CRISPR/Cas9-edited wheat plants

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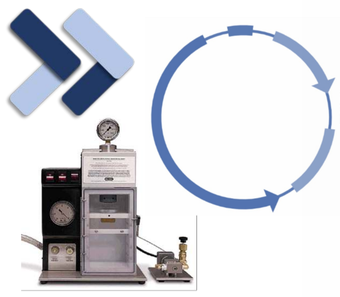
INTRODUCTION

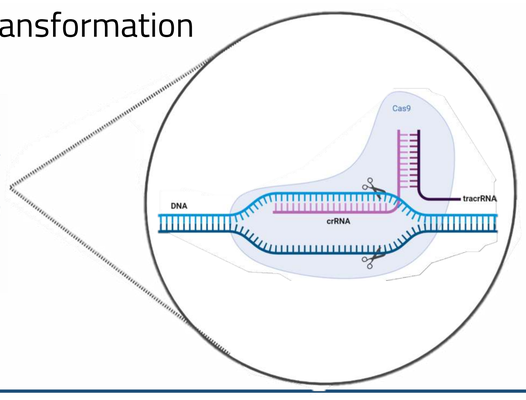
Gene editing by CRISPR (Clustered regurlaly interspaced short palindromic repeats) and associated proteins (Cas) is one of the most powerful and promising tools in biology, medicine and other life-sciences. Multiple examples of applications have been published for disease treatment and crop improvement. Wheat gluten is mainly responsible for several pathologies in humans. Gluten is made up of two large fractions: glutenins and gliadins, the latter being, particularly α-gliadins, the main cause of celiac disease. The aim of this work was to use vectors CRISPR/Cas to edit wheat α-gliadins, to generate plants with lower gluten content.

MATERIALS AND METHODS

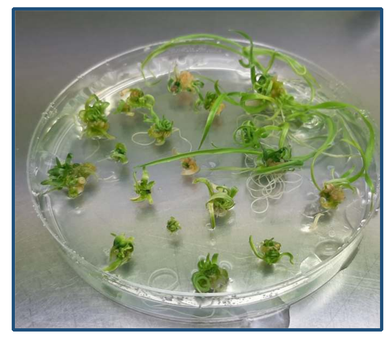
Starting material: Scutellum of bread wheat grains



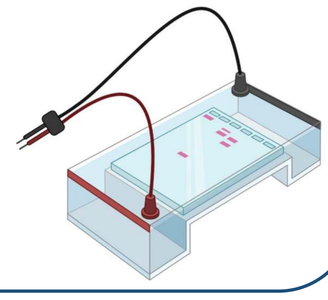
CRISPR/Cas vector transformation



In vitro culture



DNA extraction and PCR characterization



RESULTS

Figure 1. Response of bread wheat scutella to *in vitro* culture

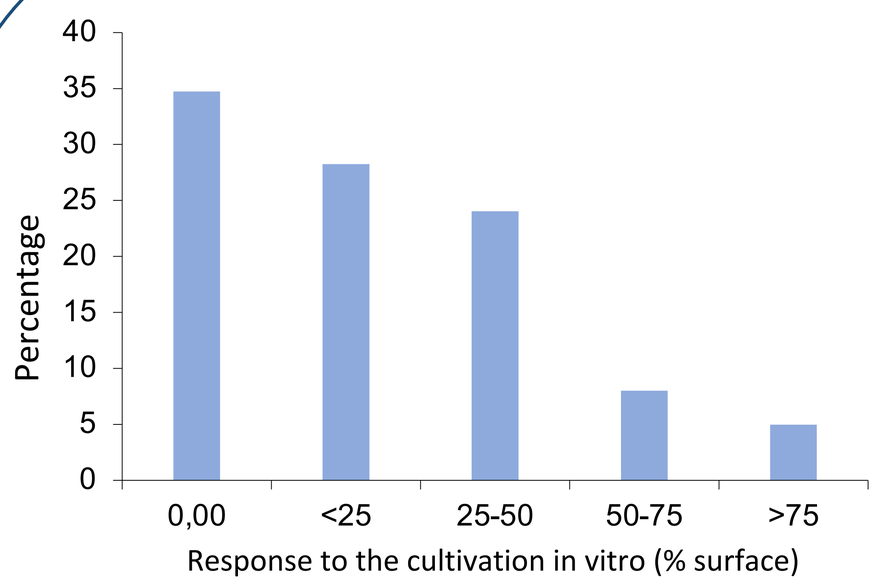
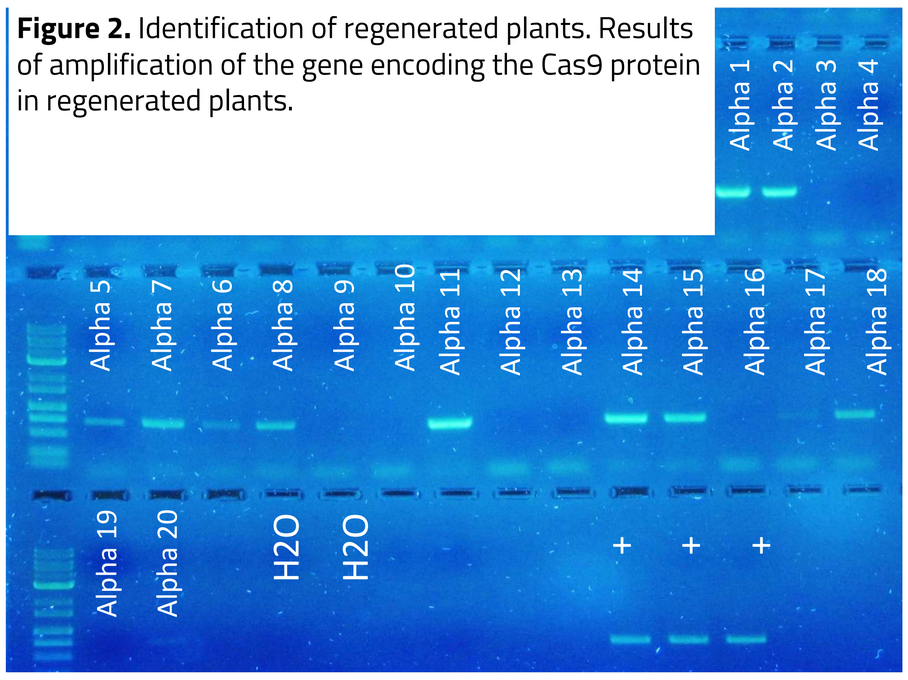


Figure 2. Identification of regenerated plants. Results of amplification of the gene encoding the Cas9 protein in regenerated plants.



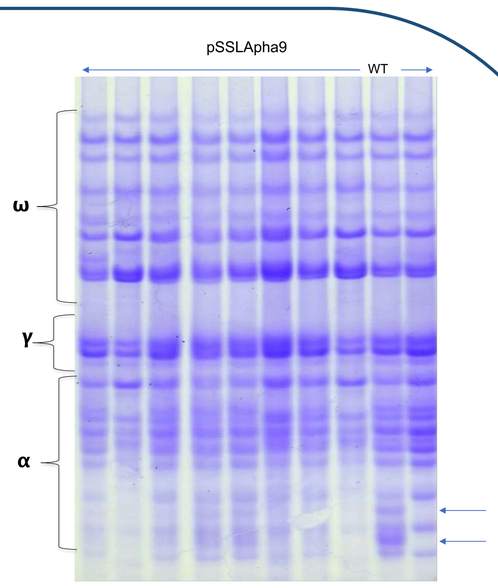


Figure 3. A-PAGE gel of wheat gliadins from lines transformed with the pSSLAlpha9 vector. The WT lane shows the protein pattern of a control wheat. The rest of the lanes correspond to grains from a line transformed with the indicated vector. It can be seen that in the alpha-gliadin region individual bands (arrows) or a set of bands (rectangles) have disappeared, demonstrating the efficiency of the guide RNAs.

Table 1. Efficiency of the regeneration and transformation of wheat scutella

Bombarded scutella	Regenerated plants	Cas9 + plants	Regeneration efficiency	Transformation efficiency
262	49	10	18.7	3.8

Figure 1 shows the response of bread wheat scutella to *in vitro* culture, measuring this response as the percentage of surface showing embryogenesis per each scutellum. So, we can confirm that more than 60% of the scutella developed embryogenesis when they were exposed to 2.4-dichlorophenoxyacetic acid (vegetable auxin). In Table 1, we show the general results of the generation of transformed plants: out of 262 bombarded scutella we managed to regenerate a total of 49 plants which implies a regeneration efficiency of 18.7%, and a transformation efficiency of 3.8

In Figure 2 we can observe the results of the PCR of Cas9 of the generated plants and its subsequent agarose gel electrophoresis for PCR product identification:10 out of 20 plants were found to have Cas9, indicating that these are transformed plants.

Finally, In Figure 3 we can see the protein profile of a line transformed with the pSSLAlpha9 vector. We can see that some gliadin bands have disappeared, confirming the efficiency of this technique.

CONCLUSIONS

- More than 60% of wheat scutella produce somatic embryos.
- The *in vitro* selection system is highly efficient and allows the regeneration of 49 putative-edited plants.
- About 20% of the regenerated plants contained the gene that codes for Cas9.
- The system CRSPR/Cas9 allows the editing of the genes that code for the proteins responsible for triggering gluten intolerances.

ACKNOWLEDGEMENTS:
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