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

A. Nieto[1*], M. Gómez[2*], N. Rodríguez[1*], P. Luque[2*]
H. Guzmán[3], F. Barro[3], E. León[1]

[1] IES Fidiana
[2] CES Lope de Vega
[3] Instituto de Agricultura Sostenible IAS-CSIC (Córdoba)
*These authors contributed equally to this work

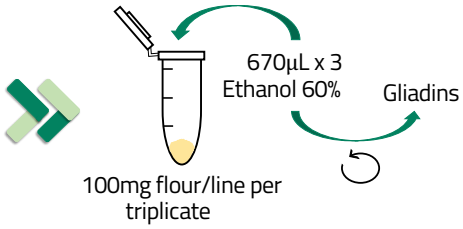
INTRODUCTION

Pathologies related to wheat consumption have increased in recent years. We can distinguish three pathologies: celiac disease, allergies to wheat and non-celiac wheat sensitivity. Wheat gluten is mainly responsible for these pathologies. Gluten is formed by two large protein fractions: glutenins and gliadins, the latter being, particularly α-gliadins, the main responsible for celiac disease. We have used two biotechnological techniques to eliminate wheat gliadins: RNA of interference (RNAi) and CRISPR/Cas. The **objective** of this work was to compare the efficiency of both technologies for the elimination of wheat gliadins.

MATERIALS AND METHODS

Genotype	Line	Technology	Target
BW208	E82	RNAi	Gliadins
	P575	RNAi	Gliadins
	V653	CRISPR/Cas	Gliadins
	V657	CRISPR/Cas	Gliadins
	BW208	NA*	NA*

*NA: Not applicable



Reverse phase High-performance liquid chromatography (RP-HPLC)

RESULTS

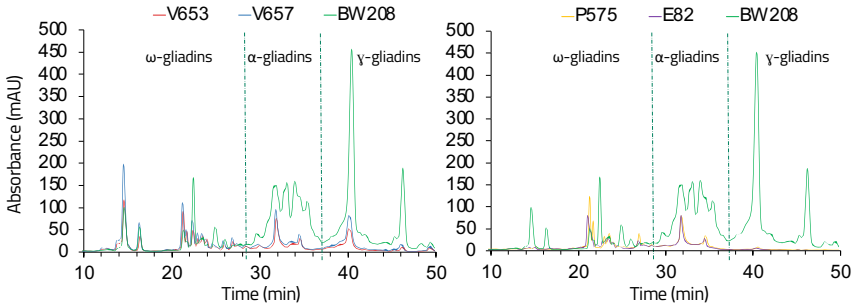


Figure 1. Chromatograms of gliadin protein extract in CRISPR/Cas (left) and RNAi (right) lines. Vertical lines indicate separation of gliadin fractions.

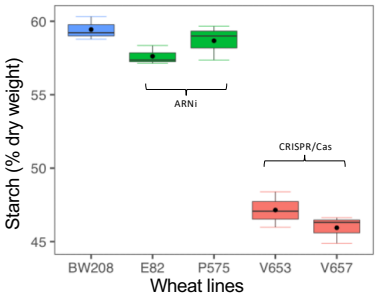


Figure 2. Starch content of grain from RNAi and CRISPR/Cas lines.

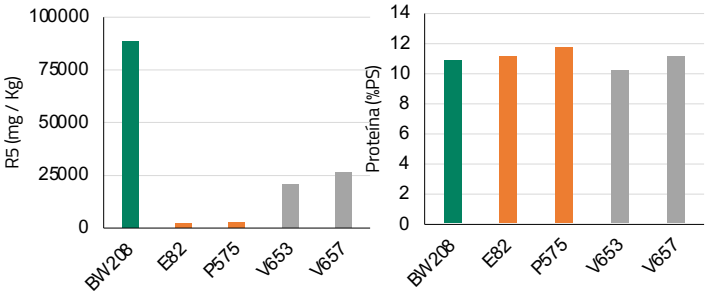


Figure 3. Gluten content determined by R5 monoclonal antibody and total protein content in the grain of the lines studied.

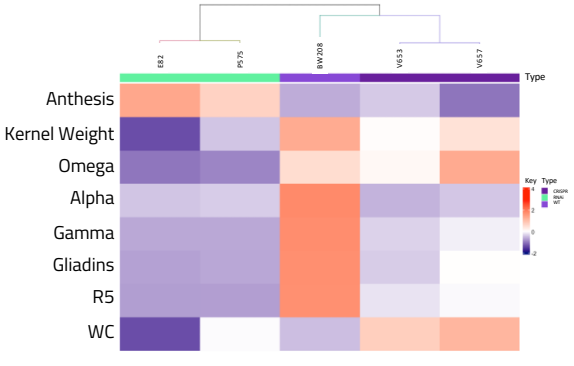


Figure 4. Heat map of the variables studied in this work.

Figure 1 shows the gliadin profiles obtained for the CRISPR and RNAi lines. In Figure 2, we observe that the amount of starch present in the grain decreases markedly in the CRISPR lines, while the RNAi lines show a starch content compared to the control line. In Figure 3, it can be seen that the gluten content determined by R5 shows large differences between the CRISPR and RNAi lines, being reduced by 97.3% in the RNAi lines while in the CRISPR lines it is only reduced by 73.2%. Although we have eliminated a large part of the gliadins, there are no significant differences in the total amount of protein present in the grain, suggesting that there is compensation with other types of proteins that do not belong to gluten. In Figure 4, we can compare all the modifications obtained in the CRISPR and RNAi lines. In general, the RNAi lines are more adapted to what we are looking for since the kernel weight is similar to the control line and all the gliadin fractions disappear in a higher percentage than the in the CRISPR lines.

CONCLUSIONS

1. The RNAi lines present a greater decrease in proteins related to gluten pathologies, since we have managed to eliminate more 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 step forward for agriculture and health, which inn the near future will help millions of people who suffer from gluten pathologies such as celiacs.

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