Testing Quality of Alpha-Gliadin Deletion Lines for Production of Reduced-Allergenicity Wheat

Maria Rottersman, Wenjun Zhang, Teng Vang, Junli Zhang, and Jorge Dubcovsky
UC Davis, California Wheat Commission, and HHMI

Celiac Disease

Celiac disease (CD) is a chronic immune disorder triggered by gluten ingestion. It results in damage of intestinal lining and causes diarrhea, fatigue, weight loss, bloating and anemia.

Limiting the abundance of CD epitopes in food products reduces the risk of sensitization of the immune system of the group of people that are genetically susceptible for CD.

Alpha-Gliadins

Alpha-gliadins are protein subunits of gluten (Fig. 1). They represent 60% of the gluten proteins with CD epitopes in pasta wheat and 45% in bread wheat. We have a list of all the gluten genes that have CD epitopes (~40 genes total) and have radiation and fast neutron mutants the eliminate complete groups of linked proteins with celiac epitopes.

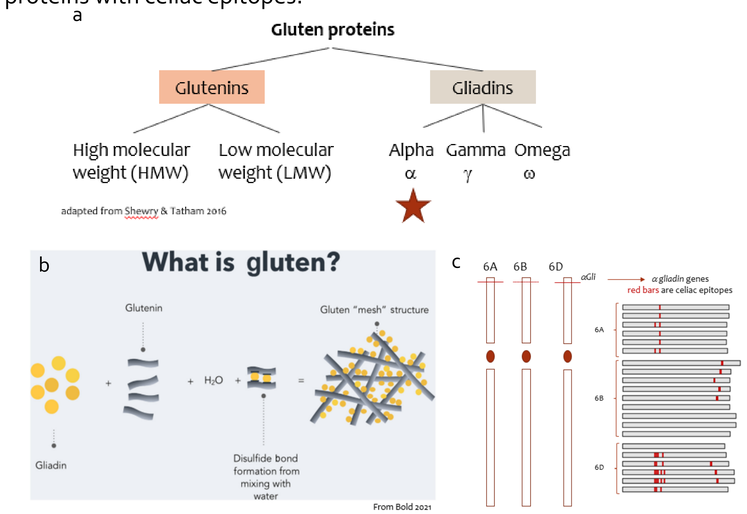

Figure 1. (a) Types of gluten proteins, adapted from Shewry & Tatham 2016. Alpha-gliadins are indicated as targeted proteins of interest. **(b)** Basic structure of gluten including roles of glutenins and gliadins. Glutenins are fibrous polymers that interact with each other through disulfide bonds forming a complex net. Gliadins can also form disulfide bonds with the glutenins, limiting the expansion of the net. Figure from Bold 2021. **(c)** Gene clusters of alpha-gliadin-encoding genes on chromosome 6.

Methods

We generated gamma-radiation deletions in recombinant inbred line 143 (RIL143) for the α-gliadin gene clusters on the short arms of chromosomes 6A, 6B, and 6D (Fig 2a). We were also able to combine that 6A and 6D deletions and generate a double mutant, but we were unable to recover a triple mutant. The estimated size of the deletions and the number of other genes deleted are in Fig 2b.

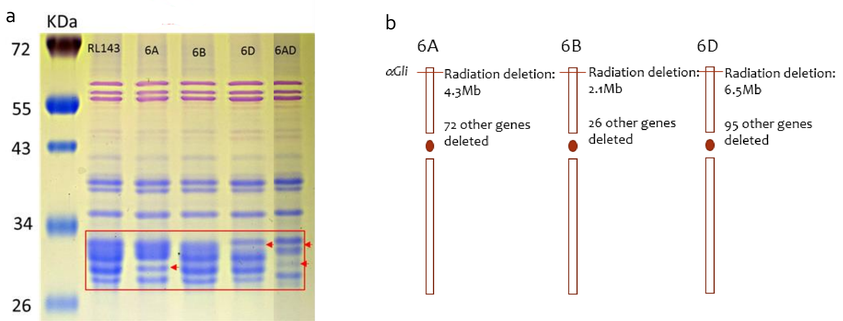

Figure 2. Gamma-radiation deletions in recombinant inbred line 143. **(a) SDS** PAGE of lines of interest. Red arrows indicate deleted alpha-gliadins. **(b)** Visual representation chromosome 6 gene deletions. Size and number of deleted genes was estimated by exome capture.

We performed replicated field experiments at UCD in 2021 and 2022 including the four alpha-gliadin deletion combinations (WT, A1, B1, D2 & AD) and tested their effects on multiple breadmaking quality parameters determined by mixograph, farinograph, and baking.

Results

Our combined ANOVA analyses for 2021 and 2022 showed no significant difference in flour protein content but highly significant differences in mixograph, alveograph and baking parameters associated with gluten strength (Figs 3, 4).

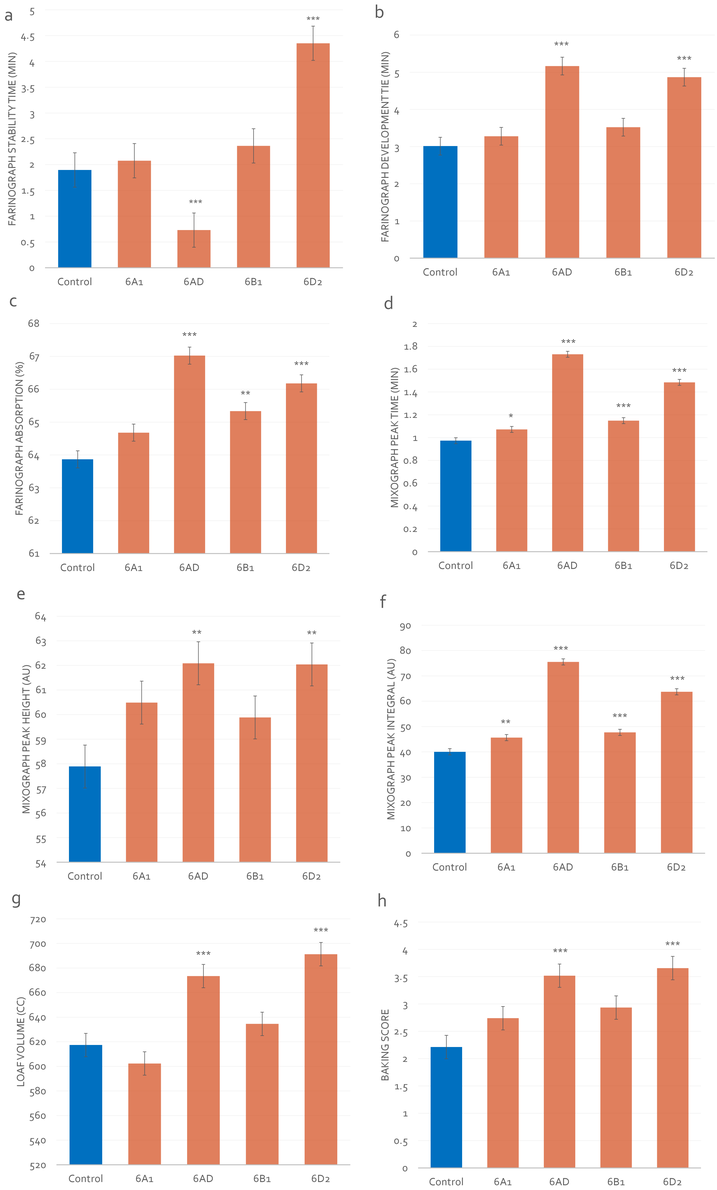

Figure 3. Wheat quality parameters for deletion lines 6A1, 6B1, 6D2, and 6AD relative to Control (RIL143, Dunnett Tests). **(a)** Farinograph development time (min), **(b)** Farinograph stability (min), **(c)** Farinograph water absorption, **(d)** Mixograph peak time, **(e)** Mixograph peak height, **(f)** Mixograph peak integral, **(g)** Loaf volume, **(h)** Baking score. Combined ANOVAs for 2021 (5 blocks) and 2022 (4 blocks). Bars are least squares means and error bars are standard errors. Multiple mean comparisons were performed using Dunnett tests against the control. * P < 0.05, ** P < 0.01, *** P < 0.001. For all these parameters higher values correlate with improved breadmaking quality.

The 6D and 6AD alpha-gliadin deletions showed significant increases in farinograph development time, stability and water absorption; mixogram peak time, height and integral; and loaf volume and baking score. All these parameters indicate a consistent increase in gluten strength and improved breadmaking quality associated with the 6D deletion. Smaller positive effects were also observed for the 6A and 6B deletion for mixogram peak time and integral, and for 6B for farinograph absorption.

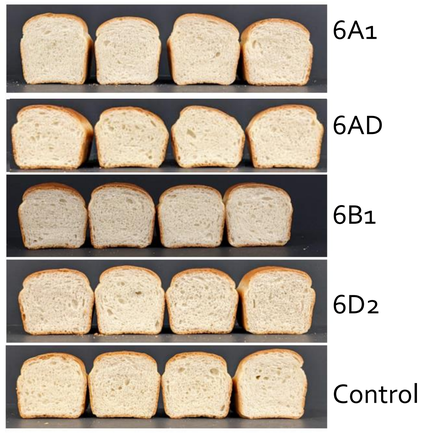

Figure 4. Final bread loaves from experimental lines of interest and RIL143 (control)

Conclusions

The 6D deletion showed beneficial effects on gluten strength and bread making quality. In addition, no reductions in grain yield were associated with this deletion during the 2021 and 2022 field trials. Taken together, these results indicate that the 6D alpha-gliadin deletion is a useful breeding tool to simultaneously reduce CD allergenicity and improve bread making quality. We have already introgressed this deletion in the UC commercial varieties Central Red and Patwin-515HP and will test its effect on quality soon.

Future directions

Our inability to combine all three alpha gliadin deletions is likely related to their relative large size and multiple deleted genes. We have recently identified smaller fast neutron radiation mutations in 6A (0.9 Mb) and 6B (0.6 Mb) in the Summit bread wheat variety. However, 2 gliadins with CD epitopes are not included in the small 6B deletion, so we plan to edit them using the novel CRISPR technology developed in our lab (Debernardi et al. 2020). We are now combining these two new smaller deletions with our current 6D deletion to try to generate the triple alpha-gliadin mutant. We are pursuing a similar strategy to eliminate gamma and omega gliadins on chromosome 1.

References

Bold, J. (2021). Gluten and its main food sources and other components of grains that may impact on health. In *Gluten-Related Disorders: Diagnostic Approaches, Treatment Pathways, and Future Perspectives* (pp. 33–48). Elsevier. https://doi.org/10.1016/B978-0-12-821846-4.00007-3

Debernardi, J. M., Tricoli, D. M., Ercoli, M. F., Hayta, S., Ronald, P., Palatnik, J. F., & Dubcovsky, J. (2020). A GRF–GIF chimeric protein improves the regeneration efficiency of transgenic plants. Nature Biotechnology, 38(11), 1274–1279. https://doi.org/10.1038/s41587-020-0703-0

Shewry, P. R., & Tatham, A. S. (2016). Improving wheat to remove coeliac epitopes but retain functionality. *Journal of Cereal Science*, *67*, 12–21. https://doi.org/10.1016/j.jcs.2015.06.005

UC DAVIS PLANT SCIENCES
California WHEAT COMMISSION
hhmi Howard Hughes Medical Institute