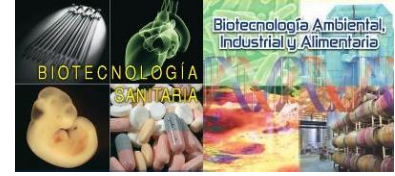


Poster

## Genomic selection of new low-gliadin wheat lines



Berlanga Torres, Jose Antonio(1), Barro Losada, Francisco\*(1)

(1)Dep. Genómica funcional. Instituto Agricultura Sostenible- CSIC. Córdoba  
Tutor académico: Francisca Reyes-Ramirez

**Keywords:** InDels; CRISPR/Cas; gluten; wheat; celiac disease; gliadins, Double Haploid/RNAi

### ABSTRACT

Gluten proteins are responsible for the quality and properties of processed wheat products. However, consuming gluten proteins (gliadins and glutenins) from wheat, barley, and rye can cause celiac disease (CD) or non-celiac wheat sensitivity (NCWS) in genetically predisposed individuals. The immunogenic epitopes that trigger celiac disease are mainly present in the  $\alpha$ -,  $\beta$ -, and  $\gamma$ -Gliadins. The only alternative currently available for this type of diseases is a strict, lifelong gluten-free diet.

The use of genetic engineering techniques such as RNA interference (RNAi) for post-transcriptional regulation of  $\alpha$ -gliadins [1] and gene editing using CRISPR/Cas for silencing the  $\alpha$ -gliadins, has resulted in wheat lines (*T. aestivum* ssp. *aestivum*), with reduced  $\alpha$ -gliadin content. [2]. These methods produce offspring with silenced, deleted and/or edited gliadins, which can reduce patients' exposure to CD epitopes.

To produce new lines with silenced  $\alpha$ -gliadins, Francisco Barro's group at the Institute of Sustainable Agriculture (IAS-CSIC), crossed lines treated with CRISPR/Cas and RNAi and, using anther culture for the production of double haploid (DH) plants, they fixed the mutations of the offspring of these crosses into homozygous lines. (Unpublished).

In this Master's thesis, a bioinformatics pipeline based on Next Generation Sequencing (NGS) amplicon sequencing (previously designed by Francisco Barro's group at the Institute for Sustainable Agriculture IAS-CSIC [3]) will be used to analyze the insertion and deletions (InDels) in target genes of lines treated by DH process. In addition, A-PAGE gels will be used to examine the protein profiles of these lines with the main objective of selecting those lines containing lower quantities of gliadins.

### REFERENCES

- [1] J. Gil-Humanes, F. Pistón, S. Tollefsen, L. M. Sollid, and F. Barro, "Effective shutdown in the expression of celiac disease-related wheat gliadin T-cell epitopes by RNA interference," *Proc. Natl. Acad. Sci. U. S. A.*, vol. 107, no. 39, pp. 17023–17028, 2010, doi: 10.1073/pnas.1007773107.
- [2] S. Sánchez-León et al., "Low-gluten, nontransgenic wheat engineered with CRISPR/Cas9," *Plant Biotechnol. J.*, vol. 16, no. 4, pp. 902–910, 2018, doi: 10.1111/pbi.12837.
- [3] M. H. Guzmán-López, M. Marín-Sanz, S. Sánchez-León, and F. Barro, "A bioinformatic workflow for indel analysis in the wheat multi-copy  $\alpha$ -gliadin gene family engineered with crispr/cas9," *Int. J. Mol. Sci.*, vol. 22, no. 23, 2021, doi: 10.3390/ijms222313076.