"Identification of hypoallergenic wheat lines using CRISPR/Cas and RNAi"

IES Fidiana
Rosa Gálvez García,
Miriam Serrano Quesada,
Ainoa Medrano Baena.

COORDINATING TEACHER:
Dra Elena León Rodríguez (IES Fidiana)

RESEARCHES Instituto de Agricultura Sostenible (IAS-CSIC):
Dr Francisco Barro Losada
Dra Helena Guzmán López
Dra Miriam Marín Sanz
Dra Marta Gavilán Camacho

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INTRODUCTION

Wheat is a cereal used in a wide variety of key food products due to its nutritional properties, its role in the global economy and its contribution to agricultural sustainability.

Pathologies related to wheat consumption have increased in recent years.

We can distinguish three pathologies:

- coeliac disease
- wheat allergies
- non-coeliac wheat sensitivity

Gluten proteins

Responsible of:

- Viscoelastic properties of bread
- Certain human pathologies

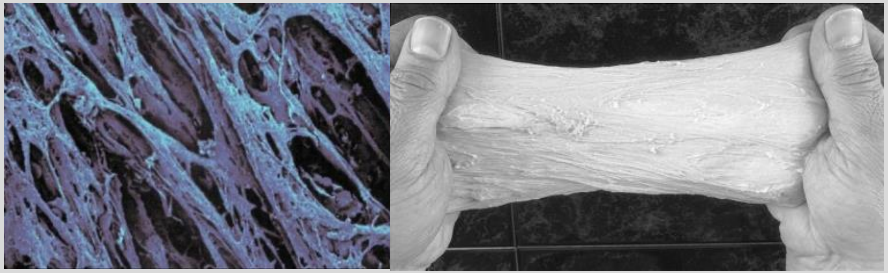
Coeliac Disease (CD)

Healthy

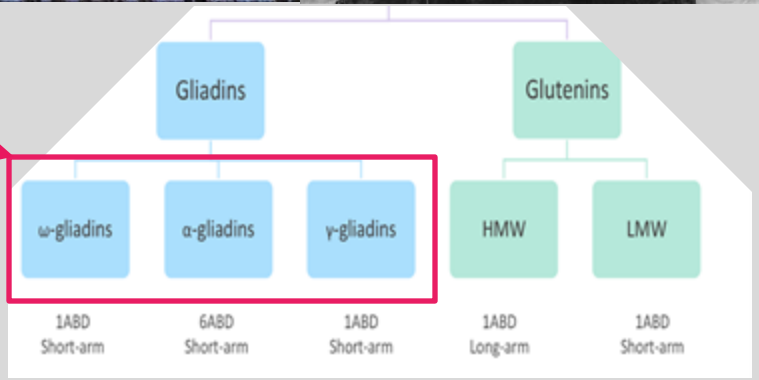
Coeliac disease

What is gluten?

The gliadin fraction is very important as it is the main fraction of gluten that is toxic to people with wheat consumption disorders.



Responsible for immunological reactions

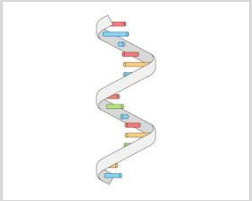


Very important to eliminate or reduce them from the wheat kernel

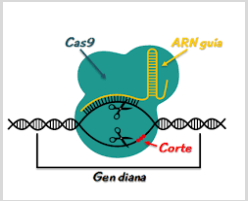
Hypoallergenic wheat (gliadin-free)

Techniques

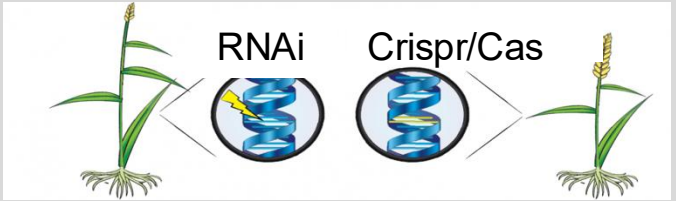
RNAi



Crispr/Cas



Combined by crossbreeding (RNAi+Crispr/Cas)



- Hypoallergenic wheat varieties with lower gluten content
- Safer foods for people with coeliac disease or gluten sensitivity
- Sustainable alternatives for wheat production without affecting its yield or agronomic properties

OBJECTIVE

The aim of this work has been to evaluate and compare a set of RNAi and CRISPR/Cas lines and to select those hypoallergenic lines with lower gliadin content

MATERIALS AND METHODS

Study variables

Independent variable: genetic treatment of each line

- RNAi
- Crispr/Cas
- RNAi+Crispr/Cas

Dependent variable: affected gliadins (α-, ω-, γ-gliadins)

MATERIALS

A Box of tips for micropipettes

B Ball mill

C Centrifuge

D Spatula

E Bottle with ethanol

F Precision scale

G Metal block

H High performance liquid chromatograph (HPLC)

I Micropipette

J Microtube rack

K Weighing capsule

DESIGN OF LABORATORY WORK

1 – Flour and sample processing

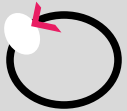
Wheat grain

50mg flour/ line in triplicate

2 – Extraction of gliadins

335µL x 3
Etanol 60%

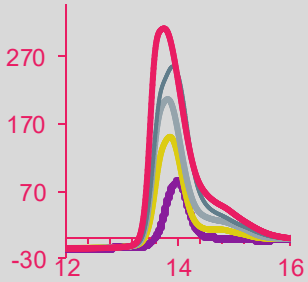
micropipette

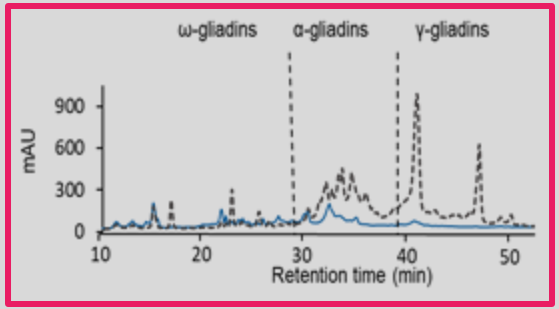


Gliadins

3 - Preparation for RP-HPLC

4 – RP-HPLC





RESULTS

			ug prot. / mg harina				Err. Est.			
Type	Target	Sample	Omega	Alpha	Gamma	Total	Omega	Alpha	Gamma	Total
WT	NA	BW208	16,9	40,2	26,6	83,8	1,39	2,89	1,59	5,82
CRISPR	alpha-gliadinas	AB343	10,8	26,9	11,1	48,8	1,66	3,90	1,38	6,87
CRISPR	alpha-gliadinas	AB344	17,4	40,8	16,4	74,5	0,89	1,84	0,78	3,39
CRISPR	omega-gliadinas	AE849	14,2	46,8	7,4	68,4	1,05	2,98	0,44	4,22
CRISPR	omega-gliadinas	AE850	9,8	29,7	4,9	44,4	1,04	1,51	0,46	2,87
CRISPR	omega-gliadinas	AE853	8,4	25,9	3,8	38,1	0,66	0,91	0,09	1,53
CRISPR	omega-gliadinas	AE900	7,9	22,1	10,2	40,2	1,13	2,77	1,19	5,04
CRISPR	omega-gliadinas	AE902	9,5	30,2	6,1	56,8	0,61	0,91	0,61	11,09
CRISPR	omega-gliadinas	AE904	9,9	32,4	9,8	52,1	0,10	1,54	1,03	2,49
CRISPR	omega y gamma-gliadinas	AH787	18,2	55,6	20,9	81,0	0,60	1,37	0,73	13,84
CRISPR	omega y gamma-gliadinas	AH788	23,9	67,2	27,1	108,0	1,35	2,01	0,91	10,54
CRISPR	alpha-gliadinas	AL893	25,1	14,6	7,8	54,1	0,88	0,13	0,24	6,68
CRISPR	alpha-gliadinas	X477	17,5	6,8	3,6	27,8	0,42	0,78	0,07	1,14
ARNi	alpha-, gamma- y omega-gliadinas	T258	9,7	10,6	1,0	21,3	0,81	0,99	0,22	1,82
ARNi x CRISPR	alpha-, gamma- y omega-gliadinas	L-004	9,9	5,5	0,1	15,5	0,48	0,27	0,04	0,77
CRISPR x CRISPR	alpha-gliadinas	L-204	19,2	8,9	3,2	31,3	1,20	0,84	0,26	2,26

TABLE 1. Content in the three gliadin fractions of each of the wheat lines obtained using the described technologies. Values are given in g protein/mg flour. The standard error associated with the 4 measurements is also indicated.

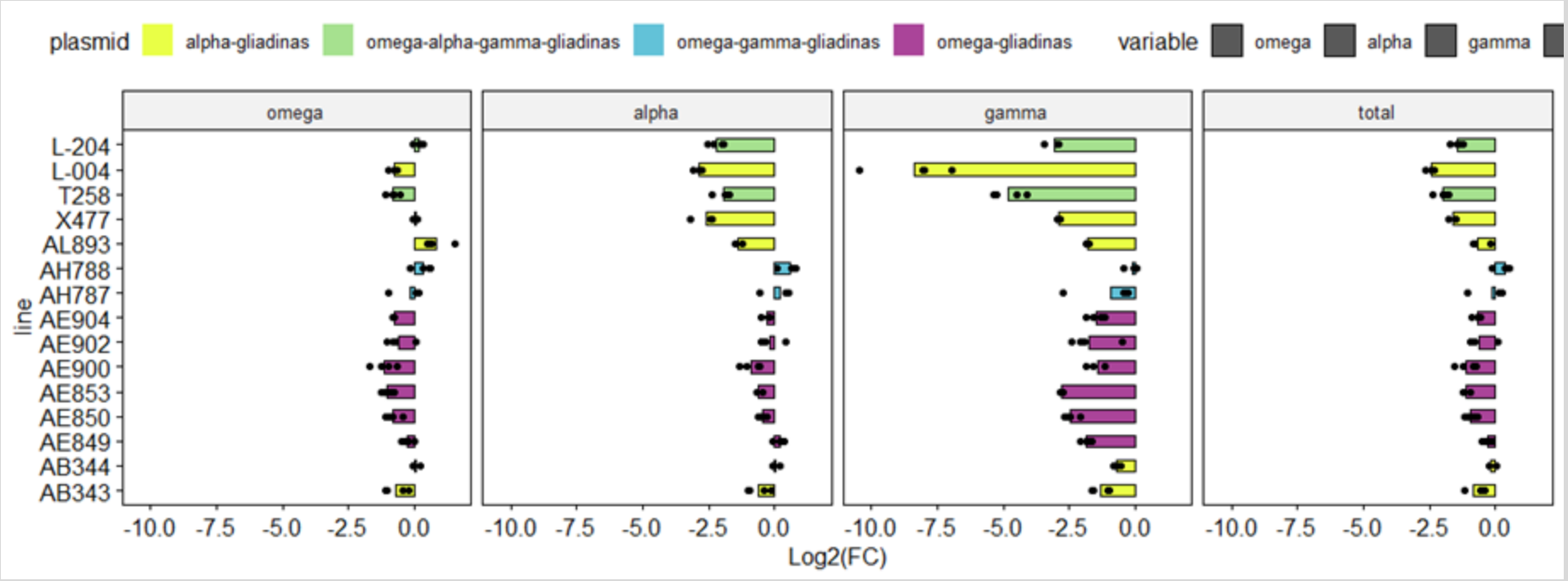


FIGURE 1. The graph shows the reduction of gliadins (omega, alpha and gamma) in different genetically modified wheat lines. The colors represent different plasmids used, and negative values indicate reduction. Some modifications affect only one type of gliadin, while others reduce several simultaneously.

Electrophoresis gel

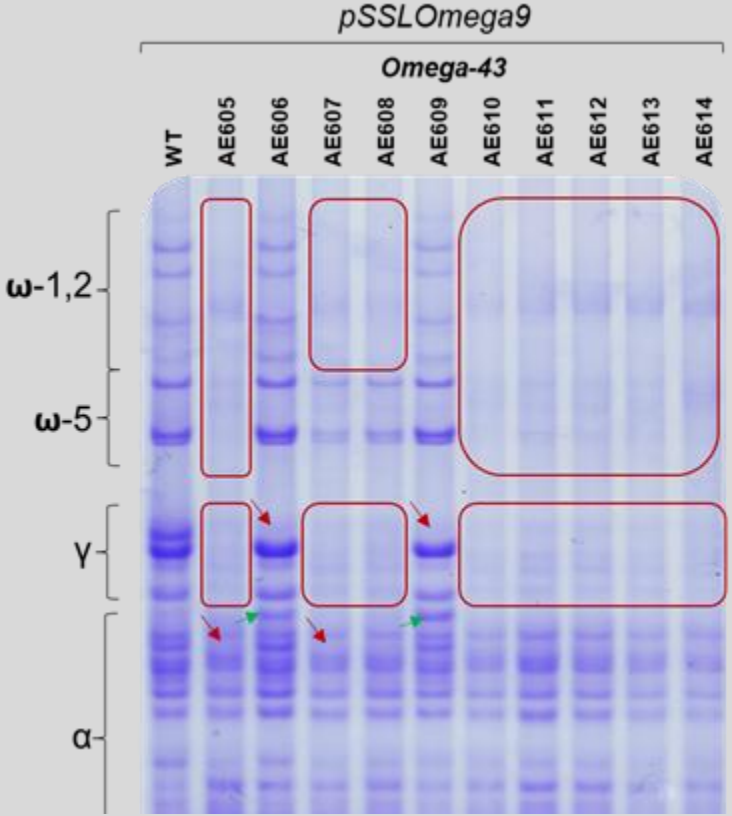
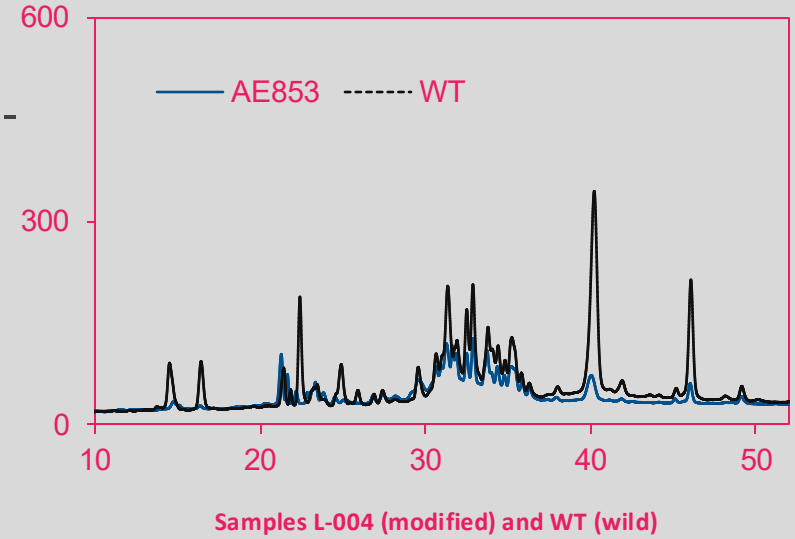


FIGURE 2. Electrophoresis gel shows the expression of gliadins (ω, γ, α) in wheat lines modified with pSSLOmega9. Bands indicate the presence of proteins, and differences from the control (WT) suggest changes in gliadin expression due to the genetic modification.



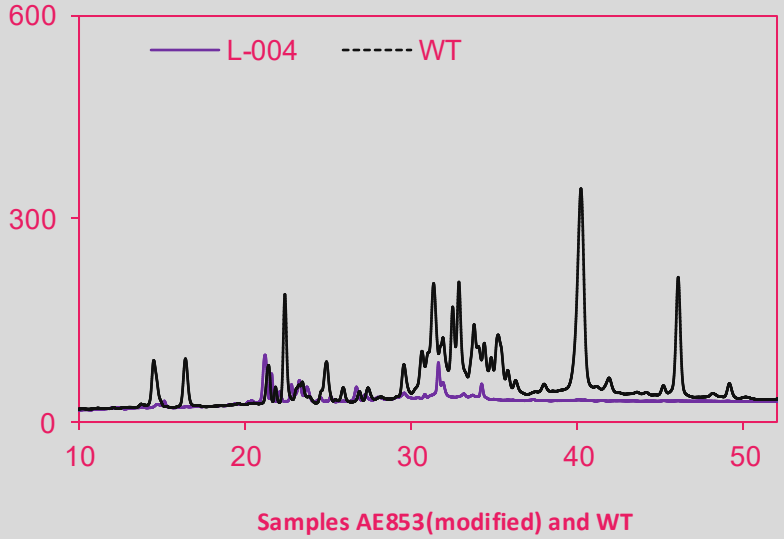


Figure 3. Chromatograms of wheat lines L-004 (RNAi x CRISPR) and AE853 (CRISPR). Left; line AE853 (CRISPR) and WT (wild-type). Right; line L-004 (RNAi x CRISPR) and WT (wild-type). The black lines are from WT (unmodified wheat), while the colored lines represent the protein samples corresponding to the modified lines.

CONCLUSIONS

- In this research it is concluded that the lines in which we found the lowest amount of omega proteins are: AE900 and AE853, which have obtained with CRISPR technology.
- The lines in which they found the greatest decrease of alpha-gliadin proteins are L-004 and X477, obtained by CRISPR x RNAi technology and CRISPR technology, respectively.
- The line with the lowest gamma-gliadin content was L-004, obtained by CRISPR x RNAi technology.
- Finally, it was concluded that the combination of CRISPR x RNAi technology is the best to reduce gliadin content in wheat.

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Instituto de Agricultura Sostenible

Researchers :
Francisco Barro Losada
María Elena Guzmán López
Marta Gavilán Camacho
Miriam Marín Sanz

Coordinating professor:
Elena León Rodríguez

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THANK YOU FOR YOUR ATTENTION!