Evaluation and Licensing Opportunities

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Tech ID: 10.512 / 12.535 / 16.604 / 19.652

Patent Literature

10.512
Grants: US 8,859,850, EP 2395089, MX 324734, CA 2,750,997, AU 2010210107

12.535
Publications: WO2014/202688
Grants: US 9,976,156, EP 3011036

16.604
Publications: WO/2018/224508

19.652
Publications: WO/2022/238443, US-2025-0283100 A1

Wheat with reduced Gliadins

RNAi of wheat gliadins produces wheat with drastically reduced gliadin fraction

Potential nutritional benefits for Gluten Intolerant consumers and Coeliac Disease sufferers

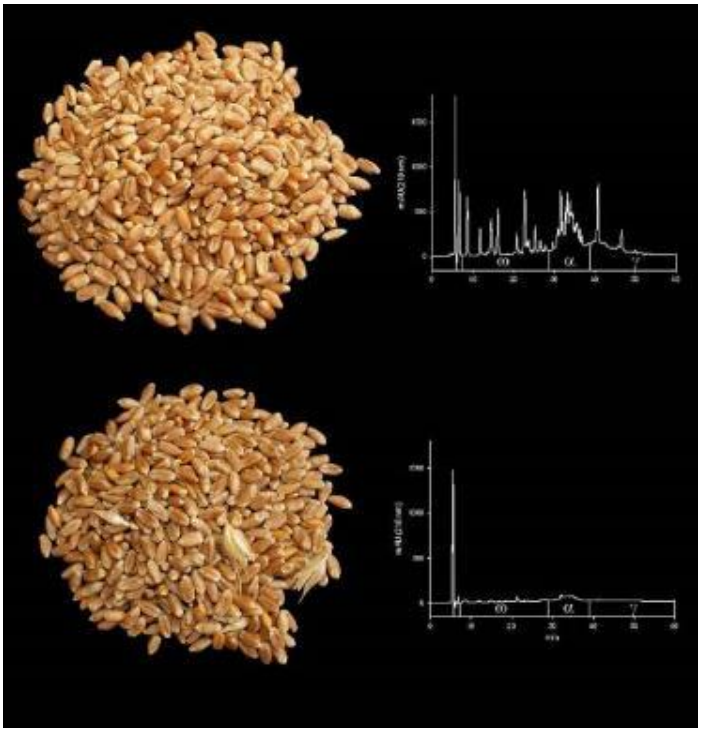
Coeliac disease (CD) is an autoimmune response derived from an allergic reaction to gluten proteins contained in wheat flour, and similar grain storage proteins in barley and rye. CD predisposition is genetically based and CD affects around 1% of the population in Western countries, and is reported to have quadrupled in prevalence since the 1950's. The only available treatment is life-long strict avoidance of gluten in the diet – which represents a significant challenge given the diverse uses of cereal-based ingredients sources in foods. An even greater number of people – estimated as being up to 15% of world population - may suffer from a milder form of gluten response known as "non-coeliac gluten intolerance".

Wheat gluten comprises families of both glutenin and gliadin proteins, but the majority of epitopes associated with CD are contained by the gliadins. The gliadin proteins are encoded by three major multigene families: α-gliadins (25-150 copies), γ-gliadins (17-39 copies) and the ω-gliadins (15-18 copies). This coupled with the fact the genes are present in tightly linked clusters, makes conventional breeding or mutagenesis approaches to reducing gliadins impractical. However, as reported in PNAS, September 2010, Franciso Barro and co-workers at the CSIC Institute for Sustainable Agriculture (Cordoba, Spain) have used a multi-target RNAi approach to successfully down-regulate the entire gliadin fraction in transgenic wheat. In tests using T-cell clones derived from CD-patients and specific for various different gliadin epitopes, protein extracted from the transgenic wheat lines showed dramatic reductions in T-cell response. For three of the transgenic lines, there was no response at all in two T-cell lines reactive to ω-gliadin epitopes.

The CSIC team designed RNAi hairpin constructs targeting conserved regions among the gliadin proteins, which are highly conserved in primary sequence. Two separate conserved domains were combined, one targeting α-gliadins and one targeting ω-gliadins, in a single chimeric construct for plant transformation. Separate versions of this construct were made for transformation experiments, expressing the chimeric hairpin element from two different endosperm-specific promoters – the wheat γ-gliadin promoter and a D-hordein promoter from barley. Transgenic wheat lines transformed with both constructs showed an average reduction in total gliadins of 85.6%. RNAi silencing of the γ-gliadins has been previously reported by the same group (Gil-Humanes et al 2008, J Cereal Science 48:656-568), however using the combined α/ω-RNAi strategy the reduction in γ-gliadins constructs was significantly greater than that previously obtained with γ-specific RNAi – with up to 99% reduction in γ-gliadins.

The use of RNAi technology to successfully down-regulate such multiple gene families represents a major technical achievement and opens the way to creating wheat suitable for consumption by non-coeliac gluten-intolerant consumers and, potentially, CD sufferers. The transgenic wheat lines are expected to retain reasonable baking properties because the high-molecular weight glutenin subunits, the major determinants of bread-making quality, are still present in these lines. SDS sedimentation analysis, a widely used assay that is highly predictive of bread-making quality, indicates that the reduced-gliadin wheats are comparable to the control bread-making quality wheats. While glutenins can elicit T-cell responses in CD patients, their stimulatory capacity is far less than gliadin proteins. Research indicates that a CD suffers can safely consume a daily gluten intake of between 10 and 100mg, which would bring these reduced-gliadin wheats within the scope of foodstuffs tolerated by CD patients.

Figure: Grain samples from control (top) and reduced-gliadin transgenic (bottom) wheat lines, with reverse phase HPLC analysis of gliadin protein fractions at the right.



UPDATE 2012:

The gliadin-reduction construct has been transformed into Triticum durum, so now pasta wheats are also available with the reduced gliadin profile.

The reduced-gliadin wheats have been extensively characterized by the CSIC researchers. The transgenic trait is being backcrossed into various elite bread wheat backgrounds and no adverse phenotype or field performance has been detected. Yield tests are on going but initial data indicates minimal, if any, yield penalty associated with the trait.

Quality tests are also being conducted. For example, Mixograph and Mixolab breadmaking quality studies indicate highly acceptable profiles foe the reduced-gliadin wheats, with eben some potential advantageous properties compared to control wheats. Data available upon request.

The CSIC researchers have conducted baking tests with partners and established that the reduced-gliadin wheats make excellent bread products, as the figure below indicates:

Sensory assessment by trained panelists have been conducted and show that many/most of the reduced-gliadin transgenic wheats make bread with very acceptable sensory profile – in marked contrast to rice flour that is a common wheat substitute in "celiac-safe" food products – see table below:

Table 6.2. Bread sensory analysis

Line	Appearance	Aroma	Crumb texture	Flavour	Springiness	Global acceptance
BW208	**7.63 ± 0.13**	**7.38 ± 0.22**	**7.06 ± 0.36**	**7.33 ± 0.25**	**7.50 ± 0.32**	**7.72 ± 0.18**
D793	6.38 ± 0.27	6.93 ± 0.21	6.07 ± 0.50	6.47 ± 0.27	8.00 ± 0.17	6.07 ± 0.30
D894	7.20 ± 0.26	7.07 ± 0.32	6.13 ± 0.39	6.87 ± 0.31	8.33 ± 0.21	6.87 ± 0.22
E82	7.25 ± 0.31	6.44 ± 0.36	6.07 ± 0.44	6.13 ± 0.33	8.44 ± 0.13	6.63 ± 0.29
E33	7.13 ± 0.22	6.38 ± 0.30	5.81 ± 0.34	6.80 ± 0.42	8.19 ± 0.19	6.93 ± 0.32
BW2003	**8.00 ± 0.18**	**7.13 ± 0.24**	**6.50 ± 0.43**	**7.07 ± 0.32**	**7.94 ± 0.19**	**7.06 ± 0.33**
D874	6.77 ± 0.41	7.00 ± 0.31	5.50 ± 0.50	7.07 ± 0.25	8.00 ± 0.21	6.85 ± 0.32
E93	7.06 ± 0.31	6.81 ± 0.28	5.50 ± 0.38	7.07 ± 0.32	7.94 ± 0.23	6.87 ± 0.34
E140	6.81 ± 0.28	6.56 ± 0.27	5.56 ± 0.45	6.38 ± 0.38	8.13 ± 0.24	6.25 ± 0.31
Rice	3.50 ± 0.73	4.38 ± 0.46	3.88 ± 0.61	2.25 ± 0.49	2.75 ± 0.88	2.43 ± 0.30

Average of 8 tasters ± SE

Further data is available on request.

The CSIC gliadin-reduction technology is the subject of pending patent applications filed by CSIC and is available for licensing from PBL.

References:

Will Europe toast GM wheat for gluten sufferers? Lucas Laursen (2016). *Nature Biotech*; 34: 369-371 doi:10.1038/nbt.3533.

The Shutdown of Celiac Disease-Related Gliadin Epitopes in Bread Wheat by RNAi Provides Flours with Increased Stability and Better Tolerance to Over-Mixing. Gil-Humanes J, Pistón F, Barro F, Rosell CM (2016). PLoS ONE 9(3): e91931. doi: 10.1371/journal.pone.0091931

Reduced-Gliadin Wheat Bread: An Alternative to the Gluten-Free Diet for Consumers Suffering Gluten-Related Pathologies. Gil-Humanes *et al* (2014). PLoS ONE 9(3): e90898. doi: 10.1371/journal.pone.0090898.

Effective shutdown in the expression of celiac disease-related wheat gliadin T-cel epitopes by RNA interference. Gil-Humanes J, Pistón F, Tollefsen S, Sollid LM, Barro F (2010). PNAS; 107(39): 17023-17028.

Silencing of γ-gliadins by RNA interference (RNAi) in bread wheat. Gil-Humanes *et al* (2008). J Cereal Sci; 48(3): 565-568.