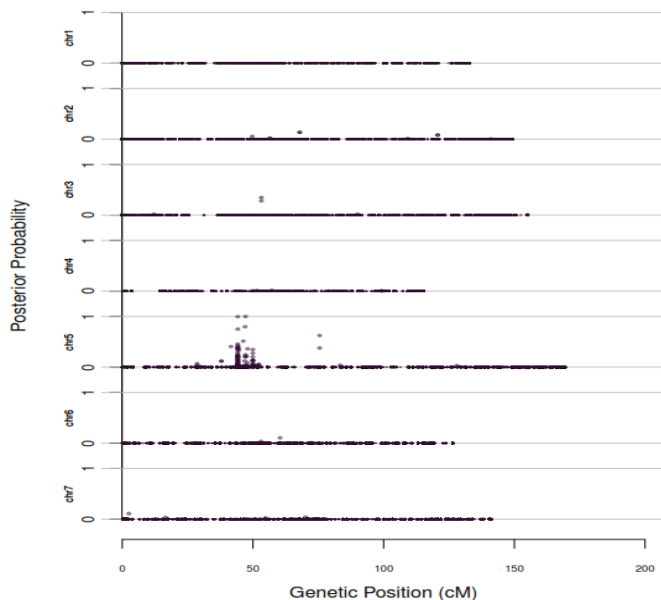


SDS-PAGE gel showing endosperm B, C and D hordeins of parental cultivar Bomi, and the *lys3a* mutant derived from it

Lys3A mutant gene identification

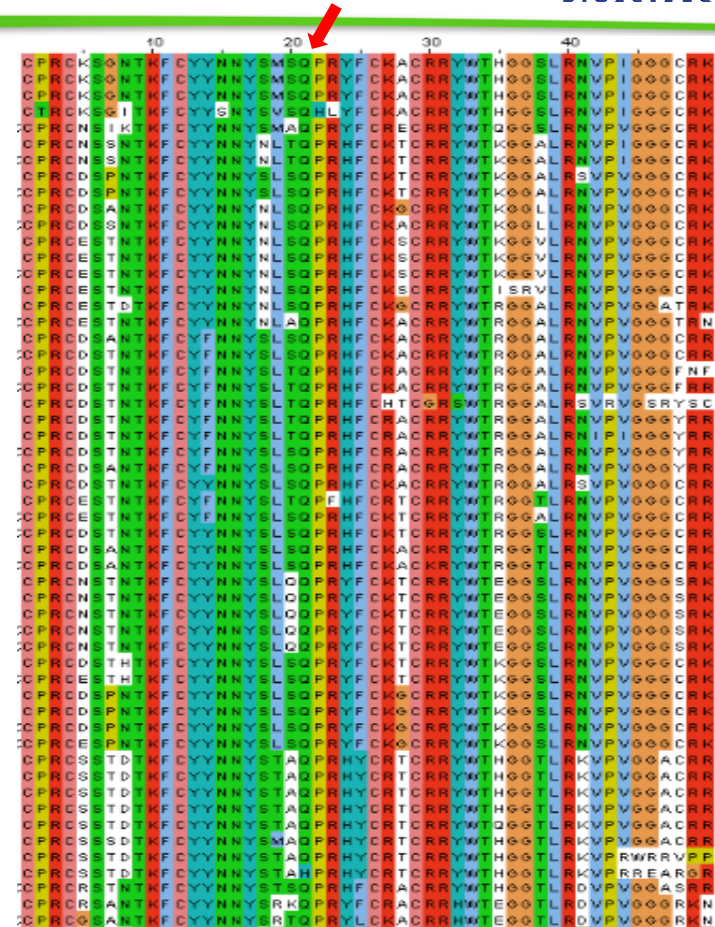


Bulked-segregant RNA-seq identified 140 out of >85,000 SNPs linked to *lys3A* mutant in 7cM interval (200 Mbp) on 5HL in mutant bulk



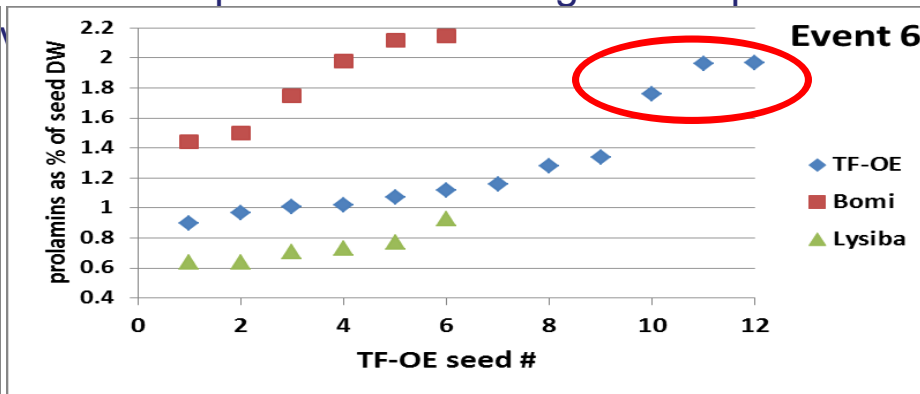
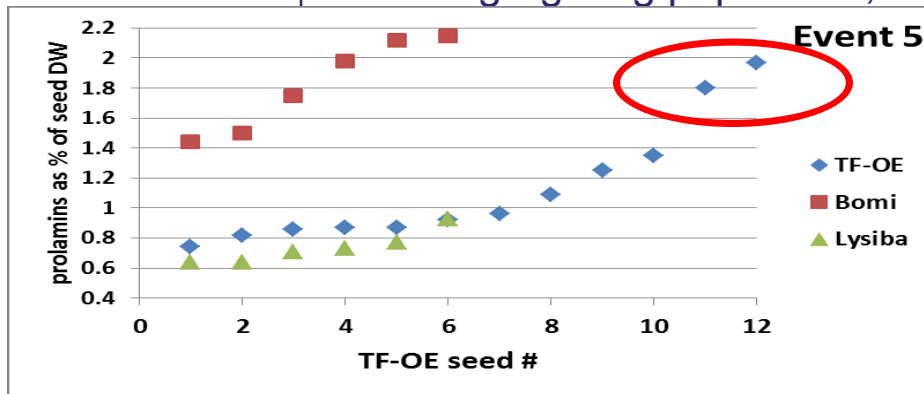
Lys3A mutant gene in barley

- Using 25 KASP genotyping probes linked to chromosome 5H, we identified two co-segregating markers 1 Mbp apart, in absolute linkage with the mutant phenotype
- Among the 60 genes we identified in this region, we searched for putative regulatory genes
- We identified one Transcription Factor (RGTF) as a likely candidate
- Comparing the sequences of the RGTF in Lysiba and Bomi identified a SNP,



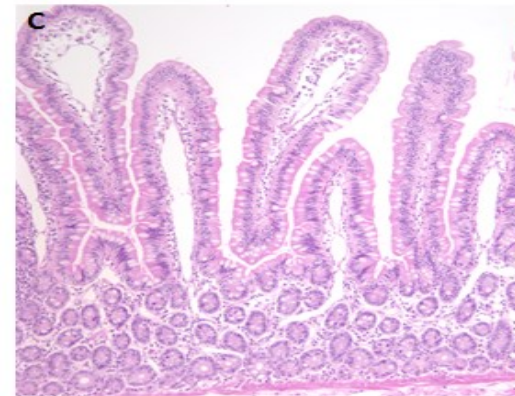
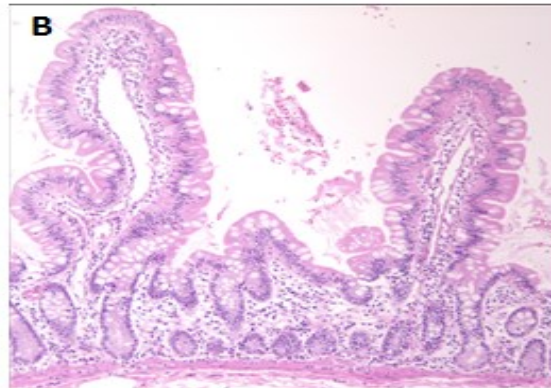
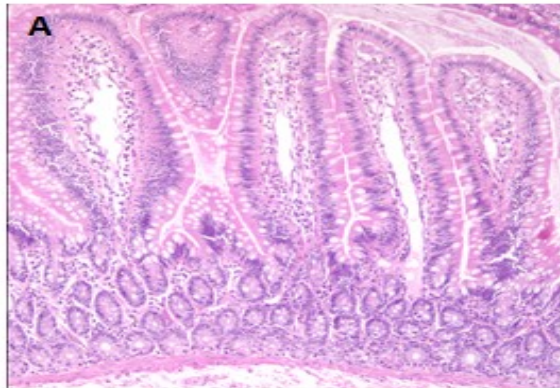
Restoring gluten levels through complementation

- *Agrobacterium*-mediated transformation of immature Lysiba seed → can low prolamin level be restored by overexpression of a *pHordein-HvRGTF* gene cassette?
- Six T₀ events were generated and grown to maturity
- T₁ seed was harvested and 12 from each event were analyzed for increased prolamin levels. T₁ is the segregating population, thus different prolamin levels might be expected



Benefits of low gluten grain (NIH-funded study)

- Pilot study using a low gluten barley variety Lysiba for monkey feeding studies
- Intestinal biopsies of control (A) and gluten sensitive (B) non human primate (rhesus macaque) model of celiac disease after consuming normal barley chow during 64 days



2015 paper: <http://www.mdpi.com/2072-6643/7/3/1657/htm>



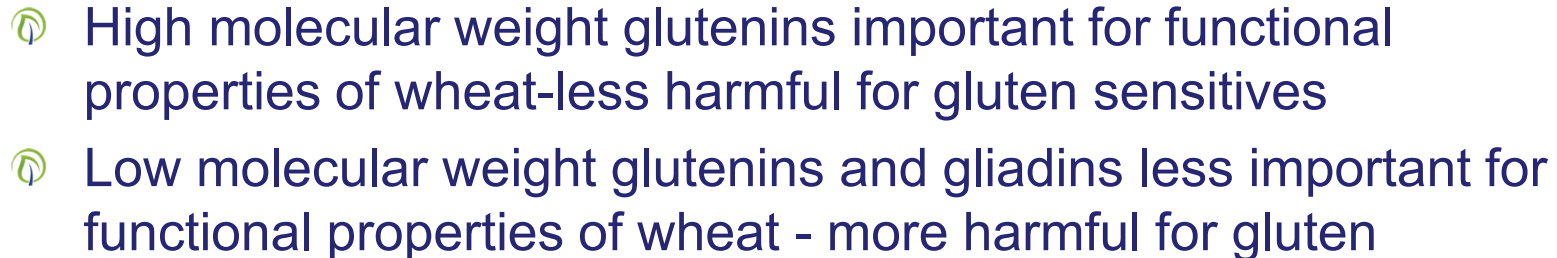
nutrients



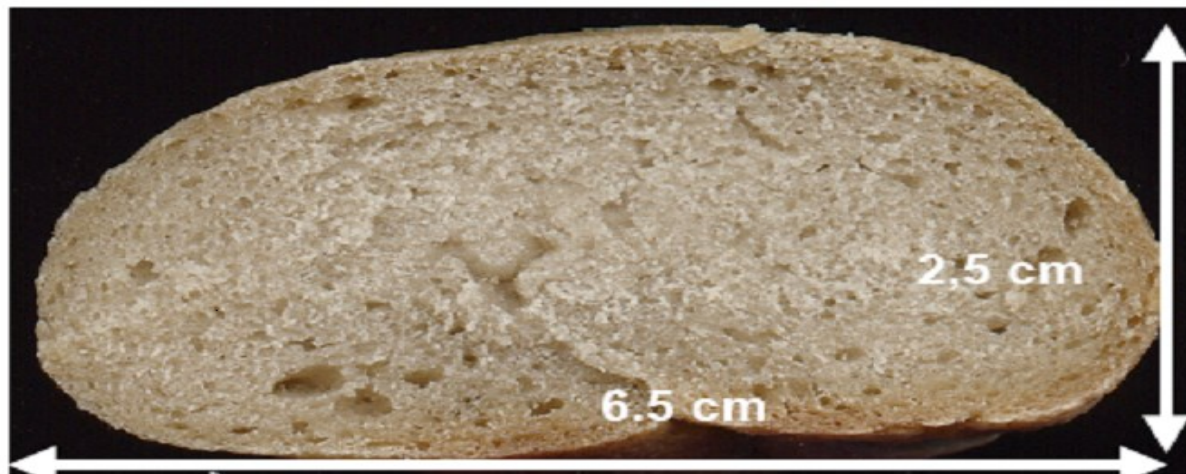
Article

Supplementation of Reduced Gluten Barley Diet with Oral Prolyl Endopeptidase Effectively Abrogates Enteropathy-Associated Changes in Gluten-Sensitive Macaques

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-  High molecular weight glutenins alone are necessary and sufficient for bread-making properties of wheat

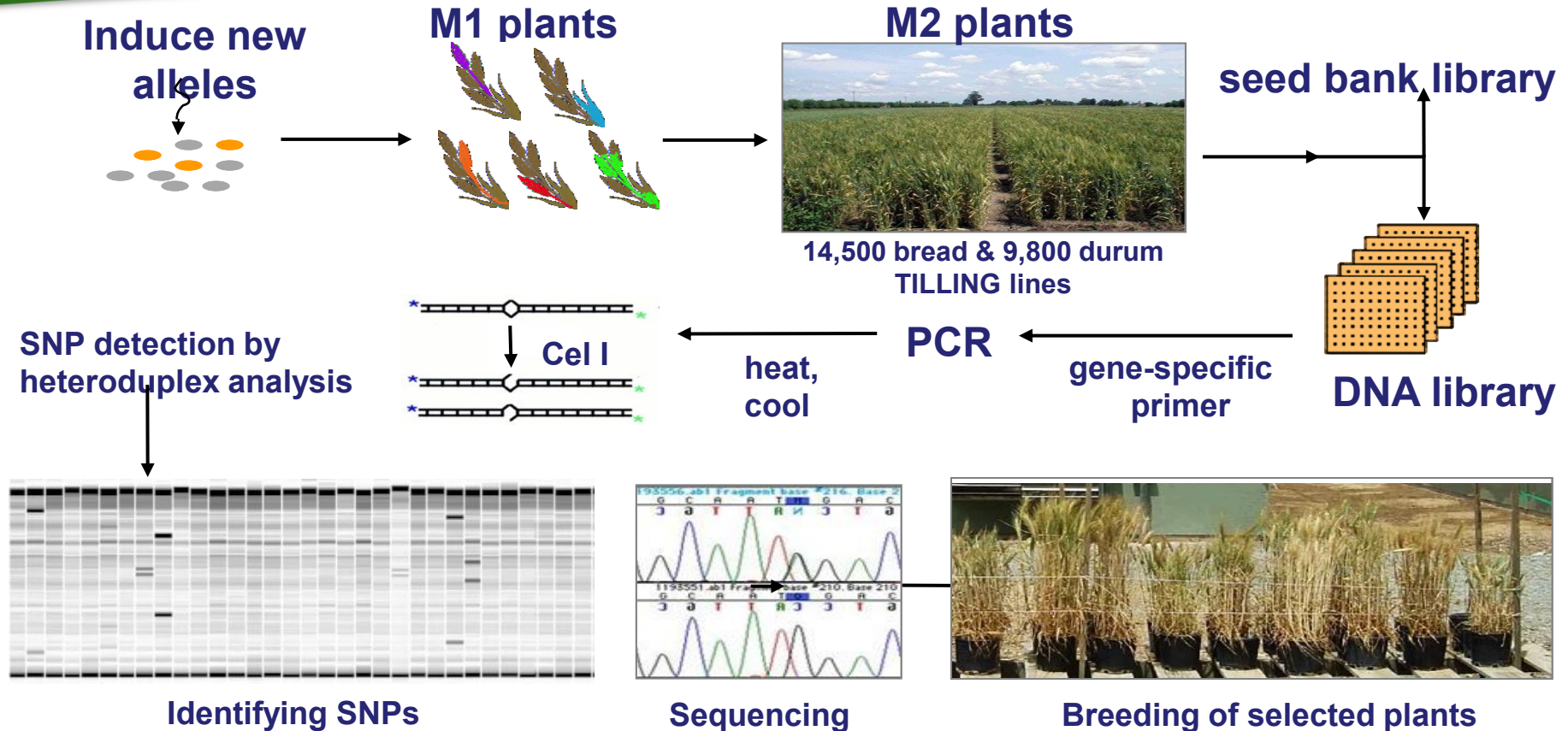


Bauer, 2005

Dough prepared with wheat flour washed to remove all gliadins and HMW + LMW glutenins.

1.4 grams recombinant HMW-1Dx5 glutenin produced in yeast was added to the gluten-free flour, the dough kneaded and baked.

TILLING overview: non-GM plant breeding



TILLING alleles in wheat homoeolog of *lys3a*

 We found 530 new alleles in our 10,000 line bread wheat TILLING library

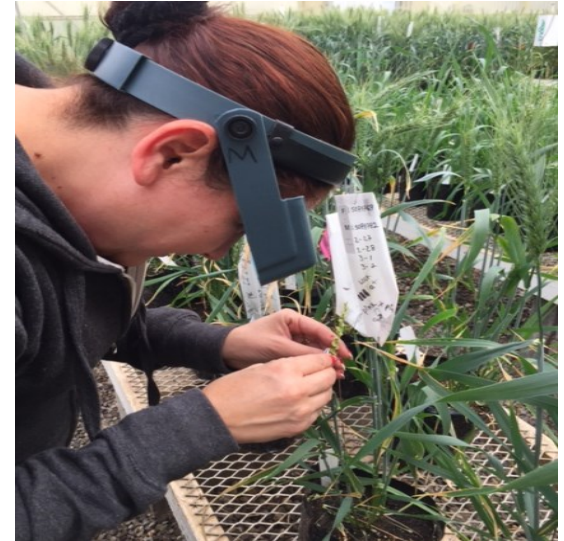
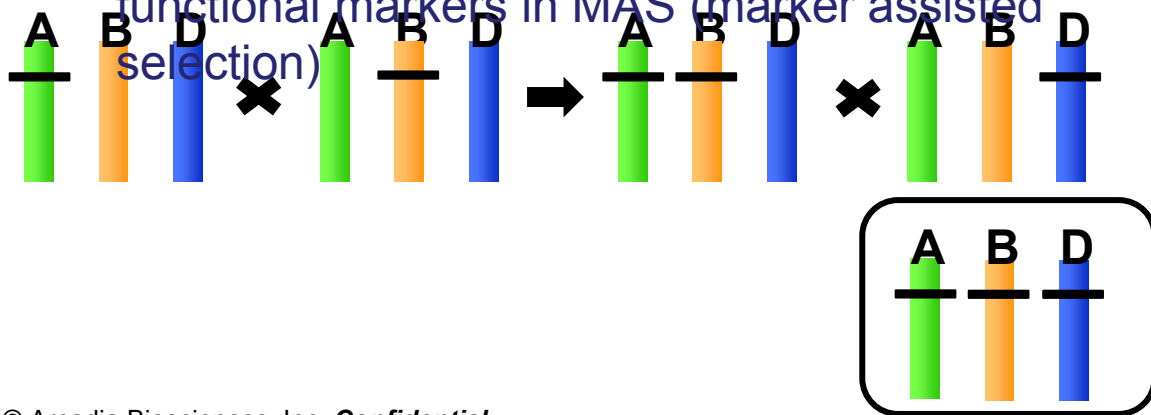
 We focused on stop and severe missense mutations

Genome:

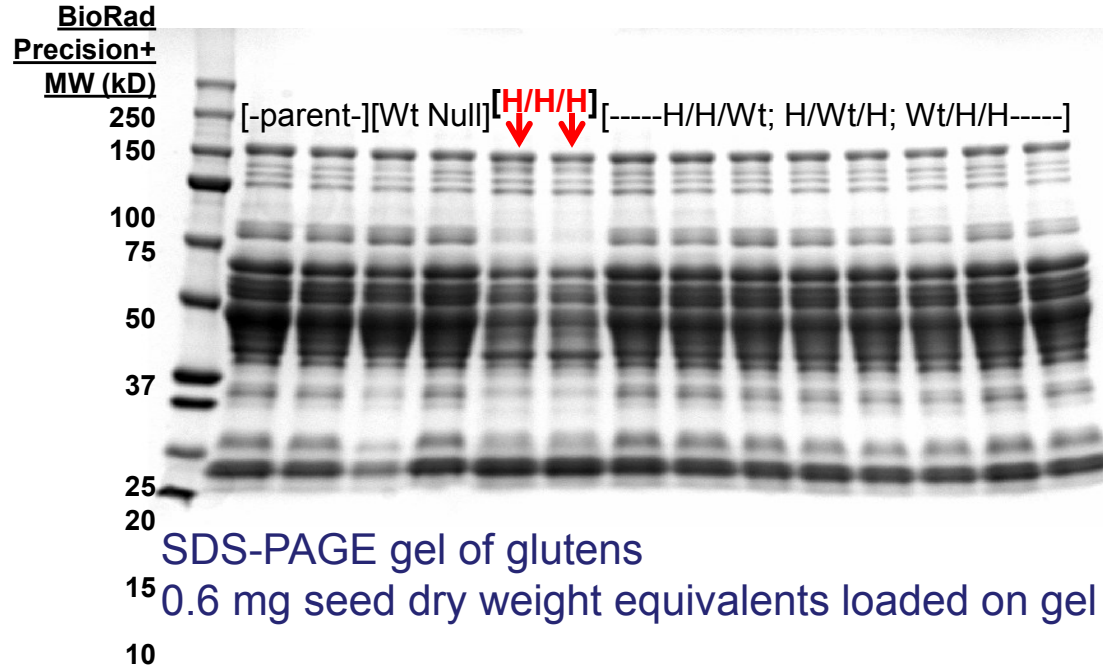


Plant breeding with TILLING alleles

- Selected for mutations that 1) predicted highest impact, 2) most similar to *Lys3A* and 3) phenotypically normal
- Transcription factor has to be inactivated in all three genomes of hexaploid wheat, A, B and D
- SNPs (single nucleotide polymorphisms) act as functional markers in MAS (marker assisted selection)



Parent cv. Express, Homozygous, Heterozygous and Wt null mutants



75% reduction in allergenic glutens

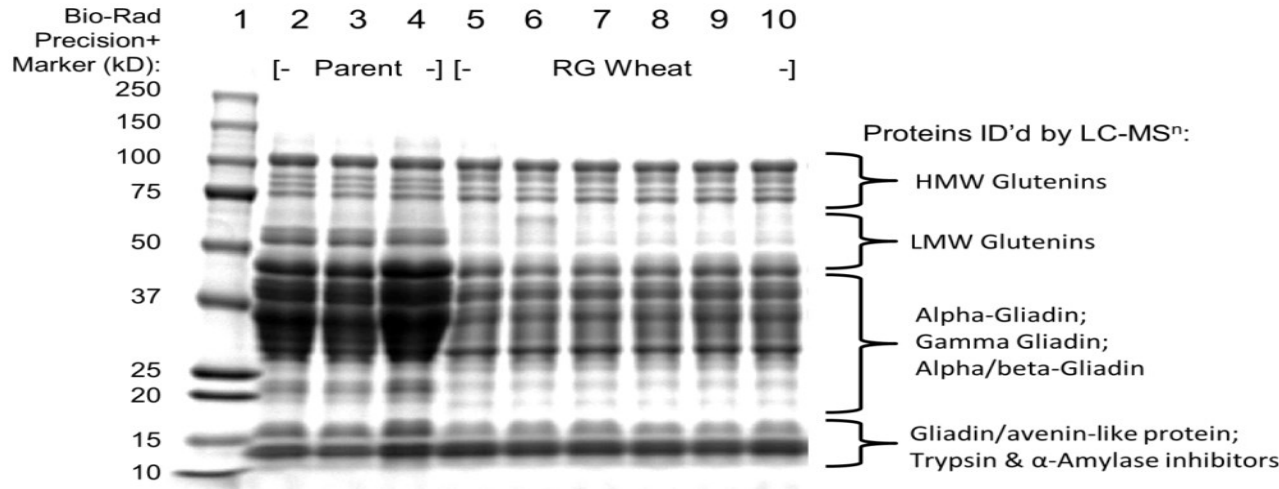
Pedigree	Sample No.	% Seed Weight as Glutens ²	% of Express Control as Glutens	Estimated ppm Glutens	% of Express Control as Gliadins and LMW Glutenins ³
Express Parental Variety	6	4.1%	100%	40623	100%
Mutants Wt siblings: Wt/Wt/Wt	3	3.5%	86%	35000	92%
Partial genome mutations ¹	6	3.1%	76%	31400	Not measured
Full genome mutations H/H/H (RG)	10	1.7%	41%	16732	24%

¹ These lines include Hom/Hom/WT; Hom/WT/Hom and WT/Hom/Hom

² Pierce 660 nm Protein assay and measurements by spectrophotometry

³ Percent gliadins and LMW glutenins are from integration of protein gel bands, 17 – 55 kDa region, using ImageJ image processing software (NIH.gov)

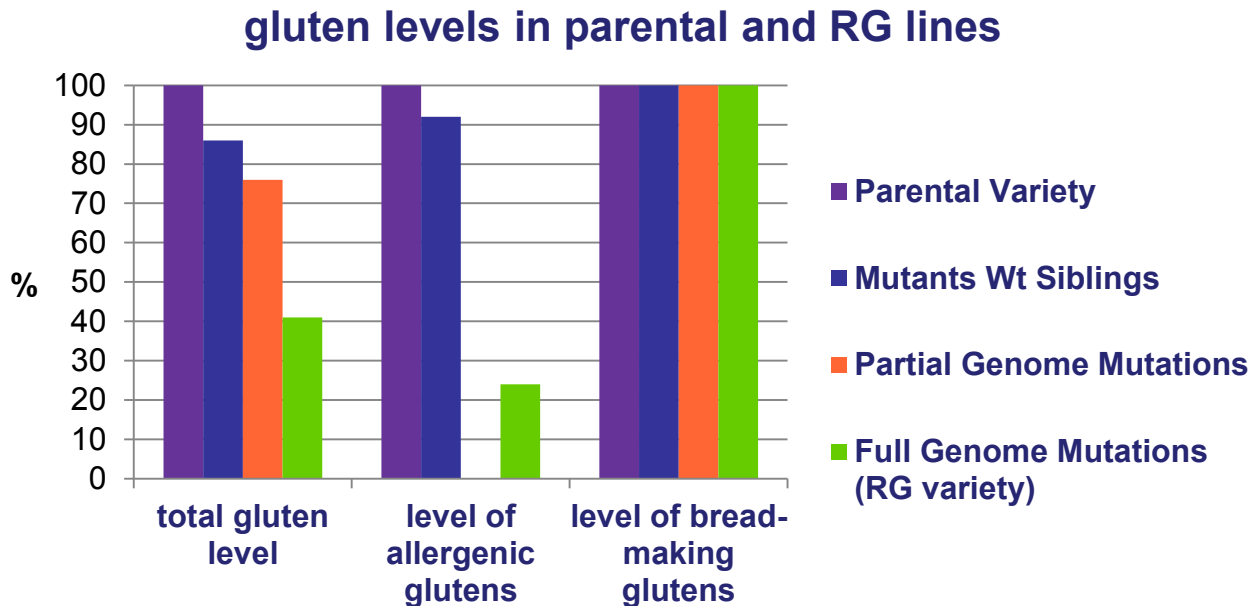
Decrease in allergenic glutens in RG wheat



 No changes in HMW glutenins, important for bread making

 Dramatic decrease in LMW glutenins and gliadins, which are prime causes for allergenic reactions

Gluten levels in each of genotypic groups



Nutritional value improvement




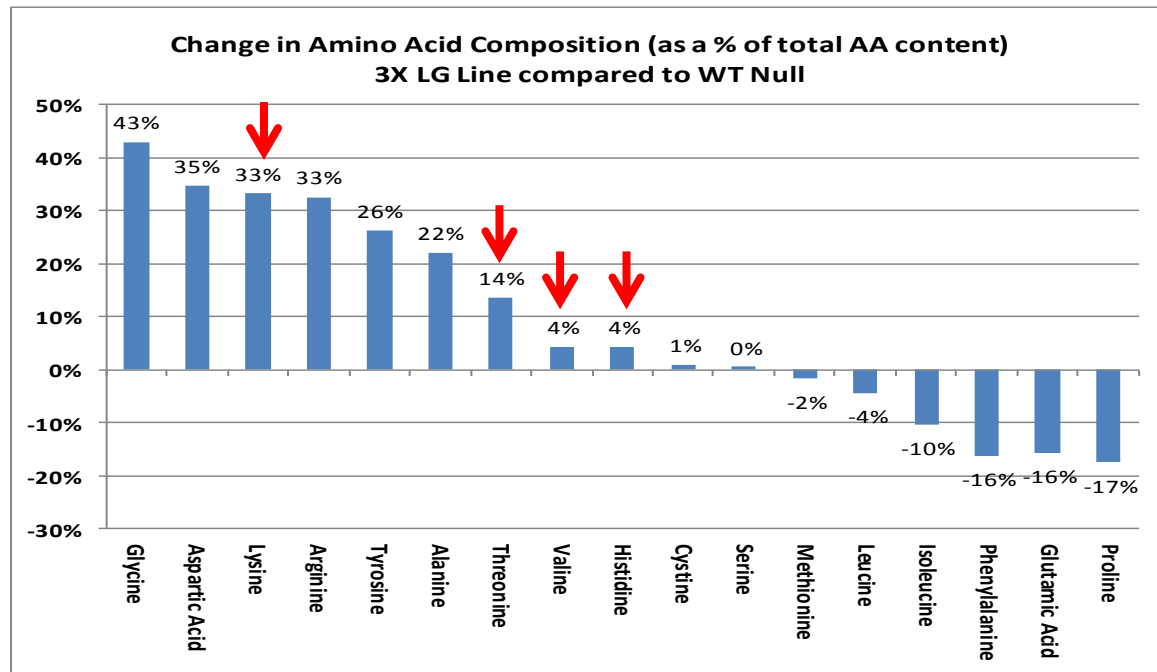
-  Cereals are low in essential amino acids
-  Reducing gluten in wheat increases some essential amino acids
-  Analogous to QPM: Quality Protein Maize



Figure 4. Growth rate advantage of pigs raised solely on quality protein maize (larger animals) versus sibling pigs raised solely on conventional maize (smaller animals). Separate feeding studies conducted in Guatemala, El Salvador, Colombia and Ghana in years indicated.

Changes in amino acids in RG wheat

- Measured by HPLC
- Lysine content up by 33%
- Also other essential amino acids that are limiting in diet (threonine, valine, histidine) are increased



Express RG Bread Wheat Conclusions

- ⑦ Only the 3x Hom RG lines display different prolamines/gluten banding pattern
- ⑦ Total measured prolamines/gluten level in 3x Hom lines is 40% of that in parental Express, and 50% of that in 3x Wt
- ⑦ Total measured **allergenic** gluten (LMW glutenins and gliadins) level in 3x Hom lines is 24% of that in parental Express, and 26% of that in 3x Wt
- ⑦ No changes in HMW glutenins, important for bread making
- ⑦ Arcadia RG Wheat products offers new options for:
 - Celiac patients when consumed in combination with oral glutenase,
 - Gluten-sensitive consumers w/o the use of oral glutenase
 - Consumers that prefer to reduce gluten all together

- Introgression of the RG trait into elite wheat varieties
- Seven generations of backcrossing and genetic fixing are needed before initiating seed scale up in the field (with MAS)
- First RG bread flour from elite wheat varieties available for testing of milling and baking ability in Fall 2018
- Further reduction of allergenic gluten by crossing with lines carrying additional mutant genes involved in gluten synthesis
- Reproducing most successful mutant combinations by CRISPR-mediated genome editing



Final Word and Thank You

Other Arcadia non-GM wheat traits

- High Fiber/Resistant Starch
- Long Shelf Life/Oxidative stability
- Heat Tolerance
- Herbicide Tolerance
- Yield Increase



Internal team

- Max Moehs
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- Valerie Wuerz
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