Identification and selection of hypoallergenic wheat lines produced by CRISPR/Cas and RNAi

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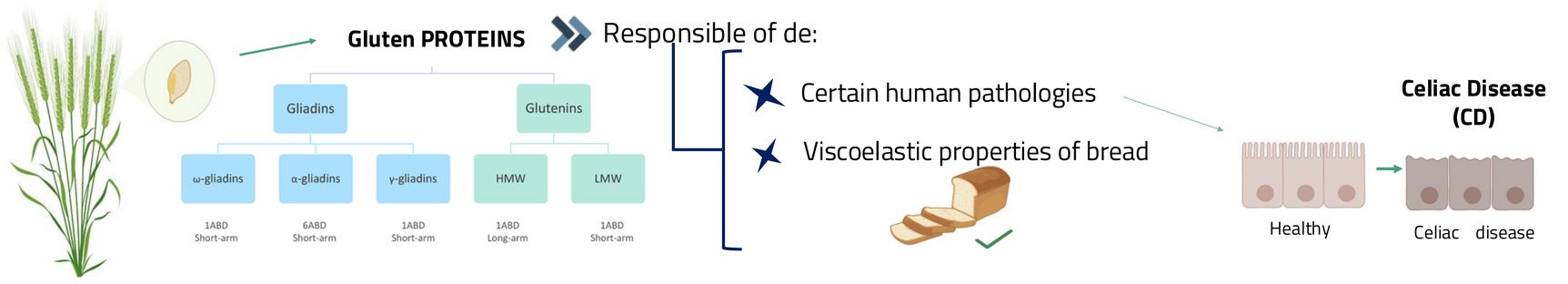
INTRODUCTION

Pathologies related to wheat consumption have increased in recent years. We can distinguish three pathologies: celiac disease, wheat allergies and non-celiac wheat sensitivity. Wheat gluten is the main cause of these pathologies. Gluten consists of two major protein fractions: glutenins and gliadins, the latter being the main responsible for celiac disease. We have used two biotechnological techniques to eliminate wheat gliadins: RNA interference (RNAi) and CRISPR/Cas. In addition, lines have been obtained by combining both technologies through crosses.

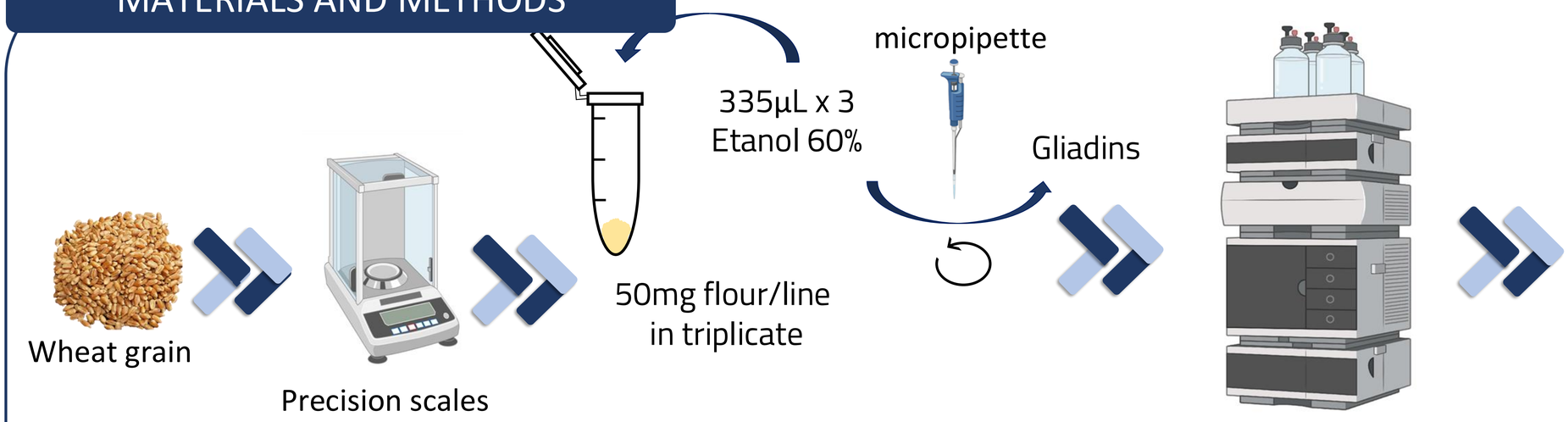


The aim of this work has been to evaluate a set of RNAi and CRISPR/Cas lines and to select those hypoallergenic lines with lower gliadin content.

Tabla 1. Genotypes used and their characteristics

Genotypes	Tecnology	Objetive
AE902	CRISPR	ω-gliadinas
AE900	CRISPR	ω-gliadinas
AE904	CRISPR	ω-gliadinas
AE849	CRISPR	ω-gliadinas
AE850	CRISPR	ω-gliadinas
AE853	CRISPR	ω-gliadinas
AB343	CRISPR	α-gliadinas
AB344	CRISPR	α-gliadinas
X477	CRISPR	α-gliadinas
AL893	CRISPR	α-gliadinas
AH788	CRISPR	ω-, γ-gliadinas
AH787	CRISPR	ω-, γ-gliadinas
L-204	CRISPRxCRISPR	α-gliadinas
T258	RNAi	α-, ω-, γ-gliadinas
L-004	RNAixCRISPR	α-, ω-, γ-gliadinas
BW208	wild-type	wild-type

MATERIALS AND METHODS



RESULTS

Figure 1. Reduction (negative values) or increase (positive values) of gliadin fractions in the different wheat lines analyzed in this work. It can be observed that line L-004 presents lower gamma and omega-gliadins content. The colors correspond to the different plasmids used.

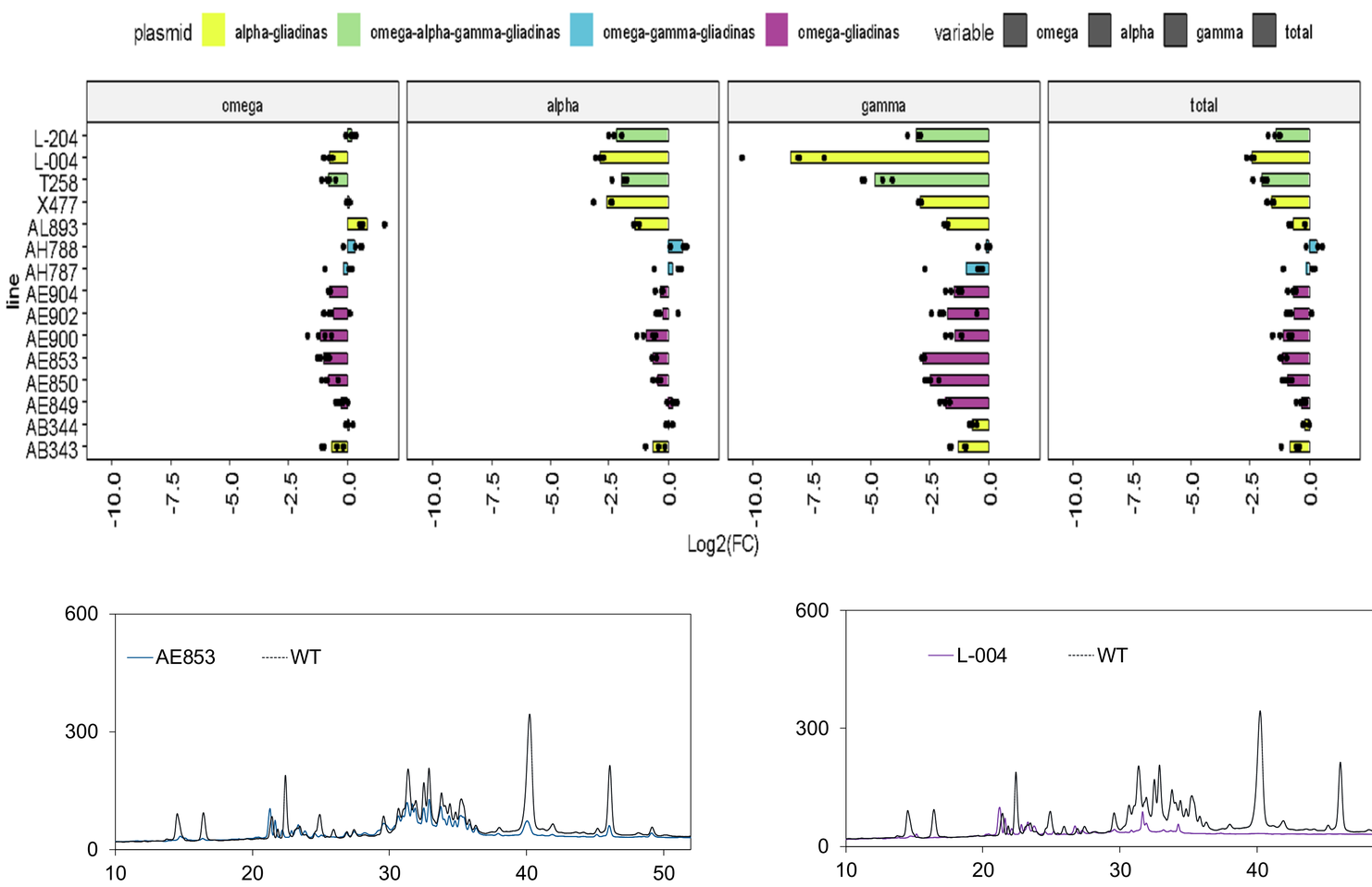


Table 2. Content in the three gliadin fractions of each of the wheat lines obtained using the technologies described. Values are given in µg protein / mg flour. The standard error associated with the 4 measurements is also indicated.

			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

Figure 2. 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.

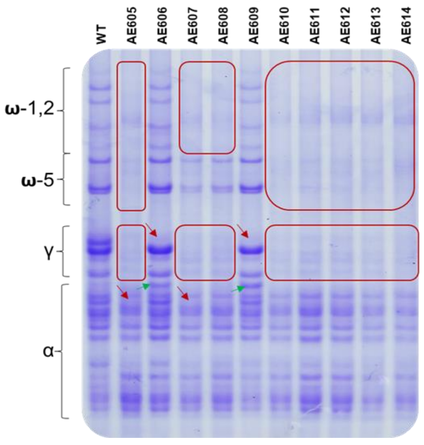


Figure 3. A-PAGE gels of wheat gliadins edited with the pSSLOmega9 plasmid. Band reduction is observed in different fractions of gliadins.

CONCLUSIONS

- The lines with the lowest amount of omega proteins are: AE900 and AE853, lines obtained with CRISPR technology.
- The lines with the highest decrease in alpha-gliadin proteins are L-004 and X477, a CRISPR x RNAi line and a CRISPR line, respectively.
- The line with the lowest gamma-gliadin content is L-004, a CRISPR x RNAi line.
- Considering total gliadins, the line with the lowest amount is L-004, which is a CRISPR x RNAi line.
- Overall, the combination of CRISPR x RNAi technology is the best technology for reducing gliadin content in wheat.

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