Biotechnology and breeding strategies to develop wheat with reduced allergenic potential

Susan B. Altenbach[1], Jong-Yeol Lee[2], Sandra Denery-Papini[3], Yong Q. Gu[1]

[1] USDA-ARS Western Regional Research Center, Albany, CA USA
[2] National Academy of Agricultural Science, RDA, Jeonju, Korea
[3] Biopolymers, Interactions, Assemblies, INRA, Nantes, France

13th International Gluten Workshop
Mexico City, Mexico
March 14-17, 2018

The gluten proteins confer the unique viscoelastic properties of wheat flour

Some of the same proteins are also responsible for human health problems

- food allergies
- celiac disease
- non-celiac wheat sensitivity (NCWS)

Research Goals

- determine which proteins in wheat flour are immunogenic

- develop strategies to reduce the levels of harmful proteins in wheat flour

Must be accomplished without negative effects on functional properties of the flour

Two strategies to eliminate proteins that cause a serious food allergy

- Biotechnology: RNA interference to silence specific genes
- Breeding: identify mutant line missing specific genes

Wheat-dependent exercise-induced anaphylaxis (WDEIA)

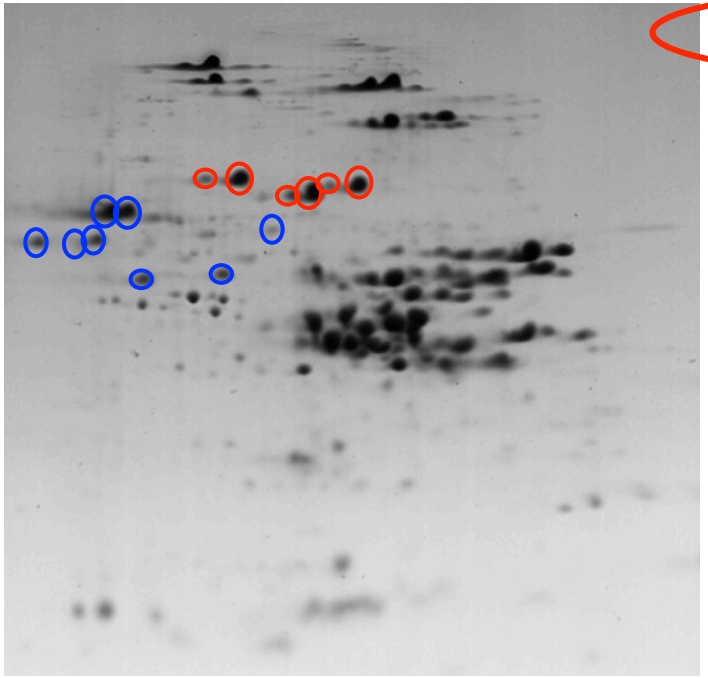
- Occurs in sensitized individuals when the ingestion of wheat is followed by physical exercise

- Major sensitizing allergens are omega gliadins

Omega gliadins

- Gluten proteins present in flour as monomers
- Comprise ~5-10% of total flour protein, dependent on cultivar and growth conditions of the plant
- Consist almost entirely of repetitive sequences with high levels of glutamine and proline but no cysteine

Omega gliadins



cv. Butte 86

Omega-5 gliadins

N-terminal sequence: SRL

Repetitive motifs: FPQQQ and QQIPQQ

Encoded on chromosome 1B

Involved in serious food allergy (WDEIA)

Omega-1,2 gliadins

N-terminal sequences: ARE, ARQ or KEL

Repetitive motif: PQQPFP

Encoded on chromosomes 1A and 1D

Contain celiac immunodominant epitopes

Omega-5 gliadins

MKTFIIFVLLAMAMNIASASRLLSPRGKELHTPQEQFPQQQQFPQPQQFPQQQI
PQQHQIPQQPQQFPQQQQFLQQQQIPQQQIPQQHQIPQQPQQFPQQQQFPQQHQ
SPQQQFPQQQFPQQKLPQQEFPQQQISQQPQQLPQQQQIPQQPQQFLQQQQFPQ
QQPPQQHQFPQQQLPQQQQIPQQQQIPQQPQQIPQQQQIPQQPQQFPQQQFPQQ
QFPQQQFPQQEFPQQQQFPQQQIARQPQQLPQQQQIPQQPQQFPQQQQFPQQQS
PQQQQFPQQQFPQQQQLPQKQFPQPQQIPQQQQIPQQPQQFPQQQFPQQQQFPQ
QQEFPQQQFPQQQFHQQQLPQQQFPQQQFPQQQFPQQQQFPQQQQLTQQQFPRP
QQSPEQQQFPQQQFPQQPPQQFPQQQFPIPYPPQQSEEPSPYQQYPQQQPSGSD
VISISGL

QQFPQQQ and QQIPQQQ identified as IgE binding epitopes

23 QQFPQQQ
4 QQIPQQQ

Matsuo et al., JBC 279 (13):12135-12140, 2004.
Battais et al., Allergy 50: 815-821, 2005.

Omega-5 gliadins

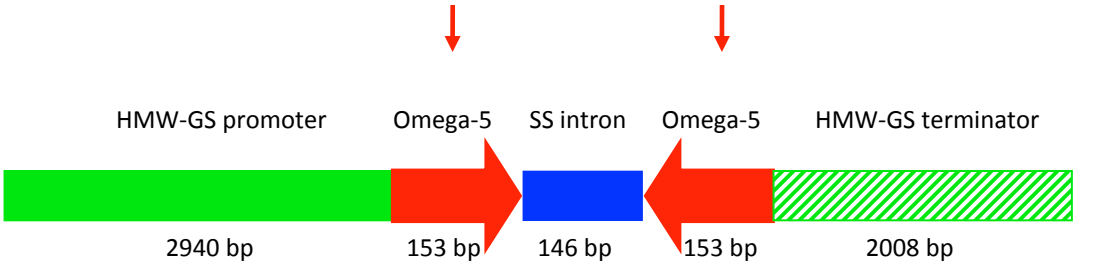
- Genes are very difficult to clone - few complete sequences in databases
- Du et al. 2016 cloned 66 genes using a PCR strategy – all had deletions of various sizes
- Only partial sequences from cv. Butte 86

Approach #1: Biotechnology

Use RNA interference to silence genes encoding omega-5 gliadins in transgenic plants

RNA interference plasmid contained a 153 bp target sequence that matched the 3' coding region

MKTFIIFVLLAMAMNIASASRLLSPRGKELHTPQEQFPQQQQFPQPQQFPQQQI
PQQHQIPQQPQQFPQQQQFLQQQQIPQQQIPQQHQIPQQPQQFPQQQQFPQQHQ
SPQQQFPQQQFPQQKLPQQEFPQQQISQQPQQLPQQQQIPQQPQQFLQQQQFPQ
QQPPQQHQFPQQQLPQQQQIPQQQQIPQQPQQIPQQQQIPQQPQQFPQQQFPQQ
QFPQQQFPQQEFPQQQQFPQQQIARQPQQLPQQQQIPQQPQQFPQQQQFPQQQS
PQQQQFPQQQFPQQQQLPQKQFPQPQQIPQQQQIPQQPQQFPQQQFPQQQQFPQ
QQEFPQQQFPQQQFHQQQLPQQQFPQQQFPQQQFPQQQQFPQQQQLTQQQFPRP
QQSPEQQQFPQQQFPQQPPQQFPQQQFPIPYPPQQSEEPSPYQQYPQQQPSGSD
VISISGL



Altenbach & Allen, GM Crops 2:1, 67-74. 2011.

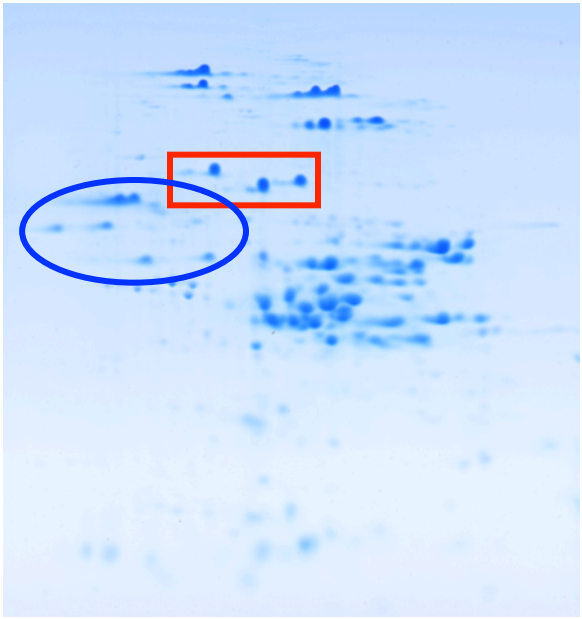
Proteomic analysis of transgenic lines

80% omega-5 gliadins

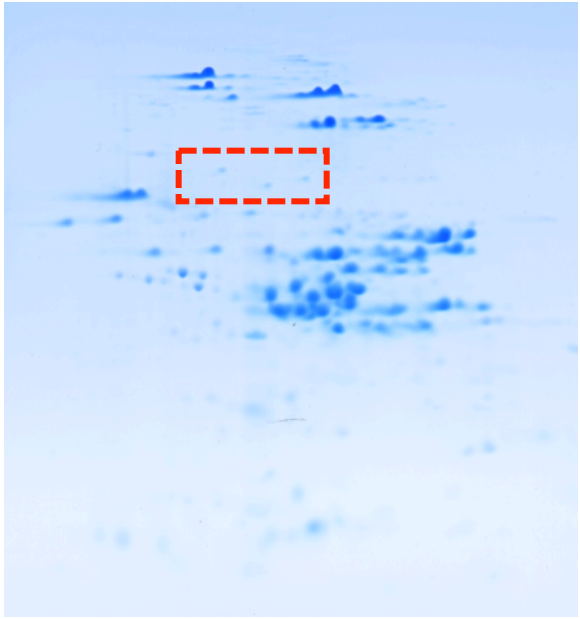
Few other changes in proteome

100% omega-5 gliadins

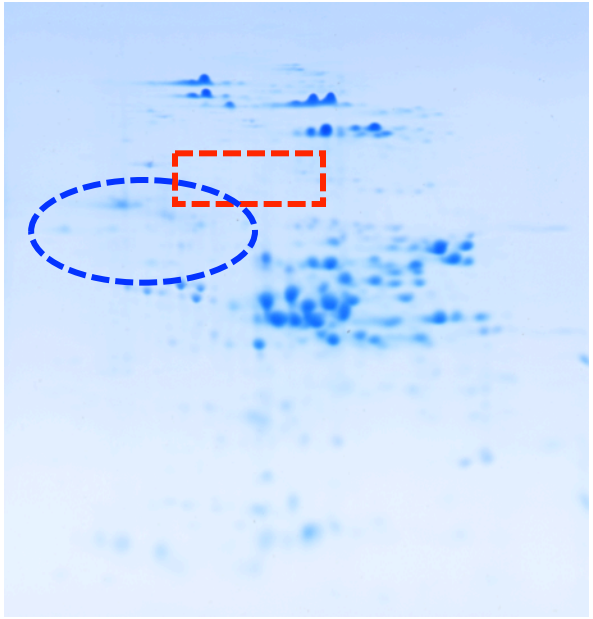
50% omega-1,2 gliadins



Non-transgenic



Transgenic 35b

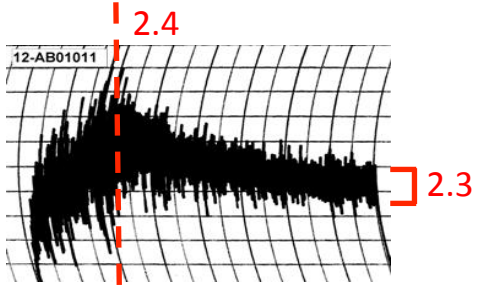


Transgenic 45a

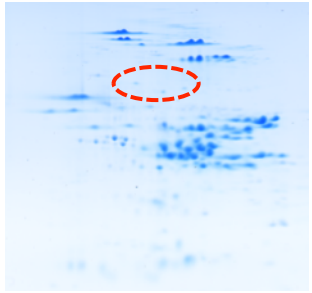
Altenbach et al. J. Cereal Sci. 59:118-125. 2014.

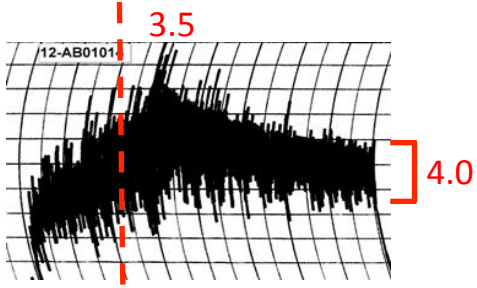
End-use quality of transgenic lines is improved

Non-transgenic



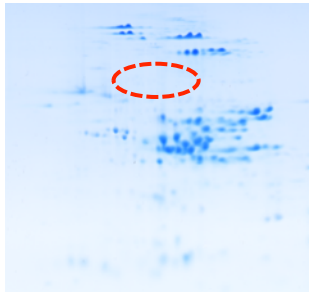
Transgenic 35b

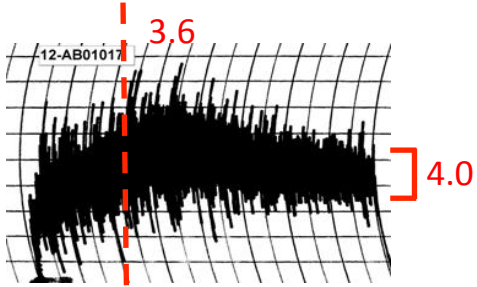




↑Mix Time
↑Mix Tolerance

Transgenic 45a

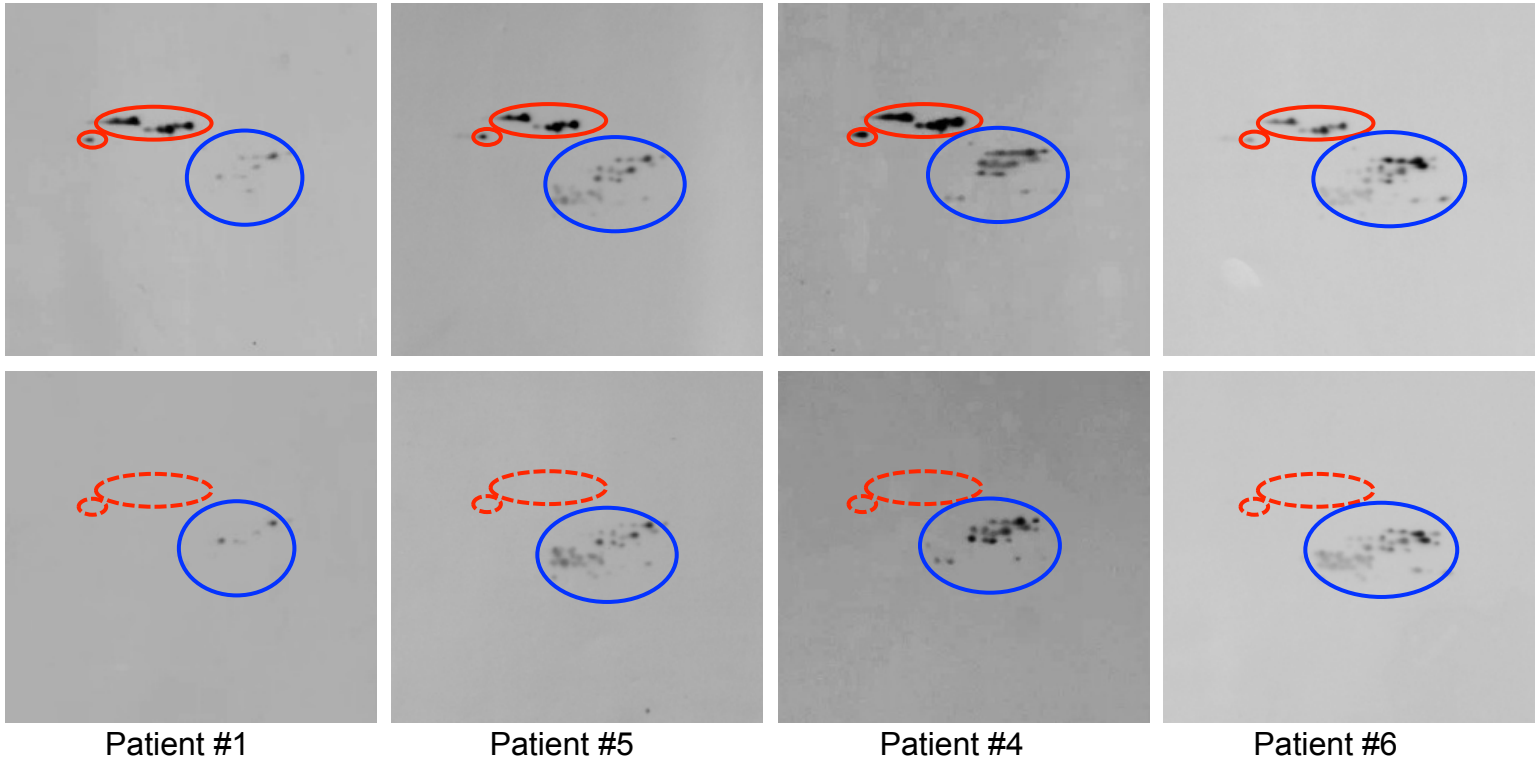




↑Mix Time
↑Mix Tolerance

Altenbach et al. BMC Plant Biology, 14:1. 2014.

IgE reactivity of flour proteins with sera from WDEIA patients is reduced in transgenic lines



(Collaboration with Dr. Sandra Denery-Papini, INRA, Nantes, France)

Altenbach et al. JAFC, 63:9323-9332. 2015.

Summary

- RNAi is an effective way to eliminate multiple closely related gluten proteins from wheat flour, even with limited sequence information
- There were few other changes to the flour proteome
- Contributions of omega-5 gliadins to quality were evaluated without confounding effects of other gluten proteins
- An important class of food allergens was removed from flour, resulting in improved flour quality and reduced IgE reactivity

- However, the plants are transgenic and subject to regulatory approval and consumer acceptance

- Transgenic wheat is not likely to be incorporated into breeding programs in the US

Approach #2: Breeding

Mutant line selected from a double haploid population resulting from two Korean cultivars

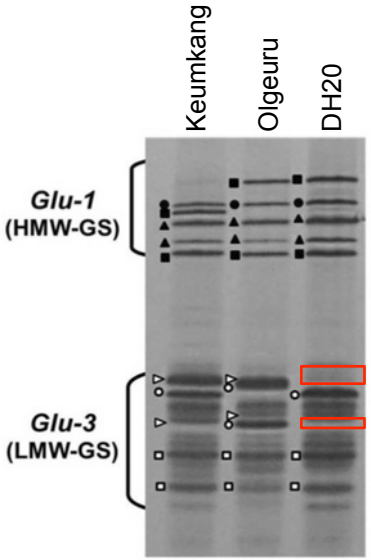
Keumkang – hard white wheat used for multiple purposes

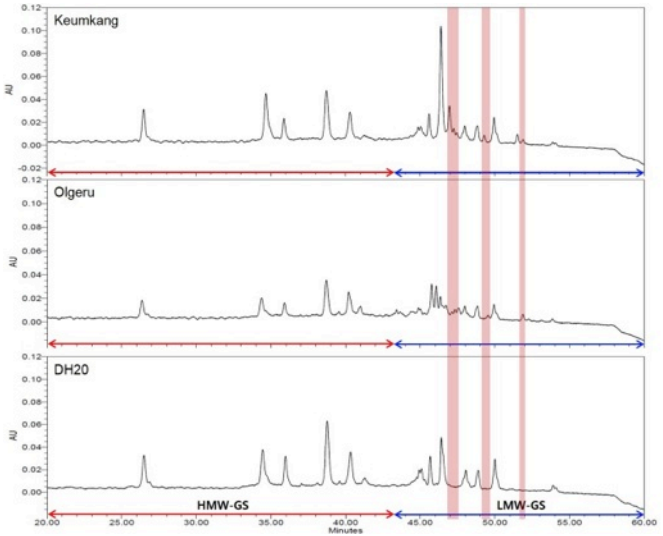
Olgeuru – soft red wheat used for noodles

(Collaboration with Dr. Jong-Yeol Lee, NIAS, Jeonju, Korea)

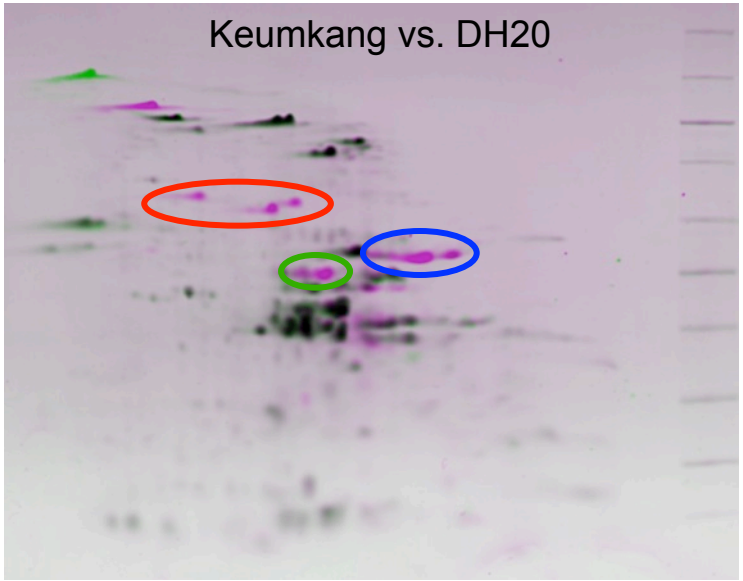
DH20 is missing LMW-GS encoded by the B genome

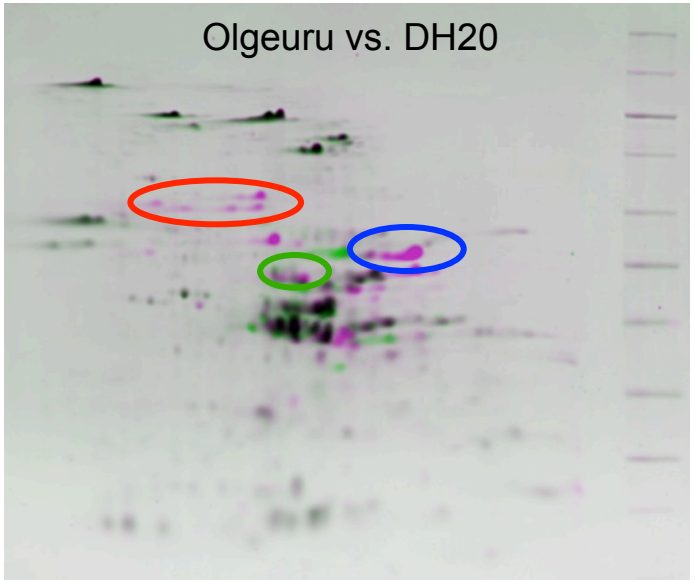
Glutenin fraction





DH20 is also missing a number of other gluten proteins

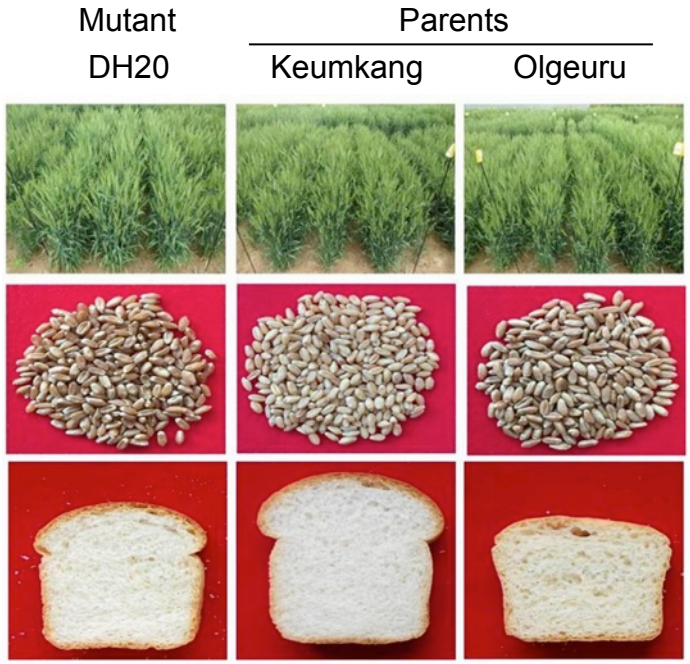




Parent = Pink
Mutant = Green
Both = Black

1B LMW-GS missing
omega-5 gliadins missing
gamma gliadins missing

Agronomic traits and flour quality



Protein content and quality characteristics

Protein %	similar to Keumkang
SDS sedimentation	similar to Keumkang
Absorption (%)	similar to Keumkang
Mixing Time	intermediate
Mixing Tolerance	similar to Keumkang
Loaf Volume	intermediate
Crumb Firmness	intermediate

Parental and mutant lines grown in the field over 2 years

Lee et al., 2017, J. Cereal Sci. 73: 158-164.

IgE reactivity of flour proteins with sera from WDEIA patients

- Reactivity with protein spots identified by MS/MS as omega-5 gliadins was observed in parental lines
- Reactivity with omega-5 gliadins was absent in the DH20 mutant
- Reactivity with two “mystery spots” was observed in both parental lines and the DH20 mutant

- The “mystery spots” also react with a monoclonal antibody prepared against omega-5 gliadin

- The “mystery spots” may be omega-5 gliadins encoded by chromosomes other than 1B.

Summary

- Mutant line was identified with acceptable agronomic and quality characteristics and reduced immunogenic potential
- However, some highly immunogenic proteins were still present in the mutant line and may be omega-5 gliadins
- It is possible that omega-5 gliadins are encoded on chromosomes in other locations in the genome

The organization of gluten protein genes was examined in cv. Chinese Spring

- Genes encoded on ~6.5 Mb region of the 1S chromosomes from the A, B and D genomes were annotated
- Two full-length omega-5 gliadin genes were clustered with six pseudogenes on chromosome 1B
- Three pseudogenes were clustered with a truncated omega-5 gliadin gene on chromosome 1D

Huo et al., 2018, submitted.

Sequences of omega-5 gliadins from chromosome 1B and 1D were compared

- Protein encoded on 1D chromosome has N-terminal sequence TRQ instead of SRL
- Protein encoded on 1D chromosome contains more copies of WDEIA epitopes
- Protein encoded on 1D chromosome has altered COOH end due to a frameshift mutation

The “mystery spots” in the DH20 mutant were identified by MS/MS

MS/MS data from the mystery spots matched the omega-5 gliadin sequence encoded by the D genome of cv. Chinese Spring

Breeding approaches are complicated

- By the linkage of omega gliadin, gamma gliadin and LMW-GS genes
- By the finding that omega-5 gliadins may be encoded on chromosomes other than 1B

Other approaches to reduce the levels of complex groups of gluten proteins in wheat flour

- Genome editing (CRISPR/Cas9) – targeted mutagenesis that is homology-based, but resulting plants not considered transgenic

- Mutation breeding – non-targeted mutagenesis, chemical or radiation

Future Directions

- Both biotechnology and breeding approaches would benefit from the detailed understanding of gluten protein genes in individual cultivars
  - numbers of genes and pseudogenes
  - sequences of genes
  - locations of genes
- The reference sequence from cv. Chinese Spring can be used to design gene capture methods to select genomic regions containing gluten protein genes from individual cultivars for DNA sequencing

Acknowledgements

USDA-ARS-WRRC
Albany, CA

Paul Allen
Charlene Tanaka
Han-Chang Chang
Bill Vensel
Anna Simon-Buss

Yong Gu
Naxin Huo

NIAS-RDA
Jeonju, Korea

Jong-Yeol Lee
You-Ran Jang
Hye-Rang Beom

Chonbuk National University
Jeonju, Korea

Chul-Soo Park

INRA
Nantes, France

Sandra Denery-Papini
Florence Pineau