Comparative study of RNAi and CRISPR/Cas techniques for the elimination of wheat proteins responsible for celiac disease

Students

Aitana Nieto Reus (1º BACH, IES Fidiana Córdoba)
Natalia Rodríguez Escobar (1º BACH, IES Fidiana Córdoba)
Pablo Luque López (1º BACH, CES Lope de Vega Córdoba)
Marcos Gómez Valverde (1º BACH, IES CES Lope de Vega Córdoba)

COORDINATOR: *Dra Elena León Rodríguez*
IES Fidiana de Córdoba

RESEARCHES: *Dra Helena Guzmán y Dr Francisco Barro*
Instituto de Agricultura Sostenible- CSIC de Córdoba

FIDI ciencia
Junta de Andalucía
Cofinanciado por la Unión Europea
CES LOPE DE VEGA
IES FIDIANA
CSIC Consejo Superior de Investigaciones Científicas
Instituto de Agricultura Sostenible IAS

Index

Introduction

Wheat is one of the central foods in the Mediterranean diet, but not everyone can tolerate it. Pathologies related to wheat consumption have increased in recent years. We can distinguish three pathologies: celiac disease (digestive disease that damages the small intestine and alters the absorption of vitamins, minerals and other nutrients that food contains), allergies to wheat (mediated by antibodies against proteins found in wheat) and non-celiac wheat sensitivity (characterized by gluten-dependent gastrointestinal and extraintestinal symptoms in non-celiac patients). Wheat gluten is the main responsible for these pathologies. Gluten is made up of two large protein fractions: glutenins and gliadins, the latter being, particularly the α-gliadins, the main responsible for celiac disease. We have used two biotechnological techniques to eliminate wheat gliadins: RNA interference (RNAi) and CRISPR/Cas

Objetives

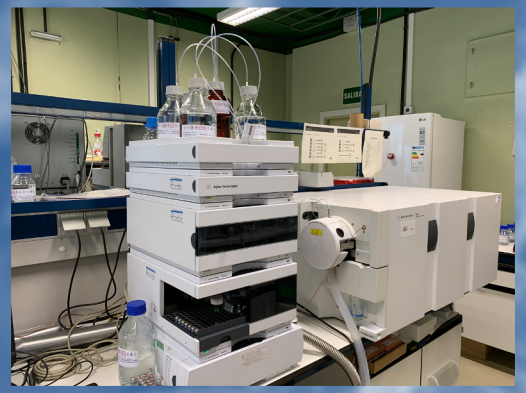
- To compare the efficiency of both technologies for the elimination of gliadins from wheat.

- To identifiy which technology would be more beneficial in the market, both for the consumer and for the supplier and the farmer.

- How the rest of the proteins are affected after the elimination of the gliadins.

Materials

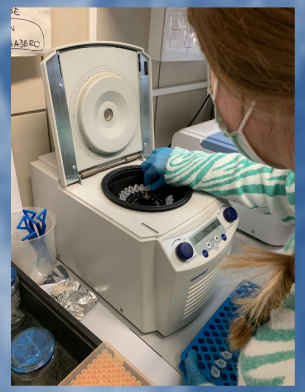
HPLC



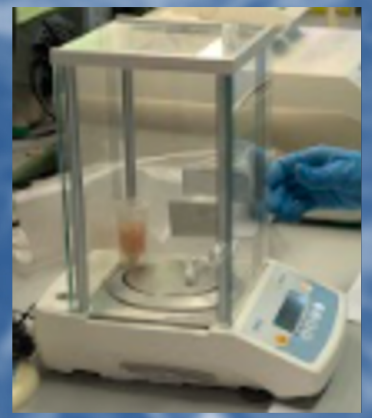
Pipette

Test tube

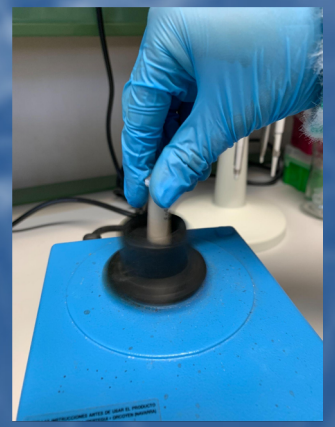
Centrifuge



Analytical precision electronic scale

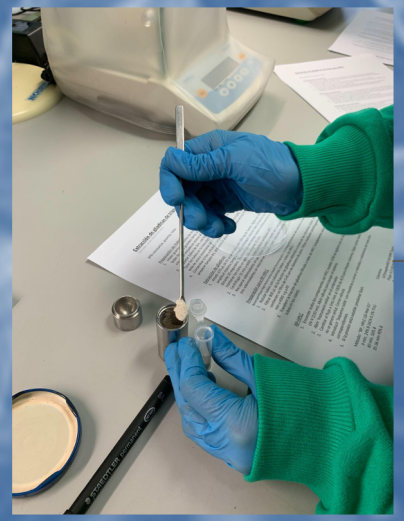


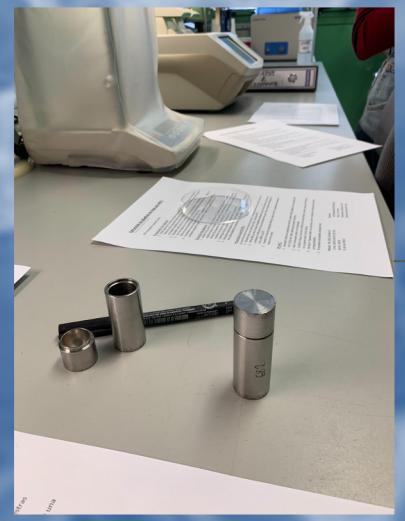
Agitator

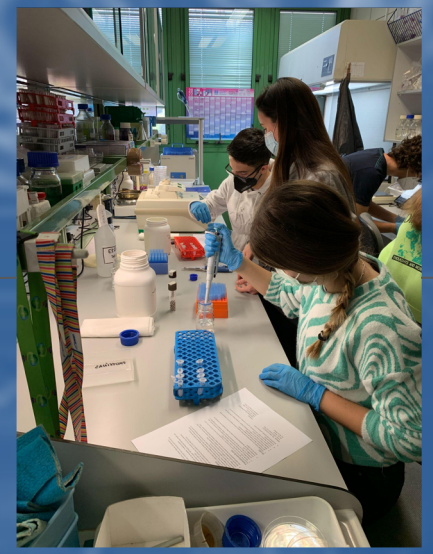


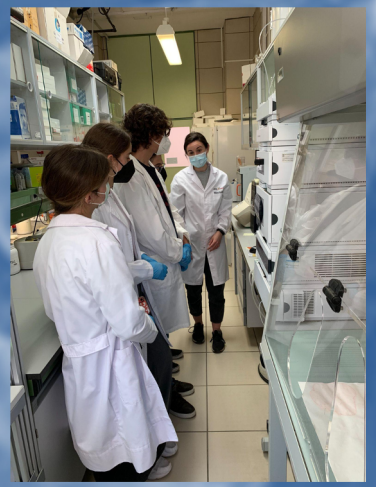
Methods

1. Preparation of the CRISPR/cas, RNAi and WT lines.
2. 100mg of flour/line in triplicate.
3. We grind the flour.
4. We add 640 µl x 3 and 60% ethanol.
5. We centrifuged to separate gliadins.
6. Reverse phase HPLC analysis.





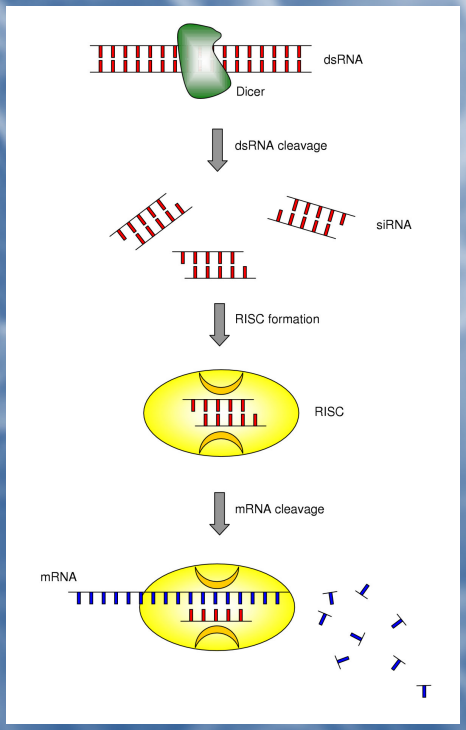


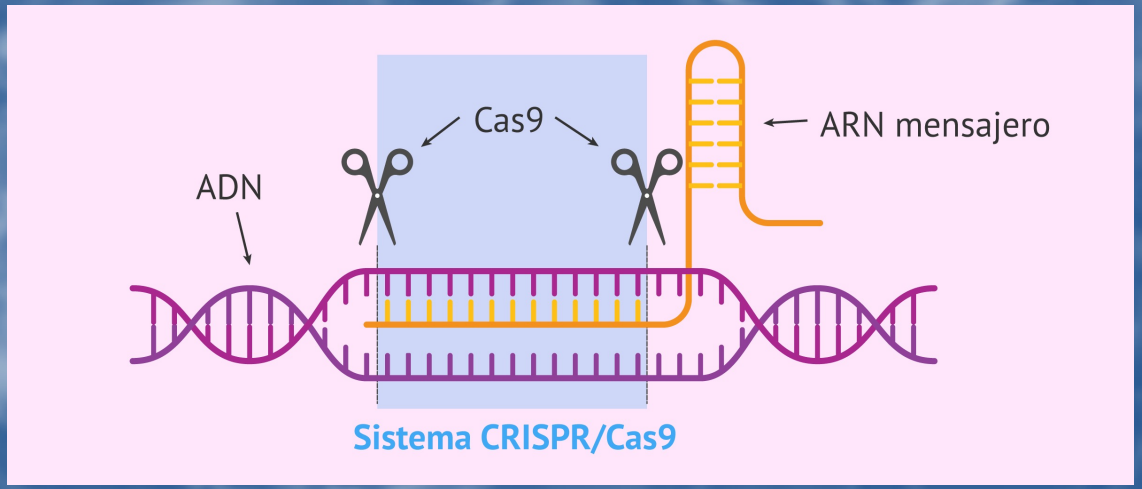


Concepts:

RNAi: Gene silencing occurs in two stages; in the first, the dsRNA is identified by the Dicer enzyme complex that fragments the dsRNA into small molecules of 20-30 nucleotides called siRNA, which serve as a template for another enzyme complex, RISC, that acts on the endogenous messenger RNA (mRNA), and if the sequences match, fragments the mRNA, preventing it from being translated into protein.

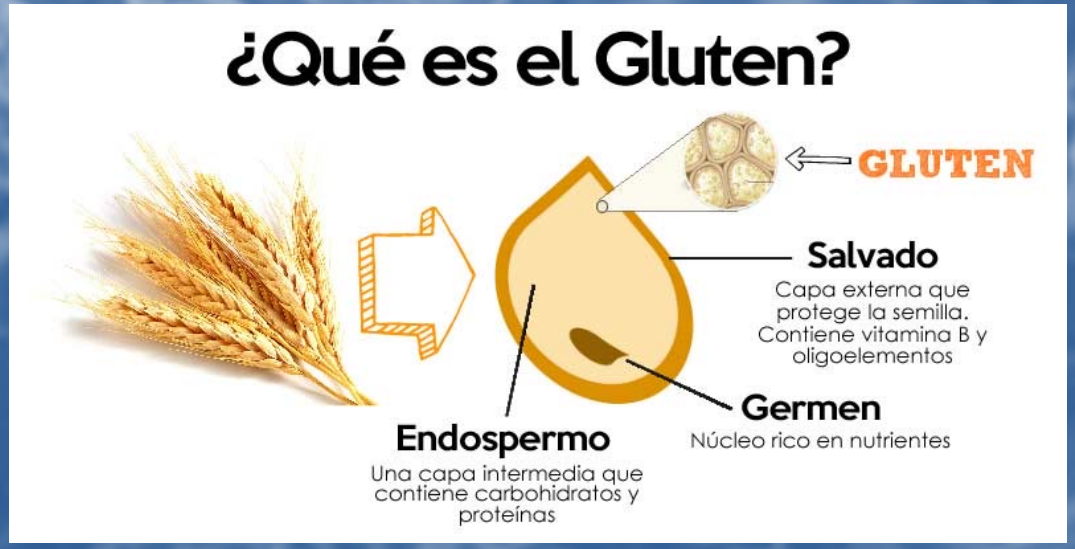
CRISPR/cas: uses RNA guides and a protein (Cas9) to target selected regions of the DNA and cleave the double-stranded DNA. From there, the cleaved ends can be joined together, a process in which mistakes are made, and the gene inactivated, or DNA templates can be introduced, allowing its 'letters' to be edited at will.



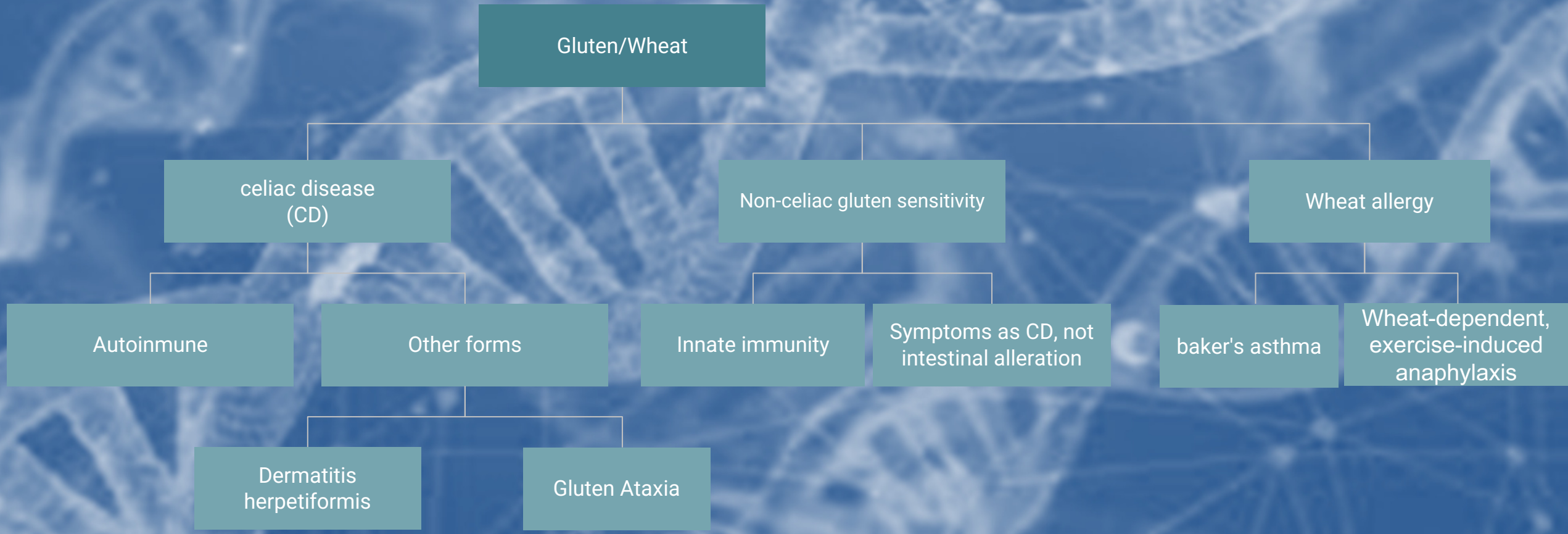


What is gluten?

It is a group of proteins of some cereals such as barley, wheat, rye, etc. It represents 80% of the total protein of the wheat grain and is made up of gliadins and glutenins. Gliadin is divided into alpha-gliadin, omega-gliadin, and gamma-gliadin. These proteins are the ones that harm celiac patients, especially the alpha-gliadins.



Gluten pathologies



Results

Figure 1:Chromatograms of the gliadin protein extracts in CRISPR/Cas (left) and RNAi (right) lines. The vertical lines indicate the separation of the gliadin fractions

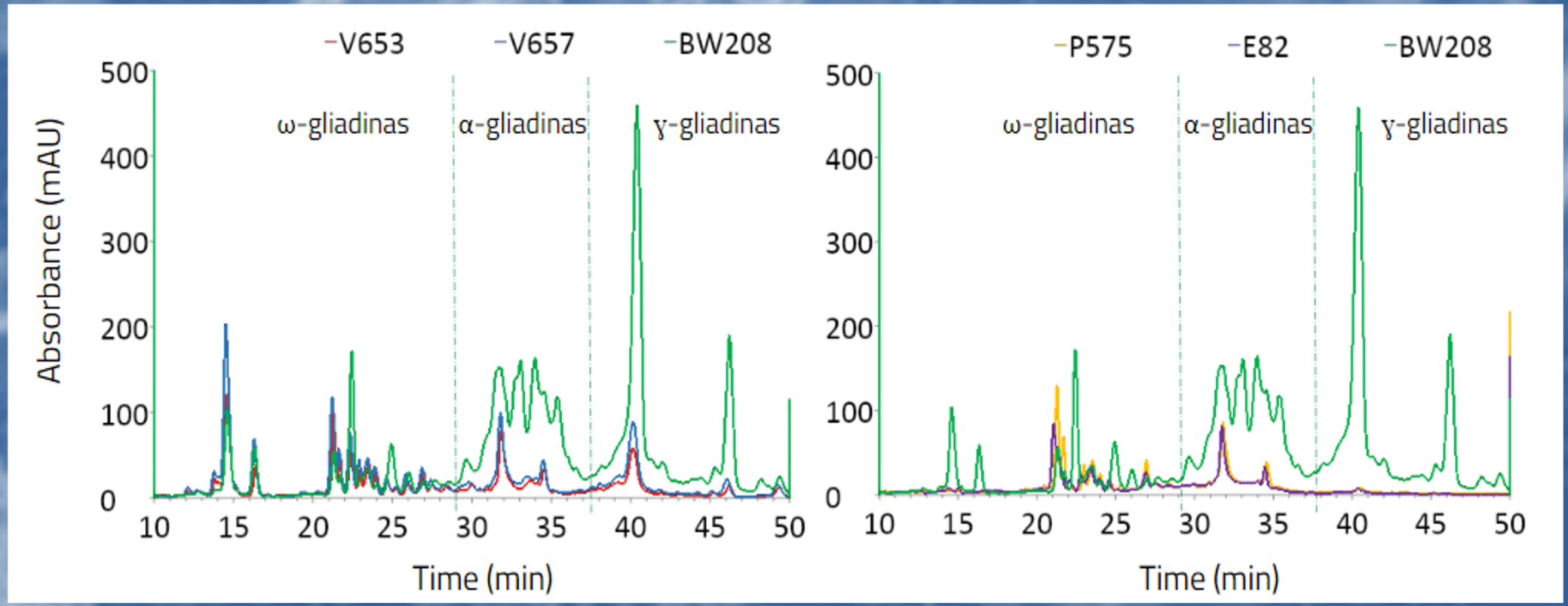


Figure 2: Boxplot of the grain starch content of the RNAi and CRISPR/Cas lines.

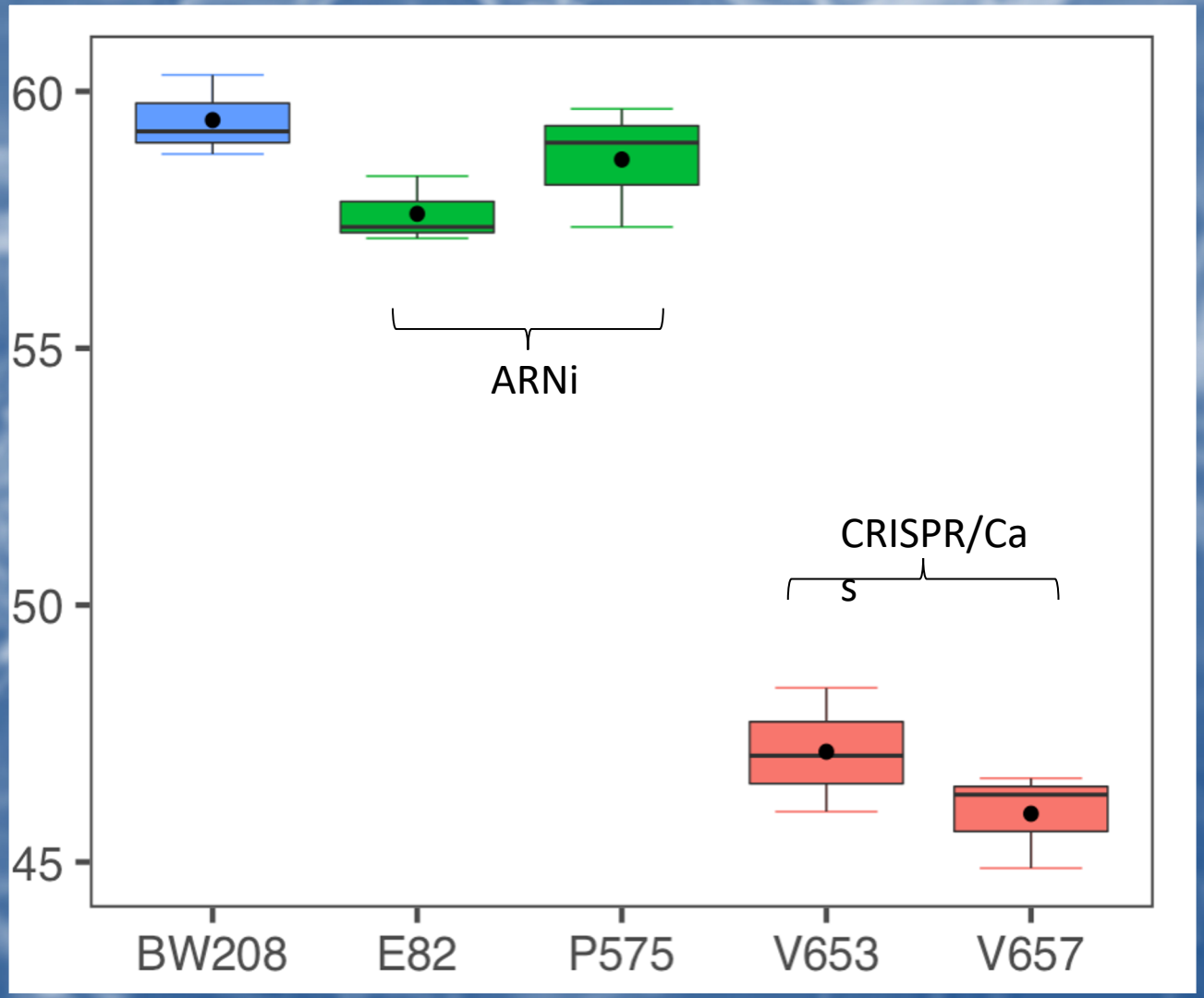
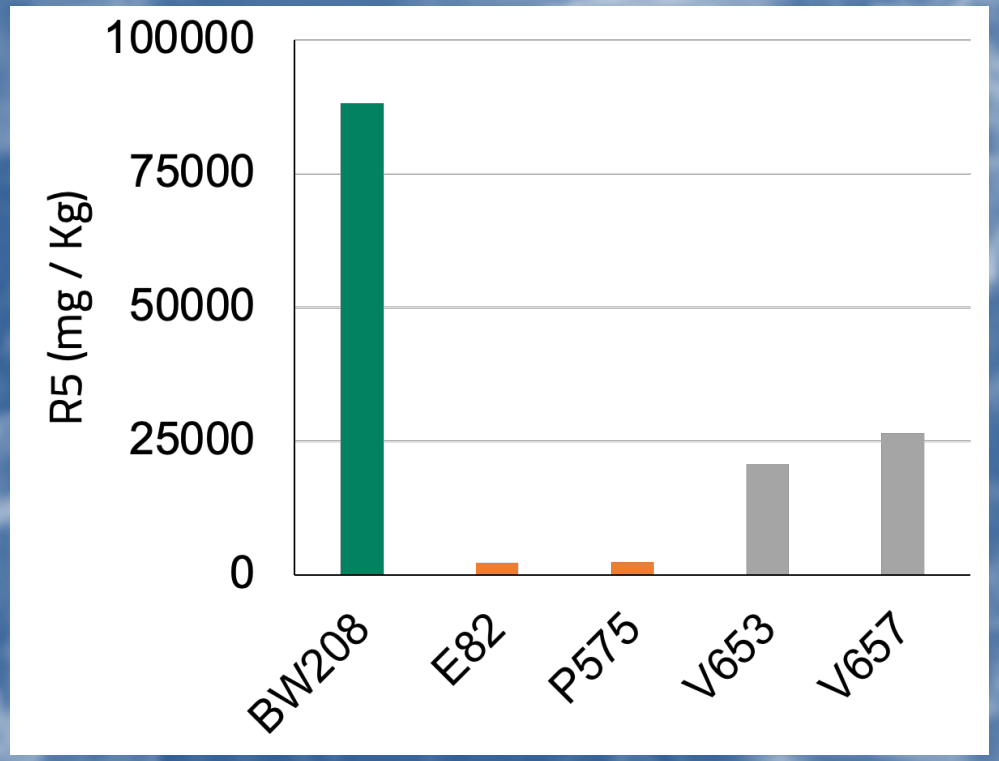


Figure 3: Gluten content determined by the monoclonal antibody R5 and total protein content in the grain of the studied lines.



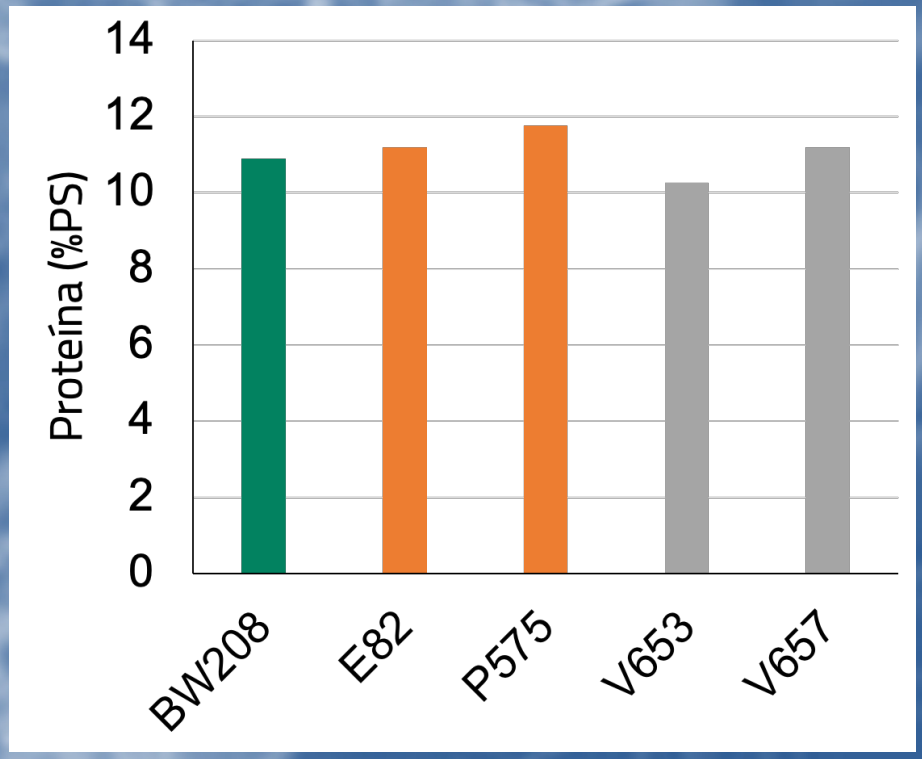
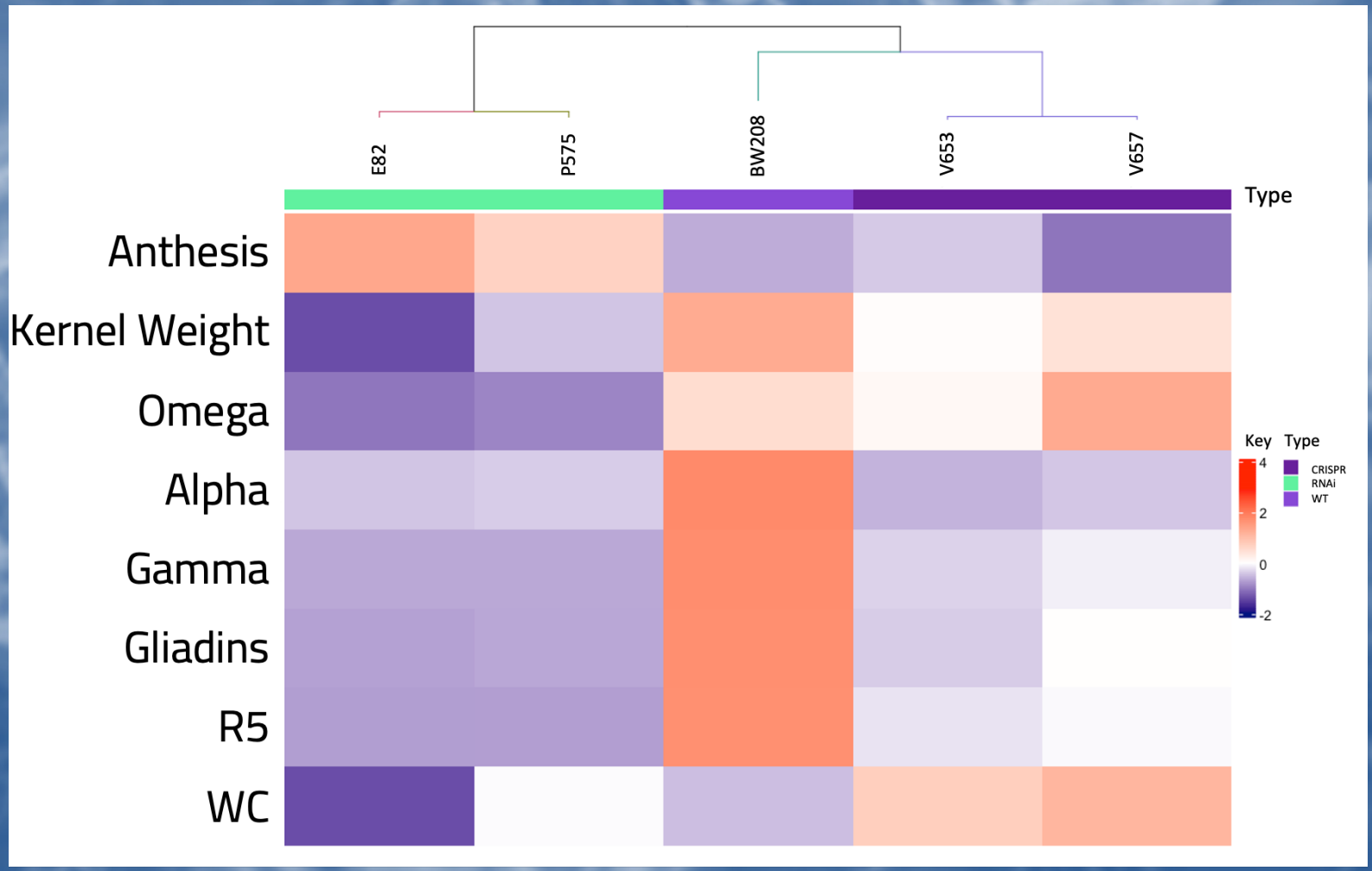


Figure 4: Heat map of the variables studied in this work in the CRISPR and ARNi lines.



Conclusions

1. **The RNAi lines present a greater decrease in the proteins related to gluten pathologies, since we have managed to eliminate higher percentage of gliadins with this technique than with CRISPR/Cas.**

2. **RNAi technology has a minor effect on other important components of the grain such as the content of starch and total protein, these quantities being higher in the RNAi lines than in the CRISPR/Cas lines.**

3. **Therefore, the development of these lines is an important advance for agriculture and health, which in the near future will help millions of people with gluten intolerance such as celiacs.**

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