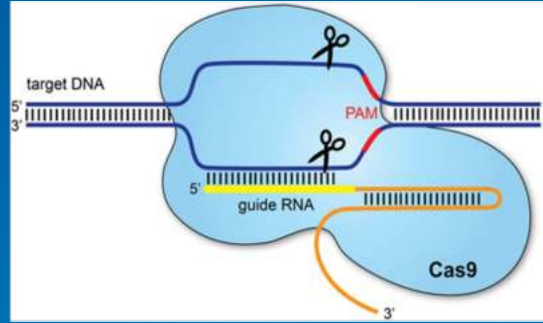
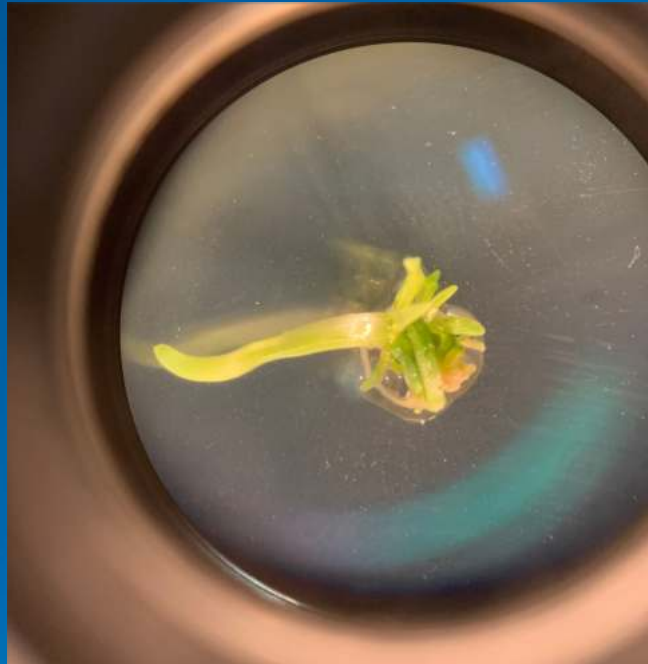


Edited wheat plants with CRISPR/Cas9



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INVESTIGATING STUDENT BODY: Paula Maestre Aguilar , Paula Resina Cruces, Paloma Garrido Berenguer, Álvaro Giménez Arcos.

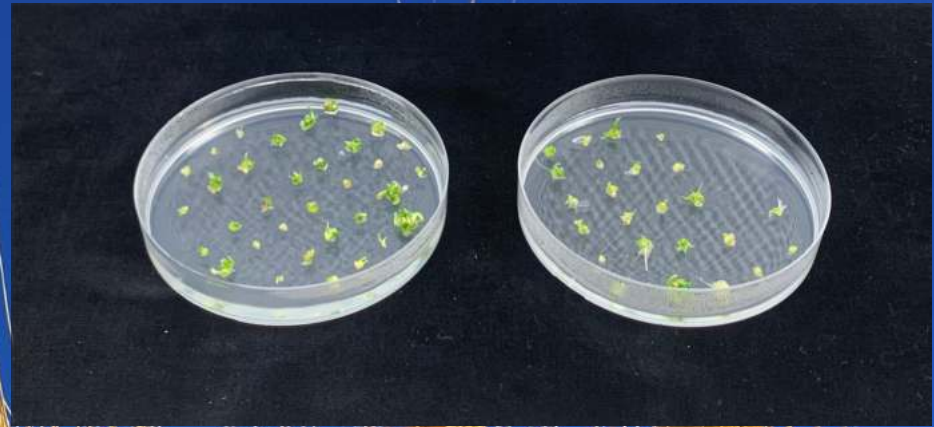
COURSE : 1-BACH

ACADEMIC YEAR: 2022-2023

CONGRESS NAME: 2-Congreso Científico Internacional Eurociencia Joven.

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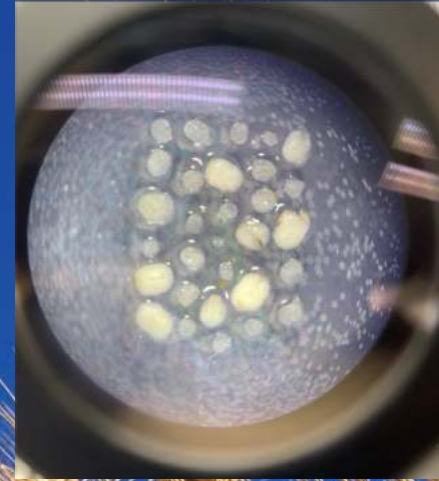
1. RESEARCH OBJECTIVES.
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INTRODUCTION

Today wheat is the most cultivated cereal in the world. In our project we have used CRISPR/Cas (Clustered regularly interspaced short palindromic repeats) which is a family of DNA sequences found in the genome of prokaryotic organisms and protein editing nucleases associated with these sequences (Cas) that cut DNA guided by RNA sequences and thus edit pieces of DNA.

- Gluten proteins are responsible of the breadmaking quality of wheat but also of some important pathologies.
- The main gluten proteins are: **gliadins and glutenins**.
- Gliadins are primarily responsible for **celiac disease**.



OBJETIVES

The main objective of this work is to use biotechnological techniques, CRISPR/Cas vectors to edit wheat gliadin genes, to test the efficiency of the RNA guides for generating wheat lines with gliadin genes mutated.



THEORETICAL FRAMEWORK

Gluten: The main storage group of grain proteins found in wheat. Being proteins they must be digested and broken down into small fractions. Some people have trouble digesting it causing damage.

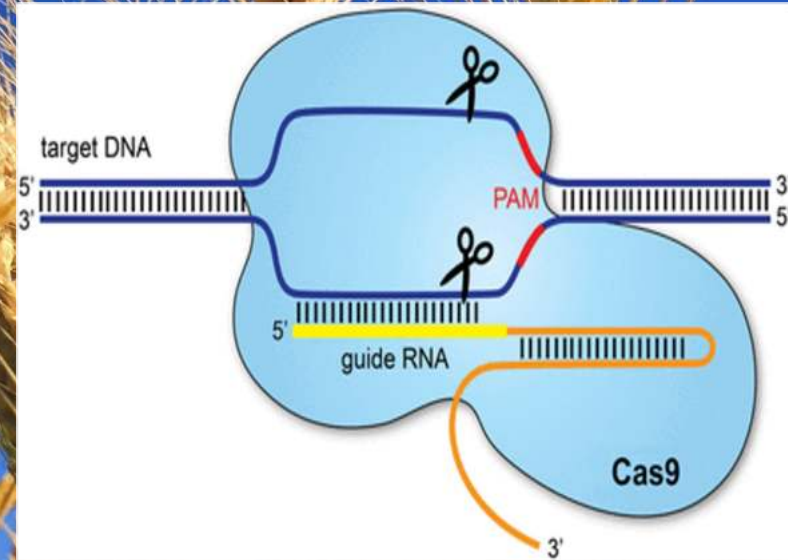
Celiac disease is an autoimmune genetic disease in which there is a permanent intolerance to gluten.

In vitro culture: growing a plant in a Petri dish, containing nutrients which are provided, and specific environmental conditions are simulated.



THEORETICAL FRAMEWORK

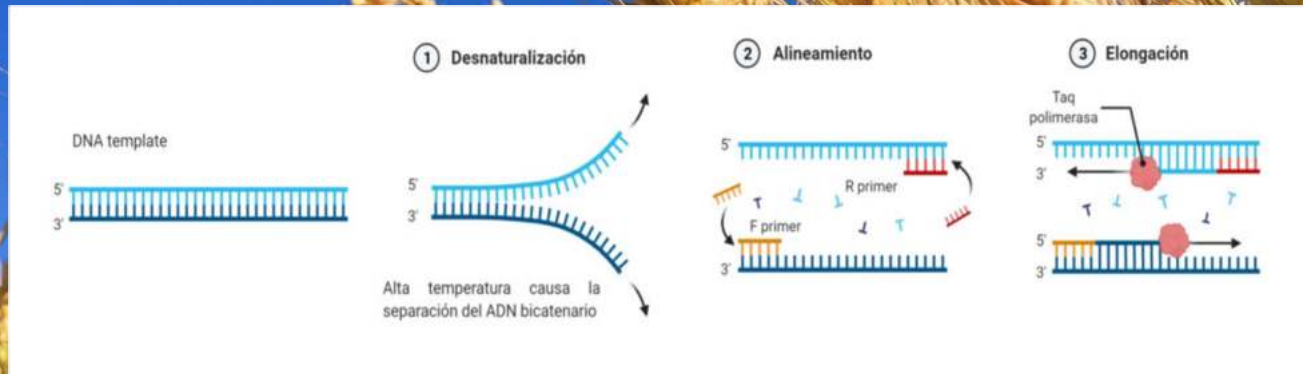
Cas9: DNA endonuclease enzyme directed by a guide RNA, capable of identifying DNA and cutting both DNA strands, inactivating it. CRISPR/Cas is used in this research to prevent the synthesis of some proteins, which are harmful to people with celiac disease, taking advantage of the DNA repair pathways that are activated after DNA cutting



THEORETICAL FRAMEWORK

Particle bombardment: a technique used to introduce plasmids containing the DNA of some genes into plant cells. Gold particles, coated with plasmid DNA, are used to disrupt the cell wall so that foreign DNA enters the nucleus of the cell.

PCR – A technique that allows the rapid production of millions of replicates of a specific segment of DNA. What is needed is a DNA polymerase, nucleotides, primers, magnesium, and short fragments of synthetic DNA.



MATERIALS AND METHODS



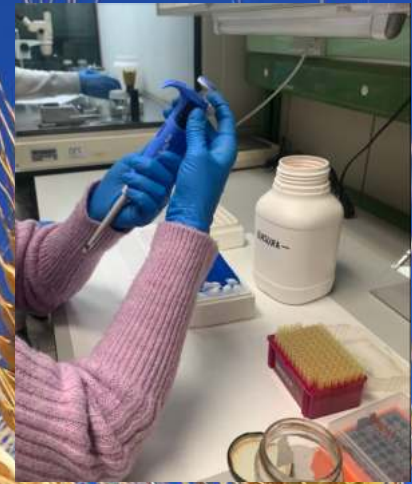
Electrophoresis cuvettes



Microcentrifuge



Flowhood chamber



Micropipettes



Thermal cycler



Petri dishes

DESIGN OF EXPERIMENTAL TOOLING

1. Isolation of scutella.
2. Bombardment with CRISPR/Cas9.
3. In vitro culture:
 - Embryogenesis
 - Plant regeneration
 - Plan in soil
4. PCR of Cas9 and Electroforesis.
5. Analyze results.



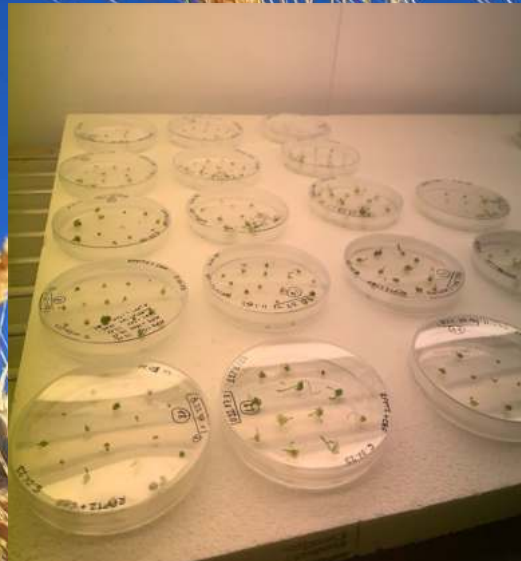
1st SESSION



The first session (23/11/22) we were explained what the work would consist of and proceeded to prepare the mixture of DNA and gold to carry out the bombardment of the previously isolated scutellum. With this, we introduced the Cas9 gene and the guide RNAs into the wheat scutellum cells.

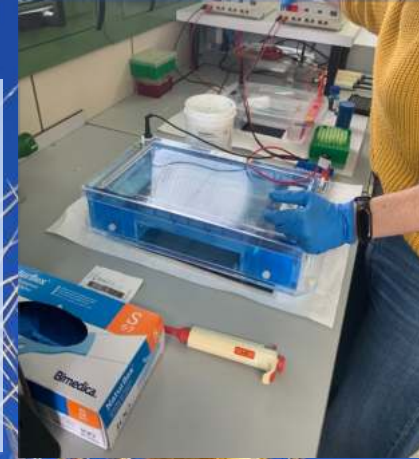
2nd SESSION

The second session (11/01/23) the embryogenic capacity of the wheat scutellum was determined and they were transferred to another medium to induce regeneration. At all times, work was carried out in a laminar flowhood chamber



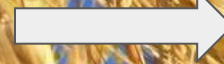
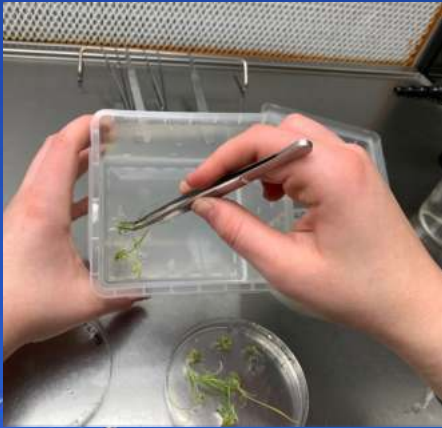
3rd SESSION

In the third session (08/02/23) the scutellum were also transferred to another new plate, or to a magenta or to pots, depending on the level of development of the explants/plantlets. In addition, DNA was extracted for PCR to detect the presence of the Cas9 gene.



4th SESSION

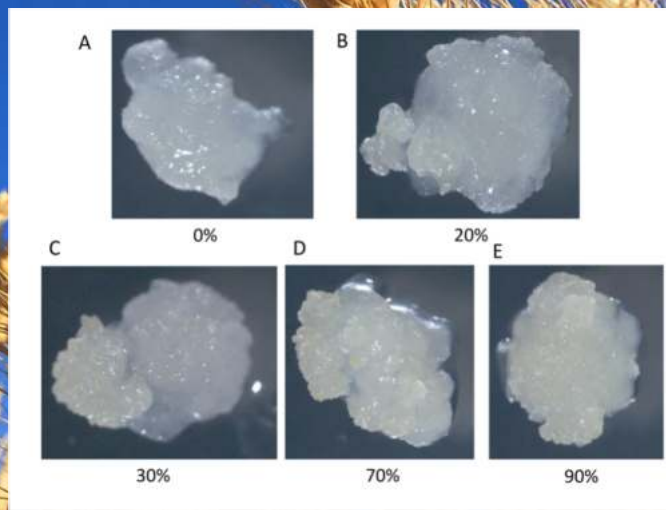
In the fourth session (08/03/23) more plants were transferred to magenta or soil pots. Afterwards, in the IAS meeting room, the data were analysed, the tasks for the report were distributed and the design of the poster was worked on.



RESULTS

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

<u>Bombarded scutella</u>	<u>Regenerated plants</u>	<u>Cas9 + plants</u>	<u>Regeneration efficiency</u>	<u>Transformation efficiency</u>
262	49	10	18.7	3.8



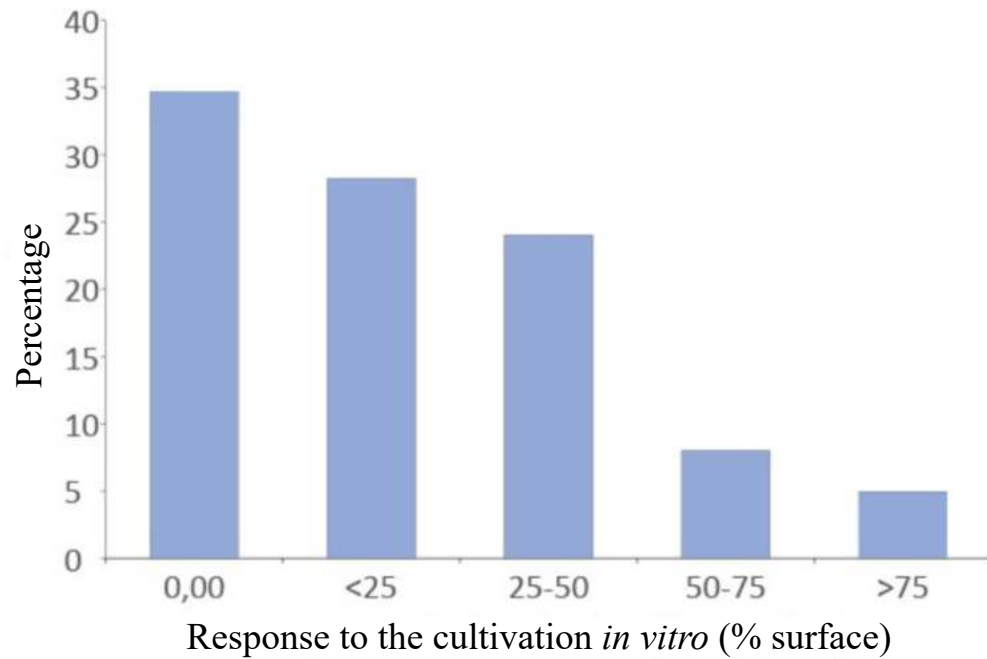


Figure 2. Identification of regenerated plants. Results of amplification of the gene encoding the Cas9 protein in regenerated plants.

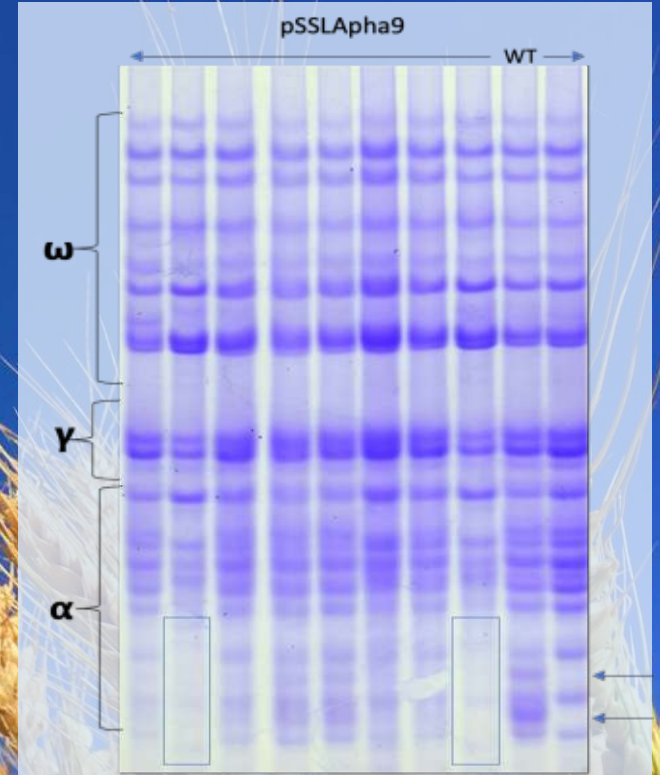
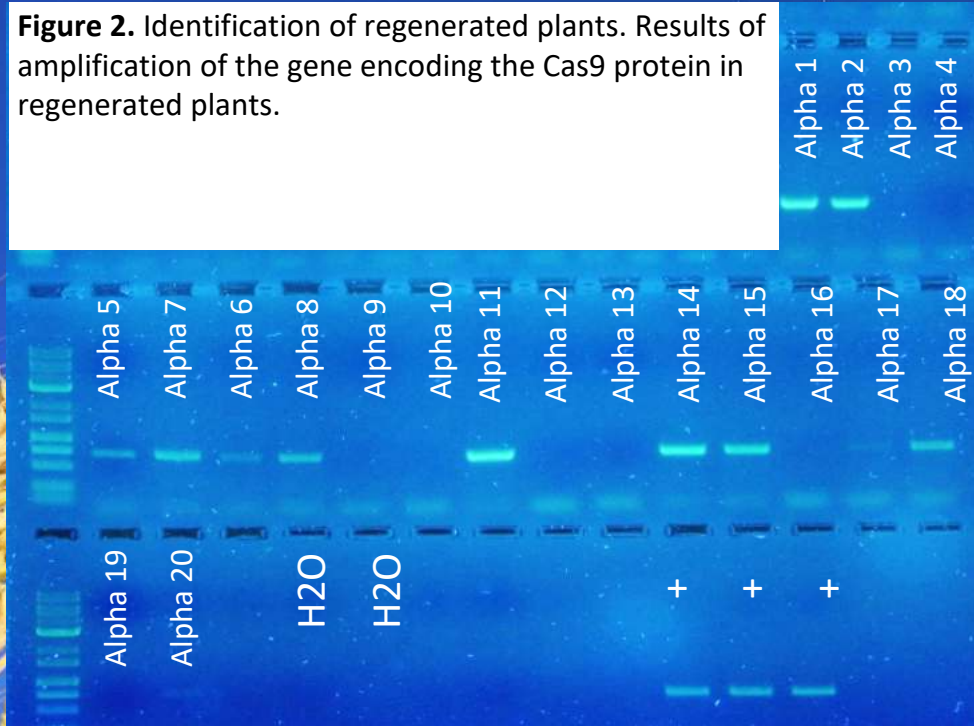


Figure 3. A-PAGE gel of wheat gliadins from lines transformed with the pSSLAlpha9 vector.

CONCLUSIONS

1. **Over 60% of wheat scutella produce somatic embryos.**
2. **The in vitro selection system is highly efficient and allows to regenerate 49 plants (18.7%)**
3. **Around 20% of the plants analyzed contained the gene that codes for Cas 9.**
4. **The CRISPR/Cas9 system allows the editing of genes that code for the proteins responsible for triggering gluten intolerances.**



ACKNOWLEDGMENTS

- To the researchers Helena Guzmán and Francisco Barro from the IAS-CSIC who have helped us so much in this process, both in the experimental phase and in the preparation of the report.
- To our tutor at the IES Fidiana, Elena León, who coordinated our research.
- To the IAS-CSIC research centre, which opened its doors to us so that we could carry out our project.
- To the fidiciencia2.0 project, which has given us the opportunity to carry out our research.



BIBLIOGRAPHY

Asociación de Biotecnología Vegetal Agrícola, Agro-Bio (22/02/2022). “Con edición genética crean variedad de trigo resistente al mildew polvoso”. Agro-Bio. <https://agrobio.org/noticias/con-edicion-genetica-crean-variedad-de-trigo-resistente-al-mildew-polvoso>

Elena Camacho (07/10/2020). “¿Qué es el CRISPR/Cas9?”. https://www.cope.es/actualidad/tecnologia/noticias/que-crispr-cas9-20201007_932436

Mojica FJ, Díez-Villaseñor C, García-Martínez J, Soria E. Intervening sequences of regularly spaced prokaryotic repeats derive from foreign genetic elements. *J Mol Evol.* 2005 Feb;60(2):174-82. doi: 10.1007/s00239-004-0046-3. PMID: 15791728.

Sapone A, Lammers KM, Casolaro V, Cammarota M, Giuliano MT, De Rosa M, Stefanile R, Mazzarella G, Tolone C, Russo MI, Esposito P, Ferraraccio F, Cartenì M, Riegler G, de Magistris L, Fasano A. Divergence of gut permeability and mucosal immune gene expression in two gluten-associated conditions: celiac disease and gluten sensitivity. *BMC Med.* 2011 Mar 9;9:23. doi: 10.1186/1741-7015-9-23.

Spiegato. “¿Qué es el bombardeo de partículas?”. <https://spiegato.com/es/que-es-el-bombardeo-de-particulas>



END

THANK YOU ALL FOR YOUR
ATTENTION