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Socioeconomic impact of low-gluten, celiac-safe wheat developed through gene editing

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Contents

Abstract

Advances in biotechnology in recent decades have led to the development of new genomic techniques (NGTs). In 2021, the *Study on the status of new genomic techniques under Union law and in light of the Court of Justice ruling in Case C-528/16* was published, as requested by the Council of the European Union (Council Decision (EU) 2019/1904). The study defined NGTs as techniques which are able to alter the genetic material of an organism and which have been developed after the adoption of the current EU legislation on genetically modified organisms (EU Directive 2001/18/EC). In addition, last year, the Joint Research Centre of the European Commission published two reports on the technological state-of-the-art and on current and future market applications of NGTs (Broothaerts et al., 2021; Parisi & Rodriguez-Cerezo, 2021).

This report presents the case study of a product developed with an NGT – CRISPR/Cas targeted mutation – namely low-gluten, celiac-safe wheat. Here, a detailed description of the gene-edited low-gluten, celiac-safe wheat products currently under development in the EU is provided. Furthermore, we illustrate the potential contribution this product would make to ensure food security, nutrition and public health if it were approved for cultivation and marketing in the EU. This report is drafted to support the impact assessment accompanying the Commission proposal on legislation for plants produced by certain new genomic techniques.

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Executive summary

Advances in biotechnology in recent decades have led to the development of new genomic techniques (NGTs). In 2021, the *Study on the status of new genomic techniques under Union law and in light of the Court of Justice ruling in Case C-528/16* was published, as requested by the Council of the European Union (Council Decision (EU) 2019/1904). The study defined NGTs as techniques which are able to alter the genetic material of an organism and which have been developed after the adoption of the current EU legislation on genetically modified organisms (EU Directive 2001/18/EC). In addition, last year, the Joint Research Centre of the European Commission published two reports on the technological state-of-the-art and on current and future market applications of NGTs (Broothaerts et al., 2021; Parisi & Rodriguez-Cerezo, 2021).

The growing human population and a changing environment pose a challenge to meeting the current and future demand for sustainable food production and consumption. This has, in turn, increased the demand for plant cultivars that are resistant to biotic and abiotic stresses and also cultivars that contribute to providing healthier and nutritious diets. The scope of this study is to assess economic, social and environmental impacts of specific crop varieties developed using NGTs, as well as any potential contribution to the European Green Deal objectives and the Farm to Fork strategy of the European Commission for sustainable food production and consumption. In particular, this report presents the case study of a product developed with an NGT – CRISPR/Cas targeted mutation – namely the gene-edited low-gluten wheat that aims to be safe for people with celiac disease (CD) and possibly also useful for those with non-celiac wheat sensitivity (NCWS).

CD is a lifelong autoimmune systemic disorder triggered by gluten and similar cereal storage proteins present in wheat, rye and barley. It has an estimated prevalence of 1–2% of the worldwide population. Individuals suffering from NCWS develop symptoms overlapping those of CD after gluten ingestion, but without the accompanying elevated level of intestinal damage and with an absence of CD biomarkers. NCWS has a self-diagnosed prevalence of about 10% and a medically estimated prevalence of 1%. Currently, a gluten-free diet is the conventional treatment for CD and recommended for NCWS patients.

Targeted mutagenesis is defined as the technique used to induce small mutations in a specific location of the genome without insertion of genetic material. The process usually results in a 'knock-out' effect, meaning the disruption of the functioning of a gene that is responsible for an unwanted effect. In this case, the knock-out effect is on the presence of the CD-immunogenic gluten (especially the gliadins) in wheat, in order to avoid the autoimmune reaction to gluten in people suffering from CD.

Wheat is a fundamental crop for the EU, which represents more than 46% of the total EU cereal production and 47% of the total EU cereal area. The EU is one of the world's top exporters, providing 15% of total wheat exports in the last marketing year 2021/22. Wheat is highly appreciated for its gluten, a group of proteins present in the grain, which enables dough production for baking purposes. However, in turn, gluten is the cause triggering gluten-related disorders in genetically predisposed individuals.

Gene-edited wheat to reduce content of immunogenic gluten

Wheat gluten proteins are classified into two main groups: glutenins and gliadins. Glutenins are essential for flour end-use quality. Gliadins include four different fractions (alpha, gamma, delta and omega gliadins) with a similar structure. Immunogenic epitopes occur mainly in alpha, gamma, and omega gliadins. Altogether, the alpha gliadins are the most immunogenic gluten fraction for CD patients and the most abundant, representing 15–30% of the wheat seed protein. Gamma and omega gliadins are associated with wheat allergy and omega gliadins with wheat-dependent exercise-induced anaphylaxis.

Conventional breeding alone cannot generate a low-gluten, celiac-safe wheat variety that only contains gliadins with non-immunogenic epitopes. Given the structural complexity of the gene coding for gliadins, the high copy number of these genes, segregating in blocks, it is extremely difficult to apply conventional breeding techniques to obtain comparable wheat varieties.

Low-gluten, celiac-safe wheat can be developed through gene editing and is characterised by the inactivation or elimination of the protein fragments (epitopes) that trigger CD in genetically predisposed individuals. The editing concerns the genes for gliadin-type gluten proteins, which carry the most important (dominant) CD epitopes, leaving the genes for glutenin-type gluten proteins that are responsible for the food technological and dough-making qualities of wheat largely undisturbed.

Currently, there are two main research centres in the EU developing low-gluten, celiac-safe wheat varieties with gene editing (SDN-1-type CRISPR/Cas targeted mutation): (1) the Plant Biotechnology group at the Institute for Sustainable Agriculture of the Spanish National Research Council of Córdoba, IAS-CSIC, and (2)

the Plant Breeding group at Wageningen University & Research (WUR). Their gene-edited wheat lines are not yet on the market and are at different levels of development.

The IAS-CSIC group designed an initial gene-edited wheat targeting alpha gliadins, which are the most reactive, to achieve a variety that is not immunogenic for patients with CD and possibly also useful for those with NCWS. These gene-edited wheat lines reached a reduction in the alpha-gliadins content from 32% to 82%, while immunoreactivity was reduced by 85%. These lines have already been cultivated in greenhouses. In addition, a second gene-edited wheat was obtained targeting gamma and omega gliadins, and achieved a reduction of 70% and 90% in the gamma and omega gliadins, respectively. These lines are not yet being tested in greenhouses. Lastly, cross-breeding between gene-edited lines to combine all gliadin mutations in a single, soft wheat genotype was carried out. These lines are now being analysed, and the final selection of genotypes containing all mutations is expected to be completed by mid-2023, and after that clinical trials are planned using the best line.

The WUR group, in collaboration with the John Bingham Laboratory, NIAB, designed a gene-edited wheat targeting alpha and gamma gliadins to achieve a variety safe for CD. These lines are at proof-of-concept stage and still need further development to reduce the content of epitopes that need to be knocked out to be safe for celiac patients.

Transgenic approaches (outside the scope of this document) based on RNA interference (RNAi) technology have been used to develop wheat varieties with low-gluten safe for celiac individuals. RNAi wheat lines produced by the IAS-CSIC group showed that the gene expression of alpha-, gamma- and omega-gliadin families has successfully been reduced by over 90%. Gluten-derived extracts and bread did not stimulate CD patient T cells, while the dough quality of their flours was barely affected.

Current alternatives to manage gluten-related disorders

The only possible treatment for gluten-related disorders is a lifelong gluten-free diet. Current options for following a gluten-free diet include (1) cereals not containing gluten – naturally free of immunogenic gluten – to substitute wheat, barley and rye and (2) processed gluten-free products. Gluten-free cereals are rice, maize and sorghum, as well as a number of species of millet and a range of pseudocereals that can be used to produce gluten-free flour. The use of cereals and pseudocereals to produce gluten-free products requires the addition of compounds that can mimic a protein network similar to that formed by gluten, so that the gluten-free dough can be kneaded. Alternatives to manufacturing new gluten-free, wheat-based products, which require gluten extraction or gliadin and glutenin separation in the lab are considered large-scale and low-tech procedures.

Social and health impact

Ensuring food security, nutrition and public health, and making sure that everyone has access to sufficient, safe, nutritious and sustainable food is the main aspect to which GE low-gluten, celiac-safe wheat can contribute. A gluten-free diet, while reducing the health impacts of CD, also has some other implications for individuals.

First, gluten-free products are more expensive – on average, 200% more than their gluten-containing counterparts. In most cases, patients and relatives do not have any financial support to cover the cost of such a diet. In the EU, the economic aid offered for buying gluten-free products is scarce and differs greatly from one country to another. The low-gluten, celiac-safe wheat is expected to have a higher price than standard wheat, 5–30% more expensive due to the increased cost of seed and additional costs of management for identity preservation. Even if gene-edited low-gluten, celiac-safe wheat were to be marketed at a significant price premium, the savings compared to the costs of gluten-free diets nowadays would still be substantial.

Second, some gluten-free products can contribute to an imbalanced diet, due to having less protein and fibre, and a higher content of saturated fat, carbohydrate and salt compared to the gluten-containing products, as these are added to compensate for the physical properties of gluten. The gene-edited low-gluten, celiac–safe wheat retains the proteins responsible for the viscoelastic properties of dough but not the immunogenic proteins. Hence, it would be a healthier choice in the production of bread and derived products included in the GFD, without the need to add fats and other less nutritious compounds to mimic gluten in certain foods. Whole grain wheat is considered a significant component of a healthy diet and non-immunogenic wheat could provide CD patients, and possibly also those diagnosed with NCWS, with the possibility of safely consuming wheat and wheat-related. Clinical trials carried out with an equivalent wheat product developed by RNAi have

previously shown that it is well tolerated by NCWS patients, and reverses the negative effects observed in the intestinal microbiota when following a GFD.

Finally, medical care costs that are incurred post-diagnosis compared to costs until diagnosis are expected to be reduced by 39% for CD patients following GFD treatment, i.e. conventional management. Up to 14 lost workdays are reported for CD patients, depending on a GFD individual's compliance and level of sensitivity to gluten. Given the importance of GFD adherence in those aspects, the mainstream adoption of the gene-edited low-gluten, celiac-safe wheat, in combination with a fully compliant and balanced GFD, could contribute to reducing the needs of medical care post-diagnosis and the lost days at work or school as it has a very low or no ability of causing adverse reactions to gluten, but still provides comparable fibre, protein and energy to standard wheat. Data are scarce and inconsistent on the use and costs of healthcare in CD patients, and therefore this assumption is difficult to quantify in terms of potential cost savings for the EU.

Economic impact

Given the lack of observable data since the product is not yet available on the market or cultivated in the EU, similarities or differences with conventional wheat and with other similar specific products already existing on the market have been explored in terms of management.

Possible impacts along the wheat value chain can be mapped from input providers to consumers. For seed providers it may increase seed sales due to higher seed prices for a premium product. Some authors have estimated that this seed premium could be around 5%. Costs will also arise from the need to keep the seeds separate from conventional wheat varieties.

For farmers, in terms of crop yield, gene-edited soft and durum wheat lines yielded comparable to that of the standard wheat in greenhouse trials. As gene editing was carried out on gliadin genes that are only expressed in the wheat endosperm during grain filling, no changes in the use of fertilisers or plant protection products are expected. Overall, the agronomic management of the GE low-gluten, celiac-safe wheat is the same as the management of standard wheat, except for the need to maintain segregation and identity preservation up to the farm gate. There is a low risk of cross-contamination since wheat self-pollinates, and therefore a small cost will be expected for farmers to segregate and preserve the GE low-gluten, celiac-safe wheat at field level. Minor costs are also expected for cleaning and inspection of farm equipment and facilities; these will likely be covered by an increased harvest price.

At farm level, considering higher seed prices of GE low-gluten wheat of about 5% and a higher producer price of 10% received by the farmer for this product, the farm gross margin could increase, on average, by 30% per hectare compared to conventional wheat for this specific niche market product. The wheat processing industry and food industry will benefit from the demand for an increased number of wheat-based products with a higher value added. Segregation costs incurred by grain handlers and processors are expected to be in the range of 5–30% more expensive than the processing of standard wheat. Nevertheless, these increased costs may be compensated by the expected benefits (current prices of gluten-free products are, on average, 200% more expensive). For EU consumers, on top of the social/convenience/health benefits previously mentioned, the main economic benefit would be the access to innovative products at a lower premium than the current price gap between gluten and gluten-free products.

Lastly, the cultivation in the EU of a differentiated, gene-edited low-gluten, celiac-safe wheat may increase the competitiveness of the agri-food system by increasing export volumes of this grain wheat in the range of EUR 0.5–2.6 billion. In contrast, high import volumes of this product may occur if eventually cultivated in other parts of the world but not in the EU (in the range of EUR 0.1–0.5 billion). This can represent a business opportunity and innovation for the wheat value chain, from farmers to end consumers, millers and bakers and other related industries, and in turn create new employment opportunities.

With regard to labelling, under the current EU regulation (Regulation (EU) No 828/2014), varieties of gene-edited low-gluten, celiac-safe wheat edited to date may not be labelled as gluten-free (less than 20 mg/kg gluten content) or very low gluten (less than 100 mg/kg), even if eventually displayed as safe for CD patients. This is because immunoreactivity is reduced by 85%, but the gluten content is still above these two gluten thresholds set in the regulation. These new wheat lines would require the development of a quantitative detection method for CD epitopes. New health claims (hypoimmunogenic, CD-safe, etc.) must be scientifically assessed by the European Food Safety Authority (EFSA) and authorised by the European Commission and Member States before a clear and unambiguously defined 'safe gluten' label can be used on the EU market.

Conclusions

A reduced gluten-related allergen content can be achieved in the gene-edited low-gluten, celiac-safe wheat to provide healthy, nutritious and affordable food to people while maintaining a neutral environmental impact. It may be a feasible alternative for promoting the European Green Deal objectives and, in particular, the Farm to Fork strategy of the European Commission. The Farm to Fork strategy aims at the creation of a favourable food environment that makes it easier to choose healthy and sustainable diets that will benefit consumers' health and quality of life, and reduce health-related costs for society. Low-gluten, celiac-safe wheat may be a healthier and nutritious alternative that is less expensive than current gluten-free products for CD patients, and possibly for people diagnosed with NCWS, for whom no other treatment is available beyond a gluten-free diet, with the possibility to safely consume wheat and wheat-related foods. In turn, it may alleviate the burden of post-diagnosis costs for the healthcare system from CD individuals that choose to consume the low immunogenic wheat in combination with a balanced gluten-free diet. Besides, the adoption of low-gluten, celiac-safe wheat could increase gross margin for farmers by an average 30% per hectare compared to conventional wheat. Lastly, the competitiveness of the EU agri-food system may be enhanced by increasing export volumes (EUR 0.5 billion to EUR 2.6 billion) if the product were cultivated in the EU, and avoiding high import volumes if eventually cultivated in other parts of the world and not in the EU (EUR 0.1 billion to EUR 0.5 billion).

1 Introduction

Advances in biotechnology in recent decades have led to the development of new genomic techniques (NGTs). In 2021, the *Study on the status of new genomic techniques under Union law and in light of the Court of Justice ruling in Case C-528/16* was published, as requested by the Council of the European Union (Council Decision (EU) 2019/1904). The study defined NGTs as techniques which are able to alter the genetic material of an organism and which have been developed since the adoption of the current EU legislation on genetically modified organisms (EU Directive 2001/18/EC). In addition, last year, the Joint Research Centre of the European Commission published two reports on the technological state-of-the-art and on current and future market applications of NGTs (Broothaerts et al., 2021; Parisi & Rodriguez-Cerezo, 2021).

The Biotechnology Unit of the Directorate-General for Health and Food Safety of the European Commission (DG SANTE) and the Economics of the Food System Unit of the Joint Research Centre (JRC) have prepared a set of case studies to illustrate the potential economic, social and environmental impacts of specific crop varieties developed using NGTs. The objective of the case studies – including the one presented in this report – is to support the impact assessment accompanying the Commission proposal on legislation for plants produced by certain new genomic techniques[1].

In particular, this report presents the case study of a product developed with an NGT – CRISPR/Cas targeted mutation – namely the gene-edited (GE) low-gluten wheat which aims to be safe for celiac patients and for people with other gluten-related disorders. Targeted mutagenesis is defined as the technique used to induce small mutations in a specific location of the genome without insertion of genetic material. The process usually results in a 'knock-out' effect, meaning the disruption of the functioning of a gene that is responsible for an unwanted effect[2].

Currently, there are two main research centres in the EU developing low-gluten, celiac-safe wheat varieties with gene editing (CRISPR/Cas targeted mutation): (1) the Plant Biotechnology group at the Institute for Sustainable Agriculture of the Spanish National Research Council of Córdoba, IAS-CSIC, and (2) the Plant Breeding group at Wageningen University & Research (WUR). Their gene-edited wheat lines are not yet on the market and are at different levels of development. In this report, a detailed description of these low-gluten, celiac-safe wheat products is provided, as well as an assessment of the potential contribution this product would make to ensure food security, nutrition and public health if were approved for cultivation and marketing in the EU.

Around one per cent of the world's population suffers from celiac disease (CD) (Gujral et al., 2012); in the EU alone, that would equate to 4.5 million people. It has been estimated that only 15% of individuals with CD have been diagnosed, while 85% remain undiagnosed or wrongly diagnosed (Csizmadia and Mearin, 1999; Hujoel et al., 2018) and keep consuming daily wheat and gluten-containing products with negative consequences for their health and quality of life (Lindfors et al., 2019). In the EU, this would correspond to about 675,000 patients with a CD diagnosis and 3.8 million CD patients without a diagnosis. In addition to CD, there is a large group of people with wheat allergy (WA) and with (self-diagnosed) non-celiac wheat sensitivity (NCWS). For instance, Igbinedion et al. (2017) estimated that 6% of the US population may be suffering from NCWS – a similar figure for the EU would equate to 27 million individuals.

Currently, the only treatment available for gluten-related disorders is a gluten-free diet (GFD), which means avoiding the consumption of wheat, wheat-based food and other nutritious cereals containing gluten (i.e. barley and rye). In the event that GE low-gluten, celiac-safe wheat were to be accepted on the market, it is expected that consumers would be willing to purchase it, not only to reduce health risks for CD patients but also to serve relatives. Restaurants may also adopt it as their standard type of wheat to provide more safety, and as an additional service for their customers. GE low-gluten, celiac-safe wheat could gradually become the mainstream standard for wheat-based food products, which is the final use of about one third of all wheat produced in Europe (Gilissen and Smulders, 2021a). A widespread shift to low-gluten, celiac-safe wheat varieties would be particularly beneficial for undiagnosed CD patients (85% of all patients or 3.8 million Europeans) who would experience considerable health and social benefits.

A reduced gluten-related allergen content can be achieved in the gene-edited low-gluten, celiac-safe wheat to provide healthy, nutritious and affordable food for people while maintaining a neutral environmental impact. It may be a feasible alternative for promoting the European Green Deal[3] objectives and, in particular, the

[1] https://ec.europa.eu/info/law/better-regulation/have-your-say/initiatives/13119-Legislation-for-plants-produced-by-certain-new-genomic-techniques_en.
[2] https://ec.europa.eu/food/system/files/2021-04/gmo_mod-bio_ngt_eu-study.pdf.
[3] https://ec.europa.eu/info/strategy/priorities-2019-2024/european-green-deal_en.

Farm to Fork strategy[4] of the European Commission. The Farm to Fork strategy aims at the creation of a favourable food environment that makes it easier to choose healthy and sustainable diets that will benefit consumers' health and quality of life, and reduce health-related costs for society. Low-gluten, celiac-safe wheat could be one of the products that allow a more affordable and healthier diet. First, it may be a healthier and nutritious alternative for CD patients and, possibly, for people diagnosed with NCWS, for whom no other treatment is available beyond a gluten-free diet, with the possibility to safely consume wheat and wheat-related foods. Second, it can provide reduced costs to the healthcare system, especially primary care, avoiding several visits and medical expenses by the CD-diagnosed individuals that choose to consume the low immunogenic wheat in combination with a balanced gluten-free diet.

The report is structured in 10 main sections including this introduction. Section 2 outlines the data and assumptions used for the analysis. Sections 3 and 4 provide the reader with basic information regarding the GE crop (i.e. wheat) and the problems solved by gene editing (i.e. CD and other gluten-related disorders). Section 5 presents a comprehensive description of the technique used for editing and the status of the gene-edited low-gluten, celiac-safe wheat products. Section 6 focuses on the current alternatives to address the problem. Sections 7, 8 and 9 illustrate the potential social, economic and environmental impacts of the GE low-gluten product if it were approved for cultivation and marketing in the EU. Lastly, Section 10 summarises the main conclusions.

[4] https://ec.europa.eu/food/horizontal-topics/farm-fork-strategy_en.

2 Data collection and assumptions

The data used to prepare this report mainly come from scientific articles published in peer-reviewed journals listed in the Web of Science™. Part of the information published in these articles has been included in the different tables and figures that appear in the report. PhD theses, accessible through the corresponding university publication services, have also been used and referenced. We have also used reports produced by different celiac and/or consumer associations, which are available on their websites. Data from the European Commission database[5] and the EU FADN[6] (farm accountancy data network) were used for trade and economic characterisation of cereal production.

For the impact analysis, we assume that the GE low-gluten, celiac-safe wheat has passed the mandatory regulatory risk assessment and is accepted for EU cultivation and market. Although this product is not yet available on the market, for the impact analysis it is assumed that GE low-gluten, celiac-safe wheat will be a niche product, likely bought by celiac disease (CD), non-celiac wheat sensitivity (NCWS) and other patients and consumers willing to reduce gluten consumption.

Given the lack of observable data since the product is not yet available on the market or cultivated in the EU, similarities or differences with conventional wheat and with other similar specific products (e.g. gluten-free products) already existing on the market have also been explored in terms of management.

[5] https://agridata.ec.europa.eu.

[6] https://ec.europa.eu/info/food-farming-fisheries/farming/facts-and-figures/performance-agricultural-policy/studies-and-reports/economic-analyses-and-briefs/agricultural-and-farm-economics_en.

3 Gene-edited (GE) crop and its importance

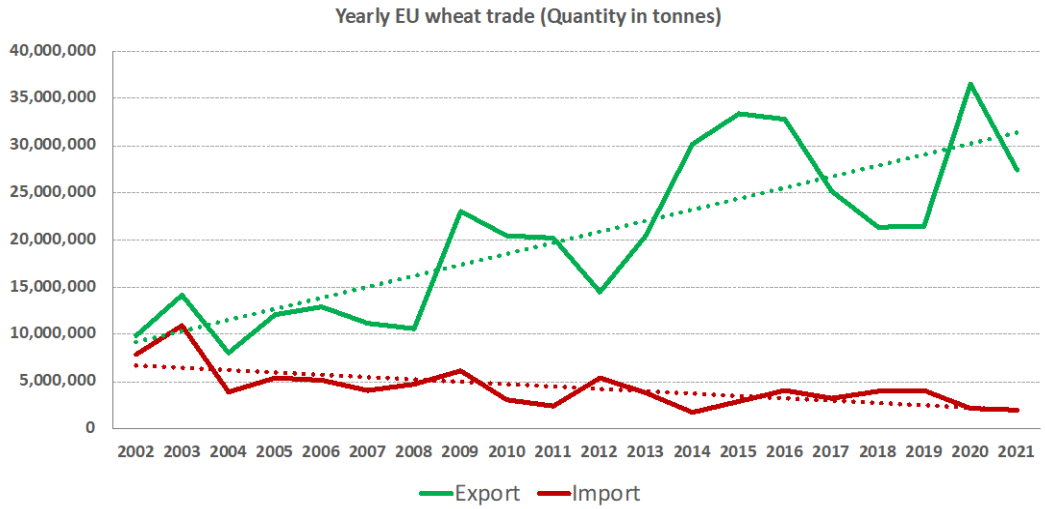
The GE crop is soft wheat, also called bread or common wheat, *Triticum aestivum ssp. aestivum*. Wheat is grown worldwide and is the third most common staple crop after rice and maize. It provides about one quarter of the global annual demand for plant proteins, carbohydrates and dietary fibre. The wheat genome has a polyploid complex involving three subgenomes – A, B and D – each containing seven pairs of chromosomes. Therefore, it is possible to find wild and cultivated species of three different ploidy levels: diploid (2n = 2x = 14), tetraploid (2n = 4x = 28) and hexaploid (2n = 6x =42). Although several species from this complex have been used throughout history, two specific species are mainly used nowadays: durum (or hard) wheat (*Triticum turgidum ssp. durum*, tetraploid, AABB genome) and soft (or bread) wheat (*Triticum aestivum ssp. aestivum*, hexaploid, AABBDD genome).

According to FAOSTAT, global wheat production in 2020 reached 760 million metric tonnes (MMT) on nearly 240 million hectares. In the period 2018–2022, the EU's total wheat production was, on average, over 130 MMT, which means more than 46% of the total EU cereal production (281 MMT on average for the same period). Of the total EU wheat production, 122 MMT is soft wheat (94%) and 8 MMT is durum wheat (6%). In 2021, EU production of soft wheat increased by 4.76% compared to the average for the last 5 years, while durum wheat production has reduced by a similar percentage (4.13%)[7].

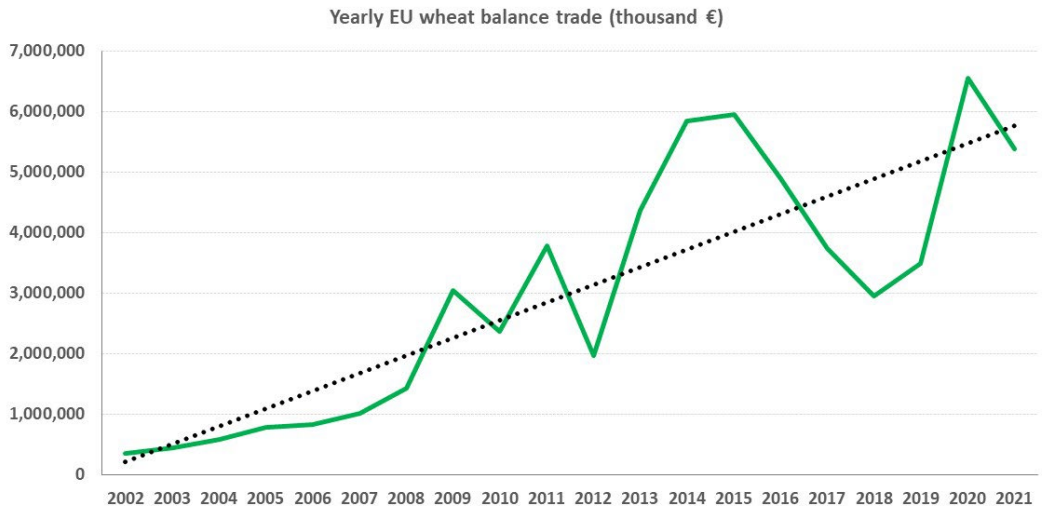
In terms of cultivated area, wheat, with 24 million hectares, represents 47% of the total EU cereal area, well above barley and maize, which represent 21% and 16%, respectively. Other wheat species, such as einkorn (*T. monococcum*, diploid, AA genome) and spelt (*T. spelta* or *T. eastivum ssp. spelta*, hexaploid, AABBDD genome) are marginal crops.

The EU is one of the world's top exporters, providing 15% of total wheat exports in the marketing year 2021/2022, as reported by the United States Department of Agriculture (USDA)[8]. Since 2002, EU wheat exports to the rest of the world have increased (**Figure 1**), reaching 28.2 MMT in 2021, of which only 0.8 MMT corresponds to durum wheat. The main wheat-exporting countries in the EU are France (7.5 MMT), Germany (4.5 MMT), Poland (3.2 MMT), Romania (3.1 MMT), Lithuania (3.1 MMT), Latvia (2.6 MMT) and Bulgaria (1.5 MMT). In contrast, wheat imports have gradually reduced over the same period, reaching 4.9 MMT in 2021, of which 2.9 MMT correspond to durum wheat and 2.0 MMT to soft wheat. Italy is the EU country with the highest wheat import volume (2.9 MMT), of which 80% is durum wheat. Belgium, with 0.3 MMT, is the second-largest importer of durum wheat from the EU.

Figure 1. Yearly wheat export and import since 2002 in millions of tonnes (top), and wheat trade balance in thousand EUR (bottom); the dotted lines are trendlines.



[7] EU Crops Market Observatory – Cereals, 2020; https://ec.europa.eu/info/food-farming-fisheries/ and https://agridata.ec.europa.eu/extensions/DashboardCereals/CerealsProduction.html - accessed April 2022.
[8] https://apps.fas.usda.gov/psdonline/circulars/grain.pdf.



Source: https://agridata.ec.europa.eu/extensions/DashboardCereals/CerealsTrade.html.

The main uses of wheat for human consumption are the manufacturing of pasta in the case of durum wheat, and baking, cakes and breakfast cereals in the case of soft wheat. Those specific uses are determined by the characteristics of each wheat species in terms of quality. In both cases, the main quality attribute is associated with the presence and properties of a group of proteins present in the grain of wheat called gluten. Wheat is highly appreciated for its gluten, which enables dough production for baking purposes (Shewry, 2019). Gluten also occurs in barley (*Hordeum vulgare*) and rye (*Secale cereale*) grains. On the other hand, wheat is also known as a cause of some human diseases (Gilissen et al., 2014). The most severe and relevant one is CD, which is triggered by the consumption of wheat, barley or rye gluten by genetically predisposed, gluten-intolerant individuals.

Due to wheat's wide applicability and versatility, it is present in many food products, feed and non-food products. In these products, wheat is applied as (fractionated) wheat flour, as wheat starch, as wheat gluten or as derivatives of these components such as glucose syrups, maltodextrin and sorbitol and as isolated gluten (vital wheat gluten, VWG; Gilissen and Smulders, 2021a). Atchison et al. (2010) found that wheat or wheat-derived components are present in almost 30% of the labelled food items of a survey of 10 235 supermarket items, and wheat, in particular, was most commonly present in pasta, breads, breakfast cereals and other baked items like muffins and biscuits. Wheat was also present in highly processed food items (sweets, frozen meals, packet soups and crisps), processed food items (pre-prepared meals, snack bars, chocolates, baking needs, cake mixes, marinades, savoury crackers and crispbreads) and, unexpectedly, wheat-derived components were found in canned vegetables, frozen poultry, cheeses and seafood.

Annually, the European starch industry produces about 17 MMT starch from cereals (maize and wheat). Wheat starch has many applications, mostly (67%) in food ('modified starch'). Vital gluten is a by-product of the wheat starch industry (Gilissen and Smulders, 2021a). In Europe, 9 MMT wheat is processed annually by the starch industry to produce over 630 000 metric tonnes VWG; much of it is used to improve the rheological properties of flours for a wide range of baking products. Gluten is also a preferred product in aquaculture due to its water insolubility and high binding capacity. Wheat starch, when not highly purified, may still contain considerable amounts of co-isolated gluten and other gluten-like (prolamin, water-insoluble) proteins (Gilissen and Smulders, 2021a).

In addition, whole grain wheat is classified as a significant component of a healthy diet (Ross et al., 2017). Whole grain foods contain the three main parts of the grain: the bran (the outer grain layer, rich in fibres), the starchy endosperm (the inner grain mass, rich in carbohydrates and proteins) and the germ (rich in vitamins and micronutrients). Several studies and health-related organisations[9] have shown that increased consumption of whole grain products (including whole grain wheat) significantly reduces the risk of several 'western lifestyle'-related chronic diseases, including obesity and diabetes, heart and vascular diseases, immune-related diseases and certain forms of cancer (Chen et al., 2016; Zong et al., 2016). Government agencies of many countries[10] therefore advise consuming whole grain foods (Kromhout et al., 2016). In

[9] https://knowledge4policy.ec.europa.eu/health-promotion-knowledge-gateway/whole-grain-3_en.
[10] https://knowledge4policy.ec.europa.eu/health-promotion-knowledge-gateway/whole-grain_en#nav_Toc479239830.

contrast, highly processed foods, including those with wheat-derived ingredients, are classified as unhealthy because they generally are too rich in sugar, salt and fat, and contain too little fibre (Lustig, 2017).

4 Definition of the problem addressed by the gene edited wheat

The main components of gluten are the cereal grain storage proteins, also named prolamins due to the high content of the amino acids proline and glutamine in them. Gluten proteins are traditionally classified into two groups: glutenins and gliadins (Wrigley et al., 2006), with glutenins being polymeric in nature, while gliadins are monomeric. Glutenins are composed of high molecular weight (HMW) glutenin subunits and low molecular weight (LMW) glutenin subunits that are linked via intermolecular disulfide bonds to form large polymers that are essential for flour end-use quality. The HMW subunits have been extensively studied and their allelic variation correlates with the baking quality of soft wheat (Payne, 1987b), whereas the LMW subunits have been associated with the semolina quality of durum wheat (Oak and Dexter, 2006). Gliadins are a heterogeneous fraction expanding four different fractions (alpha (α), gamma (γ), delta (δ) and omega (ω) gliadins), which have a similar structure, characterised by the presence of a repetitive region of variable length depending on the fraction. Gliadins are responsible for the extensibility of wheat doughs, while glutenins are responsible for elasticity.

Despite the importance of gluten proteins to the quality of wheat, and therefore for its economic value, these proteins are also responsible for triggering important pathologies related to human health. These are denoted as gluten-related disorders (GRDs), which encompasses three major types of pathologies (**Table 1**): CD, WA and NCWS. Patients suffering from GRDs may gain health benefits when consuming foods prepared using a modified wheat with a reduced content of the disease-causing gluten proteins (Shewry and Tatham, 2016; Sánchez-León et al., 2018; Jouanin et al., 2020a).

Table 1. Comparison of the main gluten-related disorders (GRDs).

	Celiac disease (CD)	**Wheat allergies (WA)**	**Non-celiac wheat sensitivity (NCWS)**
Global prevalence	0.2–2.4%	0.2–2.1%	0.6–13%
Pathogenesis	Autoimmune reaction	Allergic reaction mediated by IgE	Unknown
Antibodies	IgA EMA IgA tTG IgG DGP	IgE for wheat 50% IgA/IgE AGA	None (about 50% have positive IgG AGA)
Enteropathy	Almost always present	Absent	Absent
Symptoms	Intestinal and extra-intestinal	Intestinal and extra-intestinal	Intestinal and extra-intestinal
Complications	Long-term complications, comorbidities	No comorbidities, anaphylaxis	Unknown
HLA	95% HLA-DQ2/8	No HLA-DQ2/8 restricted	50% HLA-DQ2/8
Diagnosis	Biopsy Antibodies	Positive IgE for wheat Skin prick test	Excluded WA/CD Perform gluten challenge
Duration of GFD	Lifelong	Possibly lifelong	Possibly lifelong

([1]) Acronyms in the table: IgA, immunoglobulin A; IgG, immunoglobulin G; IgE, immunoglobulin E; EMA, Endomysial antibody; tTG, tissue transglutaminase; DGP, deamidated gliadin peptide; AGA, anti-gliadin antibodies; HLA, human leukocyte antigen; GFD, gluten-free diet.

Source: Adapted from Dale et al., 2019.

4.1 Celiac disease (CD)

CD is the best-known and most studied pathology related to wheat consumption, affecting adults and children (Tye-Din et al., 2010; Petersen et al., 2014, 2016; Sollid, 2017; Scherf et al., 2020). It is a chronic inflammation of the small intestine and is induced and maintained in genetically predisposed individuals by the consumption of gluten proteins from wheat (and also from barley and rye), especially gliadins. CD is considered an autoimmune disease.

T-cell epitopes that are relevant to CD have been identified in all gluten protein fractions (Sollid et al., 2020). Immunogenic epitopes occur in alpha, gamma and omega gliadins, and to a lesser extent in LMW glutenins, while HMW glutenins are mostly safe for CD patients and constitute the main gluten family responsible for bread dough quality (Payne et al., 1987a; Shewry et al., 2009; Shewry, 2019). Experiments with T-cell panels have determined that the adaptive response is restricted to certain gluten peptides that are predominantly mapped in gliadin regions (Arentz-Hansen et al., 2002). For wheat, the alpha-gliadin 33-mer peptide was the

main immunodominant toxic peptide in celiac patients. The alpha-gliadin genes are the most immunogenic T-cell stimulatory peptides in wheat gluten for the 95% of CD patients who are positive for the HLA-DQ2.5 haplotype (Tye-Din et al., 2010). Moreover, an additional peptide, the p31–43, also present in these alpha gliadins, has been reported to induce the innate immune response necessary to initiate the T-cell adaptive response (Maiuri et al., 2003). Altogether, the alpha gliadins are the most immunogenic gluten fraction, and also the most abundant, representing 15–30% of the wheat seed protein.

The estimated prevalence of CD worldwide is 1-2% (Scherf et al., 2020) and EU prevalence approximately 1% (**Table 2**). This would equate to at least 4.5 million people in the EU alone, although the actual rate varies between countries (Singh et al., 2018). Of these patients, it is estimated that only around 15% have been diagnosed (Csizmadia and Mearin, 1999; Hujoel et al., 2018). For both diagnosed and undiagnosed patients, the development of gene-edited low-gluten wheat may become a relevant initial step if it is CD-safe (i.e. hypoimmunogenic) wheat.

Table 2. Prevalence of celiac disease in Europe.

Prevalence	Reference
Pooled prevalence of biopsy-confirmed CD: 0.8% (95% confidence interval [CI], 0.6–1.1%); pooled seroprevalence of CD: 1.3% (95% CI, 1.1–1.5%).	Singh et al., 2018
Overall CD prevalence: 1%, likely higher in northern European countries.	Gujral et al., 2012
Overall CD prevalence (previously diagnosed plus anti-tTG and EMA positives): 1.0% (95% CI 0.9–1.1).	Mustalahti et al., 2010
Serological screening: 0.5–1%, that is from 1:200 to 1:100.	Cataldo and Montalto, 2007

([1]) Tissue transglutaminase (tTG); endomysial antibodies (EMA).

Source: Own elaboration.

In CD, gluten is a strong environmental factor, but it also has a genetic and immunological component related to human leukocyte antigen (HLA)-DQ2 and HLA-DQ8. Ninety-five per cent of European patients with CD carry the HLA-DQ2 molecule, while about 5% express HLA-DQ8 (Gujral et al., 2012). However, it should be noted that 30% of the European population carry HLA-DQ2 and most are eating wheat with no symptoms, but only 1 in 100 will develop the disease. This clearly indicates that carrying HLA-DQ2 is necessary but not sufficient to develop the disease.

Due to their high content of proline and glutamine, gluten proteins are resistant to their complete digestion in the human digestive tract. Thus, peptides produced as a result of the partial digestion of prolamins induce an autoimmune-mediated disorder that leads to small intestine inflammation, malabsorption and villous atrophy in patients suffering from CD. As a result, the most common symptom in children and adults with CD is abdominal pain, followed by diarrhoea. In addition, vomiting, abdominal distension or weight loss are also common symptoms. Extra-intestinal symptoms such as fatigue, iron deficiency or skin symptoms in the form of dermatitis herpetiformis can also be found in paediatric and adult patients. Neurological and psychiatric complications in both paediatric and adult CD patients have also been observed (Hadjivassiliou et al., 2019). Moreover, a higher prevalence of other autoimmune diseases such as diabetes mellitus type 1 was reported in people with CD (Cabanillas, 2020).

Until a few decades ago, CD was considered to be an uncommon disease mainly affecting children and limited to individuals of European ancestry. However, simplification of the diagnostic criteria and the development and use of new serology tests have made it possible to estimate the true prevalence of CD in the general population. Currently, the prevalence of CD can be established using biomarkers (seroprevalence) or by biopsy. The most commonly used biomarkers to diagnose CD are anti-tissue transglutaminase (anti-tTG) antibodies (Ab) or anti-endomysial antibodies (AEAs). There are many studies describing prevalence in different countries around the world using these methodologies. In the case of the EU, these studies do not cover all EU countries and they report considerable variations in the occurrence of CD, both within and between different European countries. However, a precise estimation of the European prevalence of CD is needed as it would be important not only for epidemiological reasons but also for insurance, agricultural and international trade and to implement effective solutions within the EU.

In a recent review using metadata analysis (Singh et al., 2018), the seroprevalence of CD in the population was reviewed as subjects having a positive anti-tTG Ab and/or AEAs. They pooled the global prevalence of CD – including Europe – by pooling data from 96 studies covering 275 818 individuals. The global seroprevalence of CD in the general population was 1.4%: 5 571 individuals were reported to be positive for anti-tTG Ab and/or AEA. For the EU, the study covers 163 700 individuals from 11 European countries, of which 2 340

were seropositive for CD, providing a seroprevalence of 1.3% with a 95% confidence interval (CI) of 1.1–1.5%. On the other hand, based on studies reporting CD based on biopsy, the prevalence for EU was estimated at 0.8% with a 95% CI of 0.6–1.1%.

The prevalence of CD varies with sex, age and geographical location. The above study also provides evidence that biopsy-confirmed CD is 1.5 times more common in females than in males and approximately twice as common in children as in adults. This could be due to the finding that men with CD were diagnosed at later in life. Several studies confirm strong differences regarding the prevalence of CD between EU countries. Considering the data reported in Sing et al. (2018), EU countries such as Hungary, Finland, Sweden, Portugal, Italy, Spain and the Czech Republic would have a high prevalence (0.9–2.4%); while other countries such as Estonia, Germany, the Netherlands and Greece would have a lower prevalence (0.2–0.8%). This is in line with other studies reporting a prevalence of CD in Finland and Sweden as high as 2–3%, whereas it is only 0.2% in Germany, although these areas share a similar distribution of causal factors (level of gluten intake and frequency of HLA-DQ2 and -DQ8; Catassi et al., 2015b).

The prevalence of celiac disease has increased over time. These findings are based on the review of prevalence studies over various time periods, or on the analysis of blood samples that are preserved frozen. However, evidence mostly comes from individual countries or regions and EU-wide estimates are lacking. In data reported by Singh et al. (2018), the global prevalence of CD increased over time by 0.2% between 1991–2000 and 2001–2016. However, a more precise study carried out in Finland showed that the total prevalence of celiac disease was 1.05% in 1978–1980 and 1.99% in 2000–2001, almost doubling over those two decades (Lohi et al., 2007).

The reasons for these changes are multiple and include improvements in the criteria for diagnosis of the disease (e.g. the use of much more effective serology tests) and environmental components of CD such as changes in the quantity and quality of ingested gluten, infant feeding patterns, the spectrum of intestinal infections, gut microbiota colonisation, etc.

The daily intake of gluten in the adult European population is between 15–20 g. In patients with CD, 50 mg gluten per day produced measurable damage to the small intestinal mucosa. To keep gluten intake below 50 mg/day, the Codex Alimentarius proposed a limit of 20 mg/kg of gluten in foods (Catassi et al., 2007). This limit was later adopted in 2008, and taken over by the European Commission regulations concerning the composition and labelling of foods suitable for people intolerant to gluten (Regulation (EU) No 41/2009 of 20 January 2009; Regulation (EU) No 828/2014 of 30 July 2014). In these regulations, the terms 'gluten-free' (not exceeding 20 mg/kg) and 'very low gluten' (not exceeding 100 mg/kg) are defined.

Until recently, the gold standard for diagnosis of celiac disease was a biopsy of the small intestine showing inflammation phenomena such as villous atrophy, crypt hypertrophy and increased intra-epithelial lymphocyte counts. As present, biological markers (of cellular and antibody type) have also been developed that can be tested in blood samples. Biological markers are the presence of antibodies against tissue transglutaminase (tTG) and endomysial antibodies (EmA). The presence of the IgG class anti-deamidated gliadin peptide also adds evidence to the diagnosis, as well as holding the human leukocyte antigen (HLA) genotypes DQ2 or DQ8. A positive response to a gluten-free diet (GDF) is an additional diagnostic marker (Lindfors et al., 2019). Unfortunately, as CD symptoms range from vague to very severe (ranging from growth retardation and weight loss to chronic abdominal pain in children, and from diarrhoea/constipation and chronic fatigue to neuropathies in adults), in practice its diagnosis is difficult. It has been estimated that 85% of individuals with CD remain undiagnosed (Csizmadia and Mearin, 1999; Hujoel et al., 2018) and keep consuming wheat and gluten-containing products with negative consequences on their health and quality of life (Lindfors et al., 2019). The actual development of the disease in a potential gluten-intolerant individual is positively correlated with gluten intake (Koning, 2012). Only a minor fraction of the CD population will end up going to the hospital.

4.2 Wheat allergy (WA)

Allergic responses to wheat are classified according to the type of immune response that triggers the reaction (Cianferoni, 2016). Similar to CD, WA leads to an adverse reaction to proteins from wheat in which the immune system is involved. However, WA is mediated by immunoglobulin-E (IgE) and is mainly elicited by proteins contained in wheat. In allergies mediated by IgE, wheat induces diverse clinical manifestations that have a differential incidence depending on age. An allergy associated with wheat ingestion has a higher prevalence in children than in adults, and can produce symptoms such as vomiting, abdominal pain, urticaria, angioedema, anaphylaxis, respiratory symptoms and skin symptoms, among others (Zuidmeer et al., 2008; Cabanillas, 2020).

In adults, the most significant reaction to ingested wheat is wheat-dependent exercise-induced anaphylaxis (WDEIA) and involves severe anaphylactic reactions to wheat when consumption is followed by intense exercise. Neither wheat nor exercise alone cause the reaction to trigger WDEIA; it is the necessary combination of both factors. The underlying mechanism of WDEIA may be related to the development of large allergenic complexes favoured by the activation of transglutaminase (TG2) in the intestinal mucosa during physical exercise (Scherf et al., 2016).

High concentrations of IgE antibodies specific to wheat protein fractions are observed in sera of WDEIA patients, particularly to the omega-5- gliadins. A number of IgE-binding epitopes have been identified in the amino acid sequence of the omega-5- gliadins: QQIPQQQ, QQFPQQQ, QQSPEQQ, QQSPQQQ, QQFHQQQ, QSPEQQQ, QQPPQQ, and YQQYPQQ. Additional epitopes were later reported in other alpha/beta-gliadin, gamma-gliadin, omega-1-/2-gliadin, HMW and LMW protein fractions (QPGQ, QQPGQGQQ, and QQSGQGQ), all mapped in the glutamine- and proline-rich repetitive domains of the proteins. Interestingly, cross-reactivity of allergens from barley, rye and oats was studied, providing that most of the patients showed IgE-binding to rye secalins and gamma hordeins from barley but not to oat proteins (Palosuo et al., 2001). Reactivity of the different wheat proteins was assayed by a microarray, providing that IgE antibodies to omega-5 gliadins were detectable in 82%, to alpha/beta/gamma gliadins in 82% and to HMW glutenin subunits in 59% of patients (Hofmann et al., 2012). Those wheat protein fractions are therefore considered major allergens triggering WDEIA.

The amount of wheat protein necessary to induce WDEIA has not been well characterised, and it would depend on the food being consumed. Tests showed that 64 g but not 45 g of bread, and 200 g but not 100 g of a Japanese noodle made from wheat, can trigger WDEIA allergic reactions (Scherf et al., 2016).

In a report by the European Food Safety Authority (EFSA), the results of several studies on the prevalence of wheat allergies in the EU were presented (EFSA Panel on Dietetic Products, Nutrition and Allergies, 2014). Results showed high variability between countries and age. In children under 3 years old, the prevalence ranged from 0.8% in Sweden to 2.1% in Finland. The lowest prevalence (0.2%) of self-reported wheat allergy was in a group of 7–13-year-olds in Greece. For the same age category, the highest prevalence was reported in France at 1.5%.

Cereal allergies can reliably be diagnosed by skin-prick tests and (double-blind, placebo-controlled) food challenges. People allergic to wheat will need to avoid it altogether. Therefore, a health benefit (reduced allergic reactions) for these patients from consuming the gene-edited low-gluten, celiac-safe wheat can be expected only if omega gliadins are reduced or eliminated.

4.3 Non-celiac wheat sensitivity (NCWS)

The first references to non-celiac gluten sensitivity date from the late 1970s to fill the gap between people suffering from discomfort after gluten ingestion who did not suffer from CD or WA (Ellis et al., 1978). This clinical entity has been termed non-celiac gluten or wheat sensitivity (NCGS or NCWS). NCWS is not particularly well understood as a condition, with a self-diagnosed prevalence of about 10% and a medically estimated prevalence of 1% (Catassi et al., 2015a; Van Gils et al., 2016). Patients suffering from this pathology develop symptoms overlapping those of CD after gluten ingestion; however, they present undisturbed duodenal mucosa and an absence of CD biomarkers (Ellis and Linaker, 2016; Losurdo et al., 2018).

Typical NCWS symptomatology includes gastrointestinal symptoms such as bloating and abdominal pain, altered bowel habits (diarrhoea, constipation or both), nausea and reflux, as well as extra-intestinal manifestations such as headaches, fatigue, fibromyalgia, anxiety, abdominal pain, disturbed sleep pattern, weight gain, depression, skin rash and dermatitis (Catassi et al., 2015b; De Giorgio et al., 2016). The mechanisms behind this pathology are largely unknown, and a multifactorial process has been proposed involved in the development of the disease besides an innate immune response (Sapone et al., 2010; Uhde et al., 2016).

All of these factors make the diagnosis of NCWS fairly complicated, and the current diagnosis is often based on the patient's self-diagnosis when there is an improvement in symptoms after excluding gluten from their diet and after exclusion of other pathologies such as CD or WA (Elli et al., 2015). Interestingly, haplotypes HLA-DQ2 and HLA-DQ8 typically related to CD have been found in up to 50% of NCWS-diagnosed patients (Sapone et al., 2012), a prevalence that is slightly higher than the normal population.

In the absence of a standard protocol, certain recommendations for the diagnosis of NCWS which are included in 'The Salerno Experts' Criteria' of NCWS have been proposed. These recommendations include a double-

blind, placebo-controlled gluten challenge, with gluten stimulation after following a GFD. If there is a variation equal to or greater than 30% in any of the main symptoms, it is considered a positive diagnosis for NCWS (Catassi et al., 2015a). A GFD is currently the best treatment to improve the clinical symptoms of patients suffering from NCWS, although the diet may not be as strict as that for patients with CD, and it has been suggested that individual gluten tolerance levels may vary in affected subjects (Volta et al., 2016). This is because several blind, placebo-controlled studies have questioned the role of gluten in NCWS, and other wheat components, such as FODMAPs (Fermentable Oligo-, Di-, Monosaccharides and Polyols) and ATI (amylase trypsin inhibitor) proteins, have been discussed as culprits (Servick, 2018; Brouns et al., 2019; Geisslitz et al., 2021) and may be responsible for gastrointestinal symptoms, especially bloating, flatulence and abdominal pain. As a result of this, it is still uncertain whether NCWS truly is a clinical entity separate from irritable bowel syndrome (IBS) as they have overlapping symptoms.

Due to the absence of diagnostic markers and population studies, the prevalence of NCWS is not well established. Previous data were primarily based on questionnaires for self-reported NCWS, with a prevalence ranging from 0.6% up to 13% of the general population. In the Netherlands, one study indicates that 6.2% of individuals reported symptoms after the ingestion of gluten-containing foods (Van Gils et al., 2016), which is substantially less than in a comparable UK study (13%) (Aziz et al., 2014). In Italy, a prospective multicentre survey involving 38 centres identified 486 out of 12 255 (3.9%) patients with suspected NCWS (Volta et al., 2014), and reported more than two associated gastrointestinal or extra-intestinal symptoms. NCWS was reported more often among women, adults between 40 and 50 years of age and individuals coming from urban areas (Van Gils et al., 2014).

Takeaway points from Section 4 – Definition of the problem addressed by the gene-edited wheat

i) Celiac disease is a lifelong autoimmune systemic disorder triggered by gluten and similar cereal storage proteins present in wheat, rye and barley. It has a prevalence estimated to be 1–2% of the worldwide population. The alpha-gliadin genes are the most immunogenic T-cell stimulatory peptides in wheat gluten for celiac disease patients.

ii) Wheat allergy is mediated by immunoglobulin E (IgE) and is mainly elicited by proteins contained in wheat. In adults, the most significant reaction to ingested wheat is wheat-dependent exercise-induced anaphylaxis, a severe anaphylactic reaction to wheat when consumption is followed by intense exercise. Gamma and omega gliadins are responsible for triggering the wheat allergy and omega gliadins the wheat-dependent exercise-induced anaphylaxis.

iii) Individuals suffering from non-celiac wheat sensitivity develop symptoms overlapping those of celiac disease after gluten ingestion, but without the accompanying elevated level of intestinal damage and with an absence of celiac disease biomarkers. Non-celiac wheat sensitivity has a self-diagnosed prevalence of about 10% and a medically estimated prevalence of 1%.

5 Genes edited and trait achieved

Currently, there are two main research centres in the EU developing low-gluten, celiac-safe wheat varieties with gene editing (SDN-1 type CRISPR/Cas targeted mutation): (1) the Plant Biotechnology group at the Institute for Sustainable Agriculture of the Spanish National Research Council of Córdoba (IAS-CSIC; Sánchez-León et al., 2018) and (2) the Plant Breeding group at Wageningen University & Research (WUR; Jouanin et al., 2019b). Their gene-edited wheat lines are not yet on the market and are at different levels of development.

The IAS-CSIC group (Sánchez-León et al., 2018) designed an initial product targeting wheat alpha gliadins, which are the most reactive, to achieve a variety that is not immunogenic for patients with CD and possibly also useful for those with wheat sensitivity. These gene-edited wheat lines achieved a reduction in the alpha-gliadin content from 32% to 82%, while immunoreactivity was reduced by 85%. These lines have been already tested in greenhouses. In addition, a second gene-edited wheat was obtained targeting wheat gamma and omega gliadins, and achieved a reduction of 70% and 90% of the gamma and omega gliadins, respectively. Gamma and omega gliadins are responsible for triggering the WA and omega gliadins the WDEIA. These lines are not yet tested in greenhouses. Lastly, cross-breeding was carried out between all lines to combine all gliadin mutations in a single soft wheat genotype. These lines are now being analysed, and the final selection of genotypes containing all mutations is expected to be completed by mid-2023 and after that clinical trials are planned using the best line.

The WUR group, in collaboration with the John Bingham Laboratory, NIAB, (Jouanin et al., 2019b), designed a gene-edited wheat targeting alpha and gamma gliadins to achieve a variety safe for CD. These lines are at proof-of-concept stage and still contain too many epitopes that need to be knocked out to be safe for celiac patients.

The targeted genes and the status of development varies across all of these lines. A summary of the GE lines of hypoimmunogenic wheat obtained by the two research groups, IAS-CSIC and WUR, is included in **Table 3** and a more detailed description is provided throughout Sections 5.1 and 5.2.

Table 3. Gene-edited (GE) lines of hypoimmunogenic wheat obtained by IAS-CSIC and WUR.

Targeted protein family (genes)	Protein-related pathology[1]	GE technique	Technique subtype	Targeted protein reduction (%)	Status of development	Reference
α-gliadins	CD	CRISPR/Cas targeted mutation	SDN-1[2]	32–82% (immunoreactivity reduced by 85%)	Tested in greenhouse	Sánchez-León et al., 2018
γ-, ω-gliadins	CD, WA, WDEIA	CRISPR/Cas targeted mutation	SDN-1	70–90%	Proof of concept	Sánchez-León et al., 2018
α-, γ-, ω-gliadins	CD, WA, WDEIA	CRISPR/Cas targeted mutation	SDN-1	n/a	Proof of concept	Sánchez-León et al., 2018
α-, γ-gliadins	CD	CRISPR/Cas targeted mutation	SDN-1	n/a	Proof of concept	Jouanin et al., 2019b

([1]) Celiac disease (CD); wheat allergies (WA); wheat-dependent exercise-induced anaphylaxis (WDEIA).
([2]) Site-directed nuclease type 1 (SDN-1).

Source: Own elaboration.

5.1 Gene-edited wheat developed by Plant Biotechnology group at IAS-CSIC

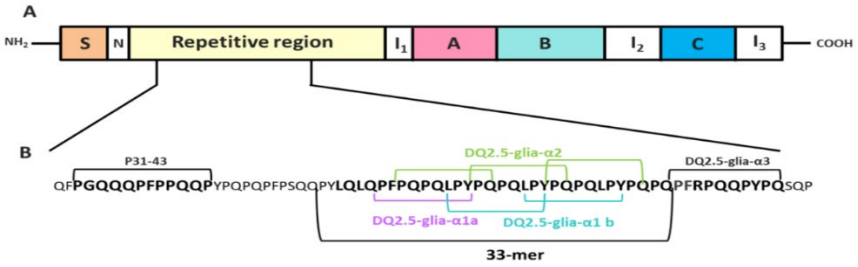
5.1.1 Gene coding for gluten proteins

T-cell epitopes that are relevant to CD have been identified in all gluten protein fractions (Sollid et al. 2020), which includes 5 distinct epitopes from alpha gliadins, 10 from gamma-gliadins, 2 from omega-gliadins, 2 from LMW and 1 from HMW glutenin subunits. However, not all gluten proteins are equally important to CD. Experiments with T-cell panels have determined that the adaptive response is restricted to certain gluten peptides that are mostly mapped in gliadin regions (Arentz-Hansen et al., 2002). Moreover, Tye-Din et al. (2010) carried out a comprehensive assessment of more than 16 000 potentially toxic peptides contained within wheat, barley and rye, particularly those in the alpha, gamma and omega gliadins, as well as wheat glutenins, and the barley and rye counterparts. Surprisingly, they found that just three highly active peptides were responsible for most of the immune response seen in patients with celiac disease after eating any of the toxic grains.

For wheat, the alpha-gliadin 33-mer peptide was the main immunodominant toxic peptide in celiac patients. This peptide is present in the N-terminal repetitive region of alpha gliadins and contains six overlapping copies of three different DQ2.5-restricted T-cell epitopes with highly stimulatory properties (**Figure 2**). The alpha-gliadin genes originated from the D subgenome of wheat – present only in hexaploid soft wheat – contribute the most immunogenic T-cell stimulatory peptides in wheat gluten for the 95% of CD patients who are positive for the HLA-DQ2.5 haplotype (Tye-Din et al., 2010). Moreover, an additional peptide, the p31–43, also present in these alpha gliadins has been reported to induce the innate immune response necessary to initiate the T-cell adaptive response (Maiuri et al., 2003). Altogether, the alpha gliadins are the most immunogenic gluten fraction, and also the most abundant, representing 15–30% of the wheat seed protein.

Therefore, wheat gliadin genes, particularly the alpha gliadins, were chosen as the target trait for CRISPR/Cas.

Figure 2. Schematic structure of the α-gliadins (A). Fragment of an alpha-gliadin repetitive region; main immunogenic fragments: peptides p31-43, 33-mer containing six overlapping copies of DQ2 epitopes and DQ2.5-glia-α3 are indicated (B). A, B, C, conserved regions; S, signal peptide, I1–I3, variant regions; N, N-terminal domain.

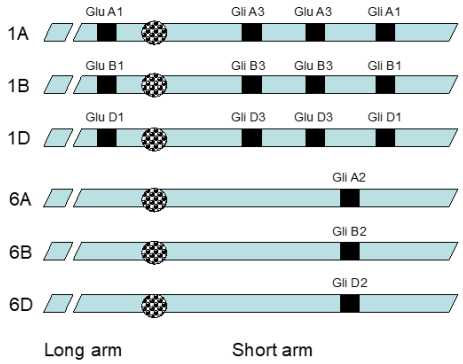


Source: https://helvia.uco.es/xmlui/handle/10396/19461.

However, targeting these genes families is highly challenging because of the high number of genes coding for gluten proteins and, specifically, gliadin proteins. The HMW glutenins are encoded by the Glu-1 complex loci located on the long arm of each of the homoeologous group 1 chromosomes, named Glu-A1, Glu-B1 and Glu-D1 in soft wheat (AABBDD), respectively (Payne, 1987b), each locus consisting of two closely linked genes (named x and y). The LMWs are encoded by the loci Glu-A3, Glu-B3 and Glu-D3, located on the short arm of the homoeologous group 1 chromosomes in soft wheat (Singh and Shepherd, 1988).

With regard to the gliadins, they are divided into four complex groups, named alpha, gamma, delta and omega gliadins, each containing numerous members with similar structures and distinct repetitive motifs. Gliadins are encoded by genes located on the short arm of homoeologous chromosomes 1 and 6 (**Figure 3**). The Gli-1 loci of homoeologous group 1 (Gli-A1, Gli-B1 and Gli-D1 in soft wheat) control the gamma, omega and delta gliadins, and the Gli-2 loci (Gli-A2, Gli-B2 and Gli-D2 in soft wheat) of homoeologous group 6 control the alpha and beta gliadins (Wrigley et al., 2006; Metakovsky et al., 2018).

Figure 3. Chromosomal location of the main loci involved in the synthesis of wheat gliadins (Gli) and glutenins (Glu). The genome of origin and chromosome number are indicated.



Source: https://helvia.uco.es/handle/10396/5233.

With the availability of a high-quality wheat genome sequence from the reference wheat Chinese Spring (CS; International Wheat Genome Sequencing Consortium (IWGSC), 2018), it has been possible to determine the complexity of these gene families in a single wheat cultivar. Thus, a complete set of genes from Chinese Spring assembled and annotated by Huo et al. (2018a, b) included 102 genes of which 47 were alpha gliadins, 14 were gamma gliadins, 5 were delta gliadins, 19 were omega gliadins and 17 were LMW glutenin subunits. Of these, 26 were alpha, 11 gamma, 2 delta, 5 omega gliadins and 10 LMW encoded full-length proteins, while the remaining genes were either partial sequences or pseudogenes containing premature stop codons or frameshift mutations. However, delta gliadins could be considered a part of gamma gliadins; therefore, from now on only three fractions will be considered: alpha, gamma and omega gliadins.

As CS is a model genotype, the IAS-CSIC group of developers used next-generation sequencing (NGS) on commercial and breeding durum and bread wheat genotypes to decipher the structural complexity of wheat's alpha, gamma and omega gliadins, especially for the BW208 (bread wheat) and Don Pedro (durum wheat) varieties targeted for trait modification (Ozuna et al., 2015; Sánchez-León et al., 2021). The results have allowed them to identify the sequences of genes and pseudogenes, their expression levels and the abundance of CD-related epitopes for all gliadin fractions.

5.1.2 Technique used for gene editing

Genome editing based on CRISPR/Cas9 in combination with biolistics for delivery of reagents were the techniques used for the modification. Plasmids for expression vectors for targeted mutagenesis, containing the single guide RNAs (sgRNAs), were constructed according to Sánchez et al. (2018) using the Golden Gate cloning technique and a modular assembly system of intermediate vectors containing the different functional elements necessary for genome engineering (Cemark et al., 2017). A wide variety of modules that allow different elements to be combined with each other are deposited on the Addgene platform. To synthesise the final expression vectors, two Gateway-compatible donor vectors, one containing TaCas9 and another containing the sgRNAs were combined in a multisite Gateway recombination reaction.

Edited lines were produced using immature scutella as explants for delivering the CRISPR/Cas9 reagents into the plant cells by particle bombardment using the methodology described previously (Gil-Humanes et al., 2011). Two bread wheat lines denoted BW208 and THA53, and one durum wheat line, cv Don Pedro (DP), were used as sources for scutellum isolation and in vitro culture. Plasmids carrying the sgRNAs were precipitated onto 0.6 μm gold particles at 0.75 pmol/mg gold. The regeneration medium was supplemented with 2 mg/L of PPT for selecting edited plants.

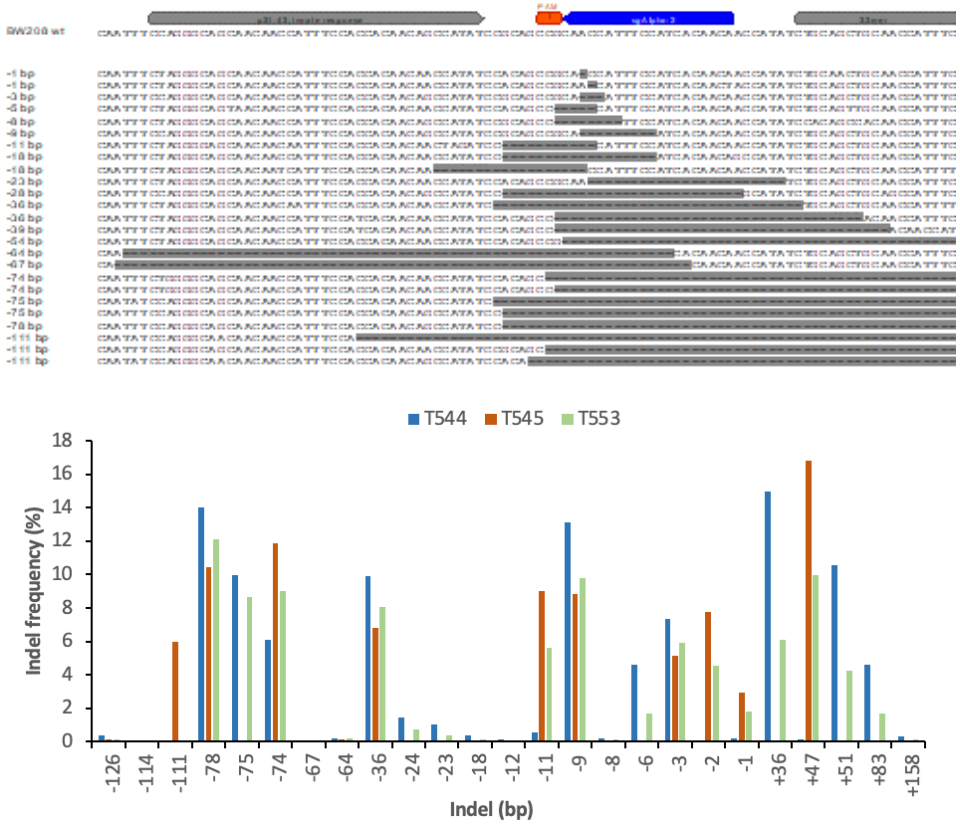
Putative edited plants were then transferred to soil and grown to maturity in the greenhouse, and the presence of transformation vectors was confirmed by polymerase chain reaction (PCR). Transgene- and insertion-free edited lines were then selected from segregating populations and analysed by PCR and Illumina sequencing, providing edited lines not containing transgenes or other DNA inserted.

5.1.3 Trait achieved after CRISPR/Cas targeted mutation

The genes coded for the gliadin fractions were mutated using CRISPR/Cas9, providing a set of wheat lines with the alpha, gamma and/or omega gliadins targeted. The first round of gene editing was focused on wheat alpha gliadins because they are the most immunoreactive fraction. Two sgRNAs (sgAlpha-1 and sgAlpha-2) were designed in regions close to the 33-mer and p31-43 immunogenic peptides. The CRISPR/Cas9 reagents were delivered into plant cells of the two bread wheat (BW028 and TAH53) and the one durum wheat (Don Pedro, DP) cultivars, resulting in 21 bread wheat and 6 durum wheat T0 edited lines (Sánchez-León et al., 2018).

Previous NGS analysis showed 45, 52 and 43 different wild-type alpha-gliadin sequences in BW208, THA53 and DP, respectively. Of these, 35, 13 and 29 were, respectively, mutated by CRISPR/Cas9 reagents. The highest mutation frequencies (62.3–75.1%) were observed in the BW208-derived lines. Edited lines of cv DP and cv THA53 showed lower insertion and deletion (indel) frequencies, ranging between 1.50% and 14.77% and 5.16–7.86%, respectively. In general, sgAlpha-2 was more effective than sgAlpha-1. Three of these T1 lines (T544, T545 and T553) had indels at the target site of between + 36 and + 158 bp and − 1 and − 126 bp, respectively (**Figure 4**).

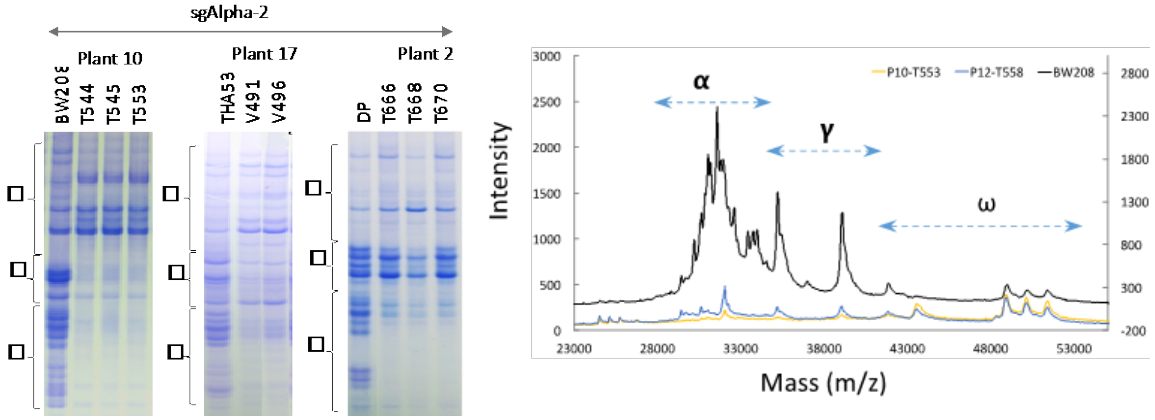
Figure 4. Gene editing of α-gliadins in bread wheat. Illumina sequencing of the α-gliadin genes and alignment of the different deletion types found at the target locus of sgAlpha-2 (top); frequency of the different type of insertions and deletions found in three BW208-derived lines (bottom).



Source: Sánchez-León et al. (2018).

To assess the impact of the observed mutations on seed protein composition, gliadin and glutenin content in T1 seeds was qualitatively assessed by A-PAGE and SDS-PAGE, respectively. A-PAGE demonstrated that alpha gliadins were strongly reduced in some of the bread and durum wheat T1 lines, and partially reduced in others (**Figure 5**). Mass spectrometry (MALDI-TOF) confirmed the sharp reduction in alpha gliadins in BW208-derived lines, with the sgAlpha-2 lines showing a greater reduction in the number of visible peaks (**Figure 5**).

Figure 5. A-PAGE of gliadins from sgAlpha-2 T1 lines T544, T545 and T553 (plant 10); V491 and V496 (plant 17); T666, T668 and T670 (plant 2); and the wild-type lines BW208, THA53 and DP (left). Matrix-assisted laser desorption/ionization (MALDI) time-of-flight (TOF) analysis of the same gliadin extracts in (left) from T1 lines T553 (plant 10) and T558 (plant 12) and the BW208 wild type.



Source: Sánchez-León et al. (2018).

Reversed-phase high performance liquid chromatography (RP-HPLC) analysis was performed to accurately quantify and characterise the different groups of gliadins and glutenins. As expected, alpha-gliadin content was significantly reduced in most of the edited lines compared to the wild type (32–82% reduction), especially in the bread and durum wheat lines edited with sgAlpha-2.

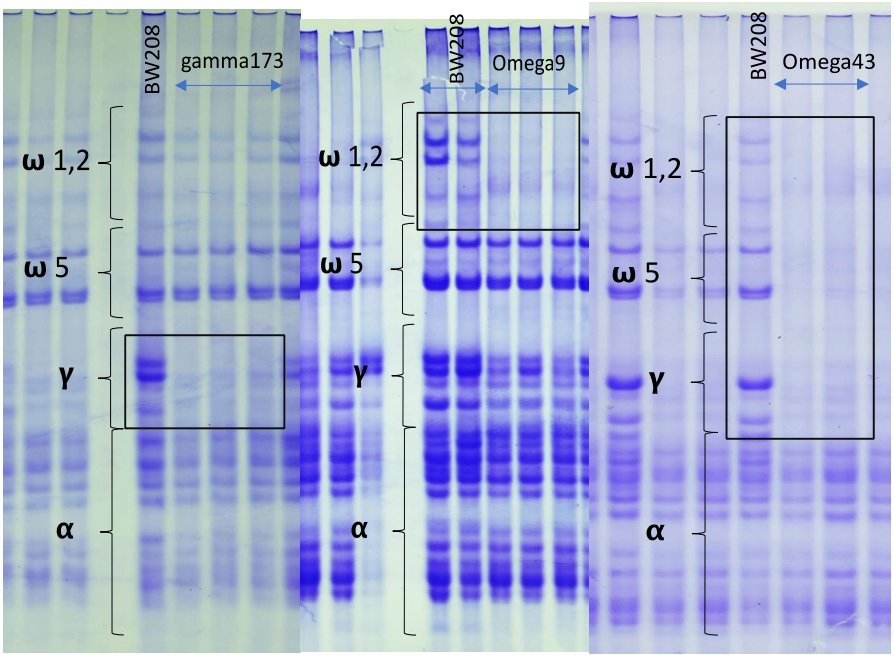
Next, the search for off-target sites was expanded to the entire bread wheat genome. Amongst all potential off-target sites, only four were annotated genes: a putative MADS-box transcription factor (Traes_7BL_F621D9B9E), two genes with unknown function (Traes_2AS_D659E88E9.1, Traes_2AS_8FCC59363.1) and one gene with homology to a gliadins (Traes_4AL_4FF5B8837). No mutations were identified in any of these genes in approximately 10 clones sequenced from each gene in the T1 mutant line T544.

The phenotype observed in the prolamin (gliadins and glutenins) content was also inherited, as assessed by evaluating 25 different T2 lines, and 16 T3 lines by RP-HPLC and A-PAGE gels. This demonstrated that the low-gluten trait is stable and heritable, and will enable its introgression into elite wheat varieties if necessary.

Finally, to identify transgene- and insertion-free (i.e. lacked insertions at the cleavage site) low-gluten, celiac-safe wheat, lines T1 and T2 were screened by PCR and Illumina high-throughput sequencing for the presence of plasmid DNA. Therefore, a number of both bread and durum wheats were selected not containing transgene or insertion at the cleavage site, and used for further characterisation.

A second product was obtained with a new round of gene editing, targeting wheat gamma and omega gliadins, to achieve a new variety to be safe for WA and WDEIA individuals. In this round of gene targeting, several sgRNAs were designed to target the gamma and omega gliadins (**Figure 6**). As for alpha gliadins, a set of edited lines was produced, and those lines not containing the CRISPR/Cas reagents were selected.

Figure 6. Gliadin trait profile achieved in soft wheat edited lines using sgRNAs to target gamma gliadins (left), omega 1-2 gliadins (middle) and gamma and omega gliadins by CRISPR/Cas. Black rectangle highlights the target gliadin region.



Source: F. Barro, unpublished data.

In addition to the single mutations, cross-breeding was carried out between lines in **Figure 6** and those in **Figure 5** aimed to combine all gliadin mutations into a single soft wheat genotype. Lines combining all CRISPR/Cas mutations are now being analysed, and the final selection of this single genotype containing all mutations is expected to be completed by mid-2023.

5.1.4 Product development stage

The gene-editing work has primarily been carried out within the framework of two research projects (AGL2013-48946-C3-1-R and AGL2016-80566-P) funded by the Agencia Estatal de Investigación (AEI), which belongs to the Spanish Ministry of Science and Innovation. The total funding for these projects was €365 000 and the main budget was for personnel costs (€117 000). The non-personnel budget includes the design and construction of the RNA guides and vectors and reagents for delivery into plant cells. It also includes reagents for in vitro culture and regeneration of edited plants, the amplicon sequencing of target genes, characterisation of monoclonal antibodies, protein extraction and high-performance liquid chromatography (HPLC) for protein fraction analysis, and the maintenance of plants in greenhouses for at least two generations. It is worth mentioning that some of the research by IAS-CSIC was carried out in collaboration with other groups, and that the cost was assumed by them and was not included in the budget. Therefore, a 20% higher cost is expected. It should also be mentioned that these costs may vary from one EU country to another. The cost does not cover the items listed in **Table 5** to place the product on the market, the full genome sequencing, if necessary, and patents and any other costs associated with any regulation or lawyers.

Compared to the transgenic approach, the costs of reagents, consumables and greenhouses are similar, as they share many of the necessary steps to develop the wheat varieties and their characterisation. In contrast, the conventional approach would require fewer reagents and consumables, molecular biology-related ones in particular, but would require much more time, and personnel costs would therefore be much higher.

The project activities took place from January 2014 until December 2019. Considering the technology readiness level (TRL), the product should be between TRL5 and TRL6.

Table 4 summarises the patent application filed so far. The patent is licensed to Plant Bioscience Limited (https://www.pbltechnology.com/).

Table 4. Patent applications for the gene-edited wheat with low gluten.

Type	Application number	Date
European patent	EP17382335	5 June 2017
International application (PCT)	PCT/EP2018/064791	5 June 2018
USA	PC928072US	21 April 2020

Source: Own elaboration.

The primary steps involved in placing the IAS-CSIC low-gluten, celiac-safe wheat product on the market are summarised in **Table 5**. Expected dates are indicative, and the main bottleneck relates to the approval process for the dossier required by the different regulatory authorities. The length and potential success of such an approval process depends on whether or not the product is considered a genetically modified organism (GMO).

Table 5. Key steps involved in placing the product on the market.

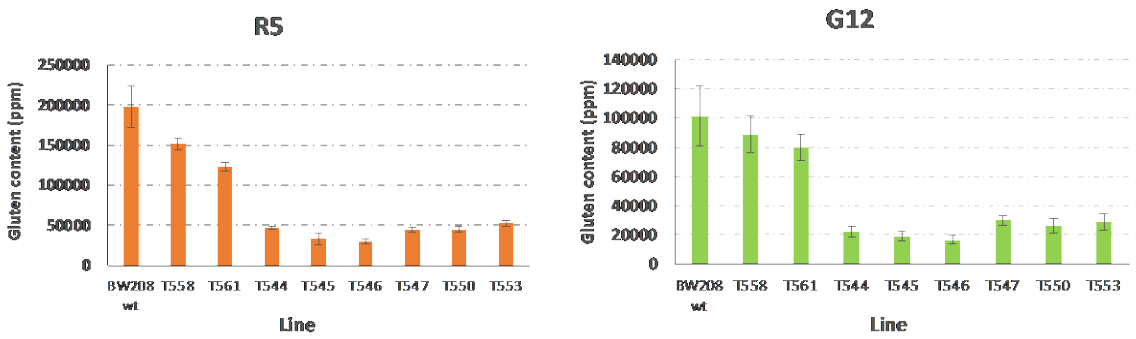
Step	Expected date	Comments
Field trial	2023–2025	Two-year field trial or two countries' field trials. Selecting the best performance line.
In vitro assays	2024–2025	[1]PBMCs/T-cell assay for choosing the best line not eliciting immunogenic response.
Variety registration	2026–2028	Variety registration according to the International Union for the Protection of New Varieties of Plants.
Clinical trial for non-celiac wheat sensitivity patients	2027–2028	A multicentre clinical trial with the best line for safety/efficacy.
Clinical trial for celiac patients	2028–2029	A multicentre clinical trial with the best line for safety/efficacy.
Dossier for regulatory authorities; USA and EU	2026–2029	Gathering all available information on lines, field trial, clinical trials, etc., for regulatory agencies.

([1]) Peripheral blood mononuclear cells

Source: *Own elaboration.*

So far, no field trials or clinical trials have been carried out. However, a number of tests and greenhouse trials were performed. These tests confirm that the altered gliadin content effectively reduced the immune reactivity of the flour; T2 seeds of the mutant lines were analysed by using the monoclonal antibodies (mAb) R5 and G12 (**Figure 7**). R5 is the mAb used in the food industry to quantify gluten content and detect a conserved domain (QQPFP) found in most gliadins (not only the immune-reactive ones). The G12 mAb is more specific, for detecting reactive epitopes, as it was developed against the 33-mer peptide. ELISA tests with both mAbs showed a strong reduction in gluten content in the sgAlpha-2-derived lines compared to that of the BW208 wild type. In those lines, a reduction in the gluten content of up to 85% was observed, and an average reduction of 66.7% and 61.7%, respectively, with the R5 and G12 mAbs (**Figure 7**).

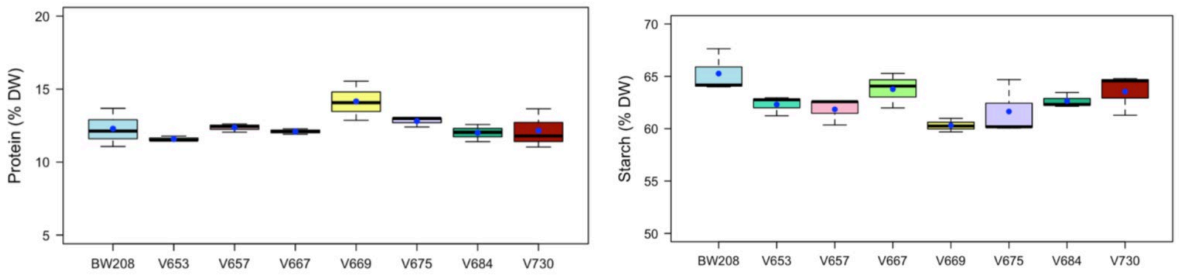
Figure 7. Analysis of immune reactivity of T2 seeds of the sgAlpha-2 mutant lines with the monoclonal antibodies (mAb) R5 (left) and G12 (right).



Source: Sánchez-León et al. (2018).

Selected lines were analysed under confined greenhouse conditions. Thousand kernel weight (TKW), total grain protein, starch grain content and days to anthesis were analysed in those lines (Figures 8, 9). For total protein content, no significant differences were found between edited lines and their corresponding BW208 wild-type line. However, starch content was significantly lower in three lines, and in one line in particular (V669), being about 4% lower than that of the wild type (**Figure 8**).

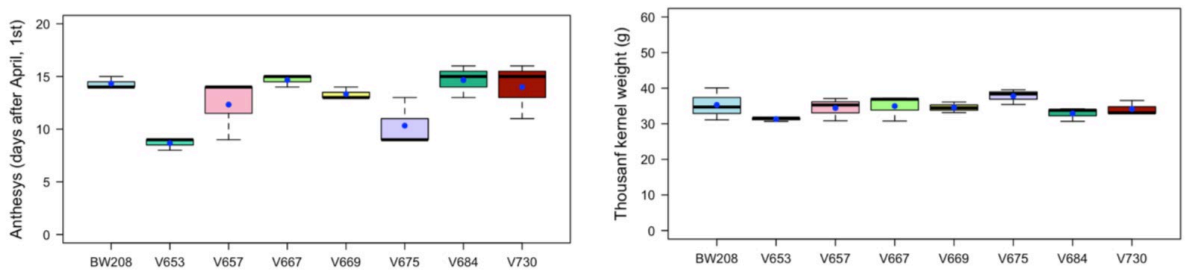
Figure 8. Total protein (left) and total starch (right) content as a percentage of dry weight (DW) in selected transgene- and insertion-free BW208-derived edited lines.



Source: F. Barro, unpublished data.

With regard to anthesis, with the exception of one line (V653), there is no significant difference between edited lines and the corresponding BW208 wild-type line (**Figure 9**). Additionally, for thousand kernel weight, all edited lines showed values comparable to that of the BW208 wild type. However, all of this data and further agronomic performance, including yield, would require confirmation in larger field trials.

Figure 9. Days for anthesis (after 1 April) (left) and thousand kernel weight (right) in selected transgene- and insertion-free BW208-derived edited lines.



Source: F. Barro, unpublished data.

Apart from the agronomic data described above, the phenotype of the plants, particularly spike morphology and grain size, was comparable between the edited lines and the BW208 wild type.

5.2 Gene-edited wheat developed by Plant Breeding group at WUR

5.2.1 Gene coding for gluten proteins

As mentioned above, immunogenic epitopes occur in alpha, gamma and omega gliadins and to a lesser extent in LMW glutenins, while HMW glutenins are predominantly safe for CD patients and constitute the main gluten family responsible for bread dough quality (Payne et al., 1987a; Shewry et al., 2009; Shewry, 2019). **Figure 3** depicts the locations of the genes on the wheat chromosomes. In total, a soft wheat variety may harbour over 90 gluten genes (Gilissen and Smulders, 2021a).

Variants of the immunogenic epitopes that are not recognised by T-cell receptors exist in hexaploid bread wheat (Mitea et al., 2010), but in all genotypes they are found in combination with other, highly immunogenic epitope variants (Van Herpen et al., 2006; Van den Broeck et al., 2010a, 2010b; Salentijn et al., 2012). Given this level of complexity, it is not surprising that conventional breeding alone has not yet succeeded in generating 'gluten-safe' wheat, containing only gliadins with non-immunogenic epitopes or even with no gliadins at all (Smulders et al., 2015; Sánchez-León et al., 2018; Jouanin et al., 2018; Jouanin et al., 2019b).

Sollid et al. (2012, 2020) have published a comprehensive list of celiac disease epitopes found in gluten. Individual patients develop a set of T cells, each of which recognises a different epitope (Vader et al., 2002; Camarca et al., 2009). Some epitopes are recognised by T cells in most patients (Tye-Din et al., 2010). Considering the large number of well-documented and clinically relevant epitopes with DQ2.5 restriction, RNA silencing (RNAi; Gil-Humanes et al., 2010; Barro et al., 2016; Guzmán-López et al., 2021) and gene-editing activities have focused on these epitopes.

5.2.2 Technique used for gene editing

The technique used for the modification was CRISPR/Cas targeted mutation. Jouanin et al. (2019b) transformed immature embryos of hexaploid soft wheat cultivar Fielder with constructs containing Cas9 and combinations of sgRNA targeting specific sites upstream of, or within, the CD epitopes in the alpha gliadin as well as the gamma-gliadin gene family. They used combinations of up to five guide RNAs targeting two positions in alpha gliadins and three sites in gamma gliadins.

5.2.3 Trait achieved after CRISPR/Cas targeted mutation

In the seeds of the offspring of regenerated plants, Jouanin et al. (2020b) found proof of small deletions that remove individual proteins as well as larger deletions, presumably of gliadin genes located between Cas9-produced double-strand breaks.

Among 117 regenerated lines in which Cas9 was expressed, seeds were found with various missing proteins. Using sequencing, Jouanin et al. (2019a) reported four plants that had both deletions of gamma gliadins on chromosome 1 and alpha-gliadin genes on chromosome 6. Using droplet digital PCR (ddPCR), Jouanin et al. (2020b) estimated that in some lines more than 20 alpha-gliadin genes were mutated. Thus, the results of this study show that in one round of gene editing plants may be regenerated in which tens of gliadins are

mutated simultaneously, in more than one gliadin gene family. These plants are not yet sufficiently reduced in gluten content to be safe for CD patients.

The random occurrence of deletions at the target sites and the large number of targets in the gliadin gene families mean that gene editing will initially produce plants with a mosaic of edited, deleted and unaffected genes. This requires efficient methods to detect the plants with edits, in order to be able to rigorously reduce the number of plants in a breeding programme through quality-directed selection steps. Jouanin et al. (2020a) describe how screening may occur at DNA level (the number of genes present and their sequences after editing), at protein level, on baking quality and on immunogenicity.

5.2.4 Product development stage

The lines of Jouanin et al. (2019b) were at proof-of-concept stage. They still contain far too many epitopes to be safe for celiac patients.

Based on the existing attempts (Sanchez-Léon et al., 2018; Jouanin et al., 2019b), the following steps could illustrate a typical breeding strategy to achieve a low-gluten wheat safe for people with celiac disease using new genomic techniques (modified from Jouanin et al., 2020a).

a. Start with lines with a deletion on chromosome 6D, so that the 6D alpha-gliadin locus with many overlapping major CD epitopes is absent to start with (Arentz-Hanzen et al., 2000; Van Herpen et al., 2006; Van den Broeck et al., 2009; Schaart et al., 2021).

b. All gliadins and LMW glutenins are targeted for deletion through gene editing. The advantage of removing all omega gliadins is that they also function as allergens in WDEIA (Gilissen et al., 2014).

c. Removing all genes by editing may require two or three rounds of gene editing using optimised gene-editing protocols (as used by Li et al., 2021; Luo et al., 2021; and as suggested above). In combination with regeneration steps, this is estimated to require 4-5 years. Advances in speed breeding may make it possible to reduce this period (Watson et al., 2018; Wanga et al., 2021).

d. HMW glutenins are maintained, possibly (in a later improvement) in a slightly edited form to remove minor (DQ8) epitopes. These are minor epitopes as only a few patients exist that have DQ8 as their HLA genotype and can recognise these epitopes (Van de Wal et al., 1999; Tye-Din et al., 2010)

e. Testing of the lines – first with ddPCR, polyacrylamide gel electrophoresis (PAGE) and antibodies, then with T cells, for baking quality, and clinical trials (Jouanin et al., 2020a).

As a result, the whole process would take between 6 and 10 years. Assuming a process duration of 10 years, a company that already has an ongoing wheat breeding programme, molecular and tissue culture labs, any required license for trials with genetically modified plants and fields for field trials, the additional costs of the elimination of toxic loci may reach EUR 3–4 million without including the cost of authorisation. It seems a reasonable estimate when compared with the estimate provided by Le Buanec and Ricroch (2021) of EUR 1 million for research in plant biotechnology and plant breeding to develop a new conventional variety. On the other hand, the cost of market approval has been estimated to vary between EUR 4–10 million for the first generation of GM crops with a single trait for herbicide tolerance or insect resistance (Schenkelaars et al., 2011). Schenkelaars et al. (2011) also concluded, from a survey on experts in bringing GM crops to the market, that regulatory compliance costs for GM crops are substantial but their estimates varied widely, from USD 10–15 million to more than USD 100 million.

Once the safe loci have been created, they may be introgressed, through crossing and selecting, into multiple varieties. If there is broad interest from breeders in low-gluten, celiac-safe wheat, they may pay for licences (provided that the low-gluten background, and/or the set of tests necessary for selection, are protected with intellectual property).

Jouanin et al. (2019b) used cultivar Fielder, a spring wheat variety, as it was one of the few genotypes in 2014 when the research was initiated for which a successful protocol for plant regeneration from cell cultures had been established. Spring wheat varieties are only a small fraction of the acreage of wheat – most varieties are winter wheat varieties as they yield more. In the last couple of years, the technology for wheat transformation and regeneration has developed fast, meaning that it is now possible to transform several commercially interesting varieties and with higher success rates (Wang et al., 2022).

Takeaway points from Section 5 – Genes edited and trait achieved

i) The wheat lines developed by the Plant Biotechnology group at the Institute for Sustainable Agriculture of the Spanish National Research Council of Córdoba (IAS-CSIC group) with gene editing (SDN-1 type CRISPR/Cas targeted mutation) achieved a reduction in the alpha-gliadin content from 32% to 82%, while immunoreactivity was reduced by 85%. These lines may be useful for celiac disease patients and possibly for those with non-celiac wheat sensitivity. They have already been tested in greenhouses.

i) A second gene-edited wheat was addressed by IAS-CSIC group to achieve a reduction of 70% and 90% of the gamma and omega gliadins, respectively. Gamma and omega gliadins are responsible for triggering the wheat allergy, and omega gliadins wheat-dependent exercise-induced anaphylaxis. These lines are not yet tested in greenhouses.

iii) The IAS-CSIC group carried out cross-breeding between all of their lines to combine all gliadin mutations in a single soft wheat genotype. These lines are now being analysed, expected to be completed by mid-2023 and after that clinical trials are planned using the best line.

iv) The Plant Breeding group at Wageningen University & Research, in collaboration with the John Bingham Laboratory, designed a gene-edited wheat targeting alpha and gamma gliadins. These lines are at-proof-of-concept stage and still contain too many epitopes that need to be knocked out to be safe for celiac patients.

v) Plant breeding programmes are costly and can reach up to EUR 1 million to develop a new conventional variety and up to EUR 3–4 million for a gene-edited variety without including the authorisation costs. Authorisation and other costs to place varieties of genetically modified organisms on the market have been reported to vary widely from EUR 4–10 million to EUR 100 million.

6 Description of current alternatives to manage gluten-related disorders (GRDs)

6.1 Gluten-free diet (GFD)

A lifelong GFD is currently the only known effective treatment for CD and WDEIA patients, and it is also recommended for NCWS patients (Lindfors et al., 2019). For the production of gluten-free products, cereals naturally free of immunogenic gluten, i.e. free of proteins that can trigger an immune system response, are used (Rosell et al., 2014). Strict adherence to a GFD also reduces the risk of developing many of the serious long-term complications related to untreated CD. However, following a GFD is not easy, as it not only involves eliminating gluten-containing foods, but there is also a sense of social isolation and pressure that accompanies the process (Jnawali et al., 2016). Current options when it comes to following a GFD include using different grain crops to substitute wheat and implementing alternative food processing methods.

Alternative grain crops

Cereals that are always considered gluten free are rice (*Oryza sativa L.*), maize (*Zea mays L.*) and sorghum (*Sorghum bicolor (L.) Moench*), which are distant relatives of wheat and are known to be safe for celiac patients. In addition, a number of species of millet, the Ethiopian cereal teff and a range of pseudocereals can also be used to produce gluten-free flours, even increasing the nutritional pattern of those products in the case of pseudocereals.

Oat has long been on the list of gluten-containing cereals, but this was most likely a result of the high degree of contamination of oat products with wheat, barley and rye (depending on the crops commonly grown in the region where the oat was produced). Pure oat is celiac safe (Londono et al., 2013; Hardy et al., 2015) and oat products containing less than 20 mg/kg gluten are now allowed to be sold as gluten-free products.

Since wheat is the only bread cereal that produces baked products with a large volume, the use of the above-mentioned cereals and pseudocereals to produce gluten-free products requires the addition of compounds that can mimic a protein network similar to that formed by gluten, so that the gluten-free products can be kneaded. A GFD is usually characterised by an imbalanced intake of different nutrients, with very low contributions to the recommended daily protein intake, and high contributions to the carbohydrate and fat dietary reference intake (Cardo et al., 2021; Vici et al., 2016). This is mainly due to unhealthy dietary habits, which can also be commonly found in non-celiac individuals, together with the difficulty of eliminating gluten from the diet that lead to increased consumption of highly processed, gluten-free products (Cardo et al., 2021). Gluten-free foods, when compared to equivalent wheat-based foods, show deficiencies in minerals, including calcium, iron, magnesium and zinc, and in vitamins, including vitamin B12, folate and vitamin D, which is coupled with an increased obesity risk due to the high glycaemic index of the GFD and the high content of saturated lipids (Vici et al., 2016).

Potential effects on gut microbiota have been examined in both patients suffering from gluten-related disorders and in healthy people following a GFD (Caio et al., 2020; Palma et al., 2009). In CD patients, gut gram-positive bacteria known for their protective effect, e.g. *Bifidobacteria, Firmicutes*, *Lactobacilli* and *Streptococceae,* are lower in number than in healthy controls, while the number of harmful gram-negative bacteria (*Bacteroides, Bacterioidetes, Bacteroides fragilis, Prevotella, Escherichia Coli, Proteobacteria, Haemophilus, Serratia, Klebsiella*) increase. These findings suggest that intestinal dysbiosis affects CD patients and contributes to persistent symptoms, even in those on a strict GFD regimen.

Clinical trials carried out with low-gliadin RNAi wheat lines – which are comparable to the CRISPR/Cas ones as the same allergen genes were knocked out – confirmed those findings as its consumption favoured a less inflammatory phenotype that could be related to the increase of *Faecalibacterium*, an anti-inflammatory genus, and a reduction in *Bacteroides,* a proinflammatory genus, thereby improving the gut inflammation characteristics of NCWS patients (Haro et al., 2018).

Alternative food processing

Sourdough fermentation. Proteolytic food processing technology was used to manufacture new 'gluten-free' or 'low celiac-toxic gluten' wheat-based products. Hydrolysis appeared to break down wheat gluten molecules into peptides that could be further degraded to further remove celiac-immunotoxic epitopes (Loponen, 2006; Greco et al., 2011a). These observations appeared promising, but still need further research to confirm the true celiac safety of the products obtained. In general, the application of sourdough fermentation may be of

general interest in Germany. With a longstanding tradition of sourdough bread consumption, the prevalence of celiac disease was remarkably low (Kratzer et al., 2013). However, more recently, prevalence has increased to 'normal' global levels, which was suggested to be caused by a diet change, especially in the younger section of the German population – embracing highly processed fast foods (Gilissen et al., 2016b). Gluten quantity in daily consumption appears to matter in the development of CD (Koning, 2012).

Reduction of gluten in foods. Apart from the alternative grain crops or foods that are naturally free of gluten, there are foods produced, prepared or processed to reduce their gluten content. The main strategy to reduce gluten in foods is using a wide range of alternative raw ingredients and additives with no gluten (e.g. starches, gluten-free flours of cereals/pseudocereals, hydrocolloids and proteins, and to a lesser extent enzymes and emulsifiers) that mimic the cohesiveness and elasticity of a gluten-containing dough (El Khoury et al., 2018). However, these processed gluten-free products are more likely to be higher in fat, sugar and salt and lower in fibre and protein than regular food products (Myhrstad et al., 2021; Fry et al., 2018).

Separation. Gliadins and glutenins can be separated almost completely at lab level. The process is more recalcitrant at industrial level but can be achieved (Bassi et al., 1997). The glutenin fraction, in particular, is important for its processing qualities, while the gliadin fraction can be omitted or functionally replaced by other proteins (e.g. avenins from oat; Van den Broeck et al., 2011). As the gliadins contain most of the celiac-relevant epitopes, separation of the glutenin fraction from the total gluten in an economically and technologically viable way may contribute to producing foods with significantly reduced celiac immunotoxicity, for the general population, including undiagnosed celiac and gluten-sensitive individuals. However, this technology is yet to be further developed.

Gluten alternatives. Another approach includes the development of alternative protein systems for visco-elastic dough preparation. One such alternative includes the use of structured suspension containing whey protein particle, similar to the particle structure of the dough gluten-starch network. Some early promising results have been extended to oat to improve the dough-making qualities of its flour (Londono et al., 2014).

6.2 Conventional breeding

Due to the structural complexity of the gene coding for gliadins, the high copy number of these genes, segregating in blocks, it is extremely difficult to apply conventional breeding techniques to obtain comparable varieties of low-gluten wheat safe for celiac people (Boukid et al., 2017; Jouanin et al., 2018; Rustgi et al., 2019).

A normal hexaploid wheat variety may harbour over 90 gluten genes (Gilissen and Smulders, 2021a). These genes are located in clusters on two chromosomes (**Figure 3**). Several genes do not contain the immunogenic epitopes that trigger CD (Mitea et al., 2010), but they are linked to tens of genes that contain several highly immunogenic epitope variants (Van Herpen et al., 2006; Van den Broeck et al., 2010a, 2010b; Salentijn et al., 2012). Given this level of complexity, conventional breeding alone cannot generate a celiac-safe wheat variety that only contains gliadins with non-immunogenic epitopes (Smulders et al., 2015; Sánchez-León et al., 2018; Jouanin et al., 2018; Jouanin et al., 2019b).

Plant mutation breeding

Mutation breeding is a method that is exempted from GMO regulations. It has recently been applied to develop the 'ultra-low gluten' barley variety Kebari (Tanner et al., 2016), which is being used to produce gluten-free beer in Germany (Howitt et al., 2018). It was used in combination with large deletions of gluten genes, which was feasible as gluten proteins are not necessary for the production of beer. Developing wheat with hypoimmunogenic gluten using a similar approach is theoretically possible, although it does constitute a great challenge (Ribeiro et al., 2018).

Ethyl methanesulfonate (EMS) mutation breeding generates many random mutations (Krasileva et al., 2017), is resource-intensive, but may result in the removal of some gliadin loci, as has been shown for bread wheat (Van den Broeck et al., 2009; Jouanin et al., 2019b). Wen et al. (2022) suggested that wheat without any gliadins and LMW glutenins may still have a reasonable baking quality based on HMW glutenins only. However, combining deletion lines, EMS mutations and crossing and selection approaches are insufficient to make one wheat variety that has so few epitopes that it is safe for CD patients.

There are projects to apply TILLING (targeting induced local lesions in genomes) to obtain mutants in the gliadin genes. A wheat line, named GoodWheat™, is commercially available in the USA that contains 50% and

60% lower gliadins and LMW glutenins, respectively, than traditional flour. It was developed by using TILLING on wheat prolamin-box binding factor (WPBF) mutants (Moehs et al., 2019).

Another strategy published was the analysis of deletion lines of Chinese Spring, a model soft wheat variety (Van den Broeck et al., 2009). These lines were developed through the addition of an alien chromosome from *Aegilops cylindrica Host* (2n = 4x = 28, CCDD genome) or *A. triuncialis* L. (2n = 4x = 28, UUCC genome) or a chromosomal segment from *A. speltoides Tausch* (2n = 2x = 14, SS genome) (Endo and Gill, 1996). As a consequence of the deletions, these lines were devoid of some of the alpha, omega and gamma gliadin, as well as LMW subunit genes.

These deletion lines were analysed using monoclonal antibodies that recognise T-cell epitopes present in gluten proteins. Lines showing partial deletion of the alpha-gliadin locus from the short arm of chromosome 6 of the D genome (6DS) resulted in a significant reduction in the presence of T-cell stimulatory epitopes but also in a significant loss of technological properties. Partial deletion of the short arm of chromosome 1 of the D genome (1DS), containing the omega gliadin, gamma gliadin and LMW, showed the disappearance of some bands recognised by monoclonal antibodies in western blotting assays. Although the authors propose that this may be a strategy to reduce gluten content, no other references have been found indicating the use of these lines in breeding programmes, or their direct use in food manufacturing.

For the above examples, no data on time and cost for obtaining those partial-gluten lacking lines are available.

6.3 Alternative genomic techniques

RNA interference (RNAi) technology

Non-conventional approaches based on GMOs, mainly on RNA interference (RNAi) technology, have been used to develop wheat varieties with low gluten (**Table 6**). RNA silencing is a sequence-specific RNA degradation system that is conserved in a wide range of organisms. RNAi is a post-transcriptional process triggered by double-stranded RNA (dsRNA), leading to gene silencing via a two-step mechanism (Watanabe, 2011). For the silencing of wheat gliadins, dsRNA is constructed using the highly conserved gliadin gene sequences.

As reported in **Table 6**, the gene expression of all three gliadin families has successfully been reduced by over 90% in soft wheat grains using RNAi to gluten genes. In turn, derived gluten extracts and bread did not stimulate CD patient T cells, while the dough quality of their flours was barely affected (Gil-Humanes et al., 2010, 2014; Guzmán-López et al., 2021). Similarly, Becker et al. (2012) significantly reduced the expression of 20 alpha-gliadin genes using RNAi; however, they also found increased expression of other storage gluten proteins. Wen et al. (2012) reduced the expression of DEMETER through RNAi, thus preventing DNA-methylation changes which are required to repress glutenin and gliadin gene expression in the endosperm.

Table 6. Transgenic approaches for the downregulation of gluten proteins in wheat.

Target genes	Method	Gluten proteins down-regulated	Reduction of target proteins (%)[a]	Reference
α-gliadins	hpRNAi	α-gliadins	63	Becker et al. (2012)
γ-gliadins	hpRNAi	γ-gliadins	65 to 97	Gil-Humanes et al.(2008), Pistón et al. (2011)
ω-5-gliadins	hpRNAi	ω5-gliadins	nd	Altenbach and Allen (2011)
ω-, α-, γ-gliadins	hpRNAi	ω-, α-, γ-gliadins; LMW-GS	60 to 90	Gil-Humanes et al. (2010), Barro et al. (2016)
Wheat DEMETER	hpRNAi	ω-,α-, γ-gliadins; LMW-GS; HMW-GS	45 to 76	Wen et al. (2012)

([a]) Determined by reversed-phase high-performance liquid chromatography (RP-HPLC).
([nd]) Not determined; LMW-GS, low molecular weight glutenin subunits; HMW-GS, high molecular weight glutenin subunits; hpRNAi, hairpin RNA of interference.

Source: Adapted from Rosell et al. (2014).

Although several approaches were initiated using this technology (**Table 6**), currently only the wheat lines, described in Gil-Humanes et al. (2010) and Barro et al. (2016) are reporting the most encouraging results on their use to control GRDs.

The potentially toxic effects of whole wheat flour from the best transgenic low-gliadin line, in comparison with the corresponding non-transgenic wild-type soft wheat line, was tested with Sprague Dawley rats for 90 days (Ozuna et al., 2017). Results showed no adverse effects and no difference between the rats that ate the whole flour and those the wild-type wheat. The low-gliadin line was then used to make bread and a dietary intervention of patients with non-celiac wheat sensitivity (NCWS) was carried out to evaluate the symptoms, acceptance and digestibility of the bread (Haro et al., 2018). Gastrointestinal symptoms were indistinguishable between the low-gliadin bread and the gluten-free bread of choice, demonstrating that low-gliadin bread does not worsen symptoms. The consumption of low-gliadin bread by NCWS patients induced potentially positive changes in gut microbiota composition (Haro et al., 2018).

Later, another crossover trial was carried out using bread made with the same transgenic low-gliadin line but with CD patients (Guzmán-López et al., 2021). This study provides evidence that bread from low-gliadin flour does not elicit an immune response after a short-term oral challenge and could help manage GFD in patients with CD.

As the transgenic RNAi construct necessarily remains in the wheat genome to silence the genes, these plants are subject to GMO regulations. It is therefore not to be expected that the RNAi lines with low gluten will be marketed soon.

Takeaway points from Section 6 – Description of current alternatives to manage GRDs

i) A lifelong gluten-free diet is currently the only known effective treatment for celiac disease and wheat-dependent exercise-induced anaphylaxis patients, and it is also recommended for non-celiac wheat sensitivity patients.

ii) Current options for following a gluten-free diet include using different grain crops naturally free of immunogenic gluten to substitute wheat (e.g. rice, maize, sorghum and a range of pseudocereals) and implementing alternative food processing.

iii) Food processing methods require the addition of compounds that can mimic a protein network similar to that formed by gluten. These processed gluten-free products are more likely to be higher in fat, sugar and salt and lower in fibre and protein content than regular food products

iv) Due to the structural complexity of the gene coding for gliadins, the high copy number of these genes, segregating in blocks, it is extremely difficult to apply conventional breeding techniques to obtain comparable varieties of low-gluten wheat safe for celiac people.

v) Non-conventional approaches based on genetically modified organisms, mainly on RNA interference (RNAi) technology, have been used to develop low-gliadin RNAi wheat lines – which are comparable to the CRISPR/Cas ones as the same allergen genes were knocked out – with positive results observed in clinical trials with non-celiac wheat sensitivity patients.

7 Social and health impacts

7.1 Accessibility to more affordable diets

Once the diagnosis is established, the cost of the GFD is passed on to the families, who in most cases do not have any financial support to cover the cost of such a diet. The prices of gluten-free products are generally much more expensive than gluten-containing products (**Table 7**). However, the range of variation depends on the type of product and the geographical region. Celiac and gluten-sensitive associations themselves carry out comparative studies of these prices and their evolution over the years.

Table 7. Gluten-free (GF) diet cost.

Cost of GF diet compared with non-GF diet	Country	Reference
+ 200% to + 900%	Spain	FACE, 2021
+ 150 to + 500%	Ireland	Celiac Society of Ireland
+ 4% to + 804%	Greece	Panagiotou & Kontogianni, 2017
+ 200%	Italy	Gorgitano & Sodano, 2019
+ 46% to + 443%	Norway	Myhrstad et al., 2021
+ 205% to + 267%	Austria	Missbach et al., 2015

Source: Own elaboration.

In Spain, the Federation of Celiac Associations of Spain (FACE) shows a detailed comparison of the prices of gluten and gluten-free food products sold in six different hypermarket chains. In the 2021 and 2022 surveys, all of the gluten-free products were significantly more expensive, respectively ranging from 200–900% and from 115–740% more expensive than gluten-containing products[11]. The biggest difference was found for bakery products. The variation found translates into an increase in the food budget for a middle-class family with at least one member who must follow a GFD. This increase in the shopping basket cost is, according to 2021 and 2022 surveys, €19.5 and €17.6 per week, €77.9 and €70.4 per month and €934.7 and €845.2 per year, respectively, compared to other families in the general population. Although the gap between the price of Spanish gluten-free and gluten products has evidently been gradually falling since 2013, the downward trend has flattened. These calculations are for one person with CD; if there are more members the cost will be higher.

A similar analysis is also reported by the Celiac Society of Ireland in its *Price Comparison of Gluten-free foods vs. Normal foods*, the gluten-free products being more expensive than the normal products, with a range of variation between 150% and 500% more.

Scientific publications are in line with the data reported by celiac associations. In Greece, 38 gluten-free product categories were analysed, all being gluten-free products available at supermarkets, except for three, more expensive compared to their conventional counterparts by 4-804% (Panagiotou and Kontogianni, 2017). They concluded that the monthly allowance given to patients for gluten-free product purchases, which is €150/month for individuals up to 18 years and €100/month for adults, is necessary and adequate to cover the additional cost of the GFD.

In Italy, the offer of gluten-free pasta in supermarkets, with respect to its ability to meet the needs of celiac people in terms of variety, price and safety, were evaluated. Results indicated that gluten-free pasta is sold at a price equal to more than twice that of conventional pasta (Gorgitano and Sodano, 2019). However, celiac people in Italy are provided with state aid to cover the extra cost they incur in buying gluten-free products. The Italian National Health Service provides state aid of over EUR 250 million annually to the approximately 250 000 celiac people living in Italy, with an annual national average of around EUR 1 200 per capita (based on 2017 data), in a monthly expense contribution for the purchase of the gluten-free products included in the list of foods suitable for celiac people of the Italian National Register.

On the Norwegian market, 423 unique gluten-free products were compared with 337 equivalents with gluten (Myhrstad et al., 2021). The price of gluten-free products was higher, ranging from 46–443% more expensive than gluten-containing products. In Austria, Missbach et al. (2015) found significant price differences between 63 gluten-free products and 126 similar gluten-containing products among 12 different Austrian supermarkets – gluten-free bread and bakery products were 267% more expensive and gluten-free cereal products more than 205%.

[11] https://celiacos.org/tratamiento/informe-de-precios/.

Although the supply and availability of these foods have expanded considerably in the last decade, the price of these products is still too high. Despite the fact that CD is a chronic disease, for which the only effective treatment is the GFD, the economic or food aid offered is scarce and differs considerably between EU countries. Recently, in April 2022, the Association of European Coeliac Societies (AOECS) published the results of a survey prepared by the the Federation of Celiac Associations of Spain (FACE) of the current economic support situation for celiac people in some European countries (**Table 8**). This was made possible through the information provided by FACE and the other European member societies of AOECS[12].

Table 8. Current economic support for celiac people in some European Countries.

Country	Aid
Andorra	No aid for celiac patients.
Belgium	Aid of €38/month.
Bulgaria	10-12 packages/month of gluten-free flour. €125/year for 50–70% disability.
Cyprus	10 kg/quarter of free gluten-free flour.
Denmark	No aid for celiac patients.
Finland	Aid of €95/month for children under 16 years.
France	No aid for celiac patients.
Greece	Aid of €100/month for adults and 150€/month for children.
Ireland	A tax refund of up to a maximum of 20% of the expense.
Italy	A monthly coupon, whose amount depends on sex and age, to buy gluten-free products.
Luxembourg	A reimbursement aid of €553.50/year.
Malta	A monthly coupon of €45 (pensioners €50).
Netherlands	A reduction of €950 in income tax.
Norway	Aids of €104.7/month for people between 5 and 30 years old, and €68.6/month for people over 30 and under 5 years old.
Portugal	Aid is around €170/year, and a possibility of deducting specific gluten-free foods.
Spain	No aid at governmental level, but the different autonomies can grant subsidies through patient associations.
Sweden	Aid for children under 16 in the form of food (€100/year) and money (€80-150/year).
Switzerland	No aid for celiac patients.

Source: Association of European Coeliac Societies (AOECS; April 2022).

In addition to GFD-related costs, consideration should also be taken of the opportunity cost for the food preparation time (often a dedicated area in the kitchen is needed), the time for other efforts related to maintaining quality of life and the participation of other family members in the GFD that will therefore strongly differ between cases (Soler and Borzykowski, 2021).

GE low-gluten, celiac-safe wheat is agronomically comparable to standard wheat, and there are therefore there no additional costs for machinery, fuel, labour, etc. compared to standard wheat from sowing to harvesting and processing. Furthermore, no additional ingredients are to be added for the production of derived products, such as fats, gums and many sugars that are used in gluten-free products to mimic the rheological properties of standard wheat. However, an additional price premium of 30% is to be expected for GE wheat flour in order to maintain the GE identity based on the example of non-GM IP soybean meal (Tillie and Rodríguez-Cerezo, 2015; see Section 8.3). Therefore, it is expected that the prices of products made from GE wheat flour will be in the range of 30% more expensive than those made from standard wheat. This increase is, however, much lower than the current prices for gluten-free products when compared to their gluten-containing counterparts (**Table 7**).

Therefore, in the event that GE low-gluten, celiac-safe wheat were available on the market, consumers might benefit from a significant saving in food expenditure. Using the Spanish case study, the total additional cost of GFD for the population can be estimated as follows. With a total population of 47.3 million, the saving would amount to (845*0.01*47.3) EUR 399 million if the shift in diet is only undertaken by individuals with CD, raising to (845*0.1*47.3) EUR 3 996 million if there was also a change in diet followed by individuals with self-diagnosed NCWS. Even if GE low-gluten, celiac-safe wheat were to be marketed at a significant price premium, the savings compared to the costs of GFDs today would still be substantial.

[12] https://www.aoecs.org/news/survey-on-economic-aid-for-coeliacs-in-europe/.

7.2 Accessibility to more nutritious and healthier diets

Another important aspect for people following a GFD is the accessibility to healthier and nutritious foods. The introduction of the GE low-gluten, celiac-safe wheat could help to alleviate two major limitations encountered among people on a GFD: the nutrient deficiencies of the GFD (Vici et al., 2016) and the negative effects that the GFD has on gut microbiota (Palma et al., 2009; Caio et al., 2020). Nutrient deficiencies are related to the necessary avoidance of grains, leading to a deficiency in alimentary fibre, eliminating the major protein sources from the diet and sticking to a high carbohydrate diet (Jnawali et al., 2016).

It is important to consider that by eliminating cereals from the diet we not only eliminate gluten proteins but also all compounds, including other proteins, which are beneficial to the diet. Miranda et al. (2014) compared the nutritional composition of the 206 gluten-free products most consumed in Spain against the composition of 289 equivalent foods with gluten. They concluded that following a GFD could result in a nutritional imbalance for celiac patients as well as for non-celiacs who follow a diet that includes many gluten-free rendered foodstuffs. Myhrstad et al. (2021) also reported that gluten-free products contained less protein and fibre, and a higher content of saturated fat, carbohydrate and salt compared to the gluten products, which could further exacerbate the economic burden of maintaining a healthy diet. Moreover, GFD products, particularly bread, have a higher content of both saturated and hydrogenated fatty acids than their normal counterpart, resulting in a high glycemic index that can increase the risk of obesity, insulin resistance and cardiovascular diseases (Scaramuzza et al., 2013). Therefore, there is an important need to develop gluten-free products that are highly nutritious and at the same time economical (Jnawali et al., 2016). In contrast, the GE low-gluten, celiac-safe wheat can provide comparable fibre, protein and energy to standard wheat, and no additional additives are necessary as they conserve excellent bread-making properties, providing products with a lower glycemic index than the gluten-free products.

On the other hand, the effect of GFD on gut microbiota was reviewed in patients with CD, NCWS and healthy people (Caio et al., 2020). CD and NCWS are often characterised by an imbalance in the microbial intestinal population composition leading to dysbiosis, a condition promoting inflammation and metabolic impairment. They reported that in all three groups, GFD was shown to reduce bacterial richness while affecting gut microbiota composition in a different manner depending on health (asymptomatic subjects) and disease state (CD and NCWS). Remarkably, in healthy subjects, GFD causes the depletion of beneficial species, e.g. *Bifidobacteria*, in favour of opportunistic pathogens, e.g. *Enterobacteriaceae* and *Escherichia coli*. This may be due to the withdrawal of gluten-containing cereal grains and associated carbohydrates.

GE low-gluten, celiac-safe wheat retains the carbohydrates of wheat but not the immunogenic proteins, which would help restore the microbiota. In fact, when low-gliadin RNAi wheat is fed to NCWS patients, there are significant changes in the microbiota aimed at restoring membrane permeability, increasing the butyrate-producing bacteria such as the *Roseburia* and *Faecalibacterium* genera (Haro et al., 2018). Butyrate is a short-chain fatty acid produced by the intestinal microbial fermentation of undigested dietary carbohydrates in the colon, playing a key role in the expression of the tight junction proteins towards preserving the gut barrier and reducing gut permeability (Wang et al., 2012). Moreover, these butyrate-producing genera are also known for having an anti-inflammatory capacity, and they could therefore play an important role in maintaining gut health (Sokol et al., 2008).

7.3 Societal health costs

A societal perspective for estimating the costs of CD includes the costs for the healthcare system, the increased costs from buying gluten-free food (see Section 7.1) and the costs related to work productivity loss, i.e. days of absence from work (Norström et al., 2021).

One of the most costly aspects for the healthcare system related to CD is the long diagnosis time. Although CD diagnosis times have been shortened due to the development of specific biomarkers, these can take up to 6 years, with numerous visits to primary care centres, hospital specialists, diagnostic tests, etc. burdening the healthcare system. A number of scientific studies have estimated these costs for the European health services, but they differ considerably according to country, age, different levels of complications and symptomatology and the time for diagnosis, from EUR 7 000 to EUR 45 000 in total or approximately EUR 1 000 per year to obtain a CD diagnosis (Greco et al., 2011b; Mearns et al., 2019; Mårild et al., 2020; Soler and Borzykowski, 2021).

The adoption impact of GE low-gluten, celiac-safe wheat on the market cannot be directly related to the costs burdening the healthcare system before diagnosis. Once the diagnosis is positive and the patient adheres to the GFD, the cost for the healthcare system declines but still remains higher than those compared of a

matched general population during the first 5 years (Mårild et al., 2020). Hospitalisation and medical visits post-diagnosis are expected to be reduced, except for those patients with poorly controlled CD or those non-compliant with the GFD, which will remain similar to before diagnosis (Norström et al., 2012; Mearns et al., 2019). Long et al. (2010) estimated a 29% reduction in outpatient costs in the year following diagnosis as well as an overall 39% reduction in the total medical cost of care. In fact, the quality of life measured as quality-adjusted life year (QALY) scores for CD patients was reported to improve after diagnosis and adequate GFD treatment (Norström et al., 2012). In the case of a positive CD diagnosis, the mainstream adoption of GE low-gluten, celiac-safe wheat could provide a new gluten-free alternative for improving the nutritional adequacy of the GFD treatment with the possibility to safely consume wheat and wheat-related foods. This, in turn, may help to avoid several medical visits and expenses post-diagnosis for CD individuals associated with nutritional deficiencies due to imbalanced GFDs or high levels of gluten sensitivity. However, data are scarce and inconsistent on the use and costs of healthcare in CD patients (Mårild et al., 2020), and this assumption is therefore difficult to quantify in terms of potential cost savings for the EU.

Besides, there are indirect costs related to time lost from work, school or other activities, and these also place a heavy burden on citizens and employers (Soler and Borzykowski, 2021). Individuals with CD may unintentionally consume foods with 'hidden' gluten that can make them physiologically sick for one or more days. Note that such sickness may occur unexpectedly. It may partly depend on an individual's compliance whether such illness occurs rarely or more frequently. This also depends on the individual's sensitivity to a certain amount of gluten; such sensitivity may change with time depending on the physiological constitution of the individual (age, condition, gender). Some CD adolescents may have periods of limited sensitivity to gluten. In Sweden, Bozorg et al. (2022) found that patients with prevalent CD had a mean of 42.5 lost workdays compared with 28.6 in the general-population comparators (an increase of over 14 lost workdays or 49%). While difficult to quantify, the mainstream adoption of the GE low-gluten, celiac-safe wheat, in combination with full compliance with a balanced GFD, could reduce the number of lost days at work or school as it has very low or no ability to cause adverse reactions to gluten.

Takeaway points from Section 7 – Social and health impacts

i) A lifelong gluten-free diet is the only treatment for celiac disease and non-celiac wheat sensitivity, but the price of gluten-free products is higher, on average 200% more expensive, than their gluten-containing counterparts and government support in buying gluten-free products is scarce in EU countries.

ii) The price premium for gene-edited low-gluten, celiac-safe wheat derived products is expected to be higher than those made from standard wheat, in the range of 10–30% more expensive, but this price premium is sufficiently compensated by the current prices for gluten-free products, on average 200% more expensive than gluten products.

iii) Gene-edited low-gluten, celiac-safe wheat could offer the possibility to safely consume wheat and wheat-related foods to celiac disease patients. It could also benefit people diagnosed with non-celiac wheat sensitivity. This would be an alternative to balance the potential fibre and protein nutrient deficiencies in gluten-free diets associated with wheat avoidance, and in turn alleviate negative effects on gut microbiota.

iv) Costs for the healthcare system and the costs related to work productivity loss are difficult to quantify due to a lack of available information. After diagnosis and following an adequate gluten-free diet treatment, medical care costs post-diagnosis for celiac disease patients, compared to diagnosis costs, are reported to be reduced by 39% and quality of life reported to improve. An increase of over 14 lost workdays is reported for celiac disease patients depending on an individual's compliance with the gluten-free diet and level of sensitivity to gluten.

v) The mainstream adoption of the gene-edited low-gluten, celiac-safe wheat, in combination with full compliance with a balanced gluten-free diet, could reduce the need for medical care post-diagnosis and lost days at work or school as it has very low or no ability to cause adverse reactions to gluten, but still provides comparable fibre, protein and energy to standard wheat.

8 Economic impacts

8.1 Changes to crop yields

Low-gluten, celiac-safe varieties of wheat obtained by gene editing are not commercially available. In addition, as they fall under GMO legislation in the EU, field trials face high time and administrative costs. Therefore, no comparative field data are available for these varieties in comparison to non-edited wheat varieties. However, trials in GMO greenhouses have been carried out in Cordoba (Spain) at the Institute for Sustainable Agriculture (F. Barro, unpublished data, 2022). In this trial, eight low-gluten, celiac-safe wheat lines edited using the CRISPR/Cas targeted mutation technology have been tested in the greenhouse using an alpha lattice design with three replications. Four lines corresponded to bread wheat and four to durum wheat, being compared with their corresponding standard non-edited bread and durum wheat lines (**Table 9**).

As shown in **Table 9**, three bread and three durum wheat varieties yielded amounts comparable to standard wheat. For the two lines yielding lower than the standard wheat, the standard deviation values were very high. Therefore, it is expected that these varieties will yield the same as the wheat from which they are derived under field conditions.

Table 9. Yield in GMO greenhouse conditions of four bread wheat and four durum gene-edited wheat varieties. The trial was carried out using an alpha lattice design with three replicates (plots) and ten plants per replicate.

	Wheat lines	**Grain yield (g / plot)** [(1)]	**SD**	**Percentage of standard wheat**
Bread wheat	**Standard wheat**	**433.6**	15.9	NA
	Low-gluten bread wheat V653	427.7	22.9	98.6
	Low-gluten bread wheat V657	445.8	58.5	102.8
	Low-gluten bread wheat V675	426.0	49.2	98.2
	Low-gluten bread wheat V730	376.9	103.3	86.9
	Average low-gluten bread wheat	**422.0**		**97.3**
Durum wheat	**Standard durum wheat**	**385.3**	42.9	NA
	Low-gluten durum wheat V520	331.0	85.6	85.9
	Low-gluten durum wheat V775	390.0	36.5	101.2
	Low-gluten durum wheat X467	419.3	30.7	108.8
	Low-gluten durum wheat X469	392.7	45.9	101.9
	Average low-gluten durum wheat	**383.7**		**99.6**

[(1)]; three plots with 15 plants per plot,
SD: Standard Deviation.

Source: own elaboration (F. Barro, unpublished data, 2022)

8.2 Changes to the use of fertilisers and plant-protection products

The effect of nitrogen (N) fertilisation during wheat cultivation on protein accumulation has been extensively studied (Zörb et al., 2018). In GE low-gluten wheat, fewer gliadins will be produced during endosperm development. Owing to physiological compensation during seed development, other non-gluten proteins will be produced at a higher rate (Altenbach et al., 2019; Bose et al., 2020), and therefore no net effect is expected on the required N fertilisation.

Sánchez-León et al. (2018) carried out gene editing on gliadin genes that are only expressed in the wheat endosperm during grain filling. These genes do not play a role in plant protection and are only expressed at the end of the agronomic cycle. Therefore, no changes in the use of fertilisers or plant-protection products are expected. Although no assays have been performed with the low-gluten, celiac-safe edited lines, assays have been performed with their counterparts obtained with RNAi technology, which has a similar protein -silencing profile to the GE wheat. The trial consisted of three N-fertiliser rates (120, 360, and 1 080 mg per pot) in combination with two S rates (8 and 30 mg per pot) using a randomised complete block design, with two plants per pot and a line for each treatment and three blocks, comprising six plants per line and treatment in total (García-Molina and Barro, 2017).

Results showed that the low-gluten lines do not differ in the use of N and S from that of the wild-type lines. Moreover, in a second trial, four wheat lines with the gliadins strongly down-regulated were characterised to

determine the effect of thermal stress and N availability on grain weight and quality, with a focus on gliadin and glutenin protein fractions (Marín-Sanz et al., 2020). Plant growth and grain filling were not affected in the low-gluten lines compared to the wild type (WT). All of these greenhouse data were corroborated with the field trial carried out under the notification B/ES/13/20, where no differences in the use of fertiliser were observed in comparison to standard wheat.

We can therefore conclude that there would be no differences in terms of fertiliser and plant-protection product use between low-gluten, celiac-safe wheat and conventional wheat.

8.3 Changes to management practices and enforcement for identity preservation

In general terms, the agronomic management of the GE low-gluten, celiac-safe wheat is the same as the management of standard wheat in the fields of any EU country. No additional new machinery and no additional agronomic work are required; therefore, fuel consumption and machinery costs can be considered to be equivalent to those of standard wheat.

The introduction of GE low-gluten, celiac-safe wheat on the market may require a system of segregation to preserve its GE identity along the entire supply chain, a system known as identity preservation (IP). In the GE low-gluten, celiac-safe wheat, the gliadin genes have been edited by introducing deletions that prevent further processing of the genes into the immunogenic grain proteins (Sánchez-León et al., 2018). These mutations have a recessive inheritance nature, and, therefore, the main modification in the agricultural management of low-gluten, celiac-safe wheat must be aimed at preserving its GE identity, preventing contamination with compatible crops, particularly other bread wheat varieties.

This would first assure the consumer that the product they are purchasing is indeed low-gluten, celiac-safe and avoid potential contamination and adverse health reactions. The development of IP systems is a measure to implement private standards and public policies, and these systems usually cover commodities with small volumes of production and trade. For instance, the EU has set rules regarding the labelling and the traceability of GM products, as well as the placing on the market of food and feed containing GM crops – Regulation (EC) No 1829/2003 and Regulation (EC) No 1830/2003 (Tillie and Rodríguez-Cerezo, 2015). As a result, a higher price than the non-segregated product is needed to cover the additional cost due to the implementation of this segregation.

Several procedures must be applied to segregate and preserve the identity of an agricultural product throughout the entire production, harvesting, handling and marketing process (Sundstrom et al., 2002; Gilissen and Smulders 2021b). These procedures may include, among others: ensuring co-existence in the field to avoid cross-contamination; cleaning and inspection of equipment and facilities for planting, harvesting and transport; certification and testing to detect traits introduced into plants through biotechnology; maintaining and handling IP documents and proper labelling of segregated products (Sundstrom et al., 2002; Tillie and Rodríguez-Cerezo, 2015). In order to maintain GE identity and avoid cross-contamination with other gluten-containing grasses, dedicated standard machinery is needed for the GE low-gluten, celiac-safe wheat, both for sowing and harvesting, as well as dedicated storage and processing sites (Maaß et al., 2019). In this respect, GE low-gluten, celiac-safe wheat should be treated like any other inherently gluten-free grain, seed and flour that can become contaminated with wheat, barley and/or rye while being harvested, transported and/or processed to avoid contamination (Thompson et al., 2010).

The most obvious route for transferring the genetic material between compatible organisms is through pollen dispersal. At field level, IP and segregation costs are expected to be low, especially if GE low-gluten celiac-safe wheat is the only crop grown under contractual agreement between farmers and buyers in which the buyers commit to purchasing the product from the farmers at fixed prices (Maaß et al., 2019), as is usually the case for such niche products (Bullock and Desquilbet, 2002). Wheat flowers are developed in such a way that they do not favour cross-pollination. Under natural conditions, the pollination of wheat is mainly achieved by self-pollination (99–97%). Wheat pollen is dispersed by wind, but being very heavy, its dispersal distance is short and it remains viable for a short period of time, usually no more than 30 minutes. In recent studies, Waines and Hedge (2003) considered that an isolation distance of 3–6 m is sufficient to avoid wheat gene flow. This is in agreement with another study which showed that outcrossing among wheat cultivars occurs at a frequency from 0.029–0.337 % at a 0 m distance, with decreasing outcrossing values at increasing distances, and with low outcrossing events detected up to 100 m from the pollen source (Loureiro et al., 2012). In case of other crops that cross-pollinate, such as maize and, in particular, for seed production, these were found to be very expensive to maintain the spatial and temporal isolation of fields, and to enforce

compliance by neighbouring farmers and elevators (Bullock and Desquilbet, 2002). In conclusion, wheat has a low risk of cross-contamination at field level since the pollination of wheat is mainly achieved through self-pollination, and therefore the co-existence measures will be less strict and less costly.

Potential costs relating to the cleaning and inspection of equipment and facilities for planting, harvesting and transport are usually covered by the contractual agreement between farmers and buyers with an increased and fixed price received by the farmer (Maaß et al., 2019). Bullock and Desquilbet (2002) estimated that the steps farmers take to clean planting and harvesting equipment are a small fraction of farmers' total segregation costs and, relatively small relative to the premium prices they receive for growing these products, usually more than 10% higher than conventional ones. The segregation cost for the IP system will be expected to be higher at other stages in the food chain after harvest, in particular, to grain handlers, millers, processors or traders, and those will in turn be reflected in increased consumer prices (Bullock and Desquilbet, 2002; Wilson and Dahl, 2002). Smulders et al. (2018) also reported that the steps required to develop and certify food safety of the Dutch gluten-free oat led to higher costs predominantly due to traceability and labelling requirements.

An example of products in the agri-food sector experiencing segregation costs is that of the soybean-derived products. In 2013, the consumer price premium for non-GM IP soybean meal was 30% higher, resulting in a 10% increase in producer price received by the farmer, operators tending to specialise production in order to reduce the segregation costs (Tillie and Rodríguez-Cerezo, 2015). Buckwell et al. (1999) found that for genetically modified crops of soybeans, maize and other oilseeds, IP costs fall broadly in the range of 5–15% of the farm gate price of the mainstream crop, but these costs will largely vary according to the nature and the value added of the trait to the processor and the consumers. If the direct beneficiary of the modifications is the purchaser of the product, which may be the final consumer or a food processor, some derived costs are expected to be transmitted to them.

Based on the literature reviewed above, we can conclude that given the low risk of cross-contamination since wheat self-pollinate, a small cost will be expected for farmers to segregate and preserve the GE low-gluten celiac-safe wheat at field level. Minor costs are also expected for cleaning and inspection of equipment and facilities for planting, harvesting and transport, and these are usually covered by an increased price received by the producer at the farm gate of about 10% more than standard wheat. Segregation costs are expected to be higher after harvest in other phases of the food chain, for example, to grain handlers and processors. These will be transmitted to consumers as higher prices for purchasing the GE low-gluten, celiac safe wheat and derived products; consumer prices will range from approximately 5–30% more expensive than standard wheat. Nevertheless, these increased costs are minor in comparison with the current costs of gluten-free products that are, on average, 200% more expensive than their gluten-containing counterparts.

8.4 Changes to farm gross margin[13]

Potential changes are expected in the costs and benefits of farmers adopting the GE wheat variety with reduced gluten safe for celiac patients. Observational data on impacts on production and price are not available since the product is not yet on the market. However, Jones et al. (2017) elicited, via expert consultation, the likely effect of adopting GE low-gluten, celiac-safe wheat on production costs and product prices. Experts elicited changes in the seed prices of GE low-gluten, celiac-safe wheat to increase by 5.47%, and an expected producer price of 9.5% more than conventional wheat. In fact, the percentage of increase of the producer price at the farm gate is very similar to those reported by Bullock and Desquilbet (2002) and Tillie and Rodríguez-Cerezo (2015) for crops that need an IP system. Maaß et al. (2019) also reported that additional costs for cultivating the GE celiac-safe wheat will arise from higher seed prices and from the separate harvesting, transporting and storage of celiac-safe wheat. The last one usually covers a higher and fixed producer price guarantee by a contractual agreement between farmers and buyers in which the buyers commit to purchasing this production.

We have estimated the potential impact of the adoption of the GE low-gluten, celiac-safe wheat on gross margins by comparing with a standard farm budget for conventional common wheat (**Table 10**). Gross margins are calculated as outputs minus production operating costs. The most recent and detailed EU economic data on common wheat were available from and gathered by the European Commission – EU FADN

[13] The farm gross margin is an estimate of the income generated by farming enterprises and is the value of the output less the variable costs directly attributable to the enterprise. It does not include fixed or overhead costs such as depreciation, interest payments, rates, or permanent labour. The gross margin budgets are intended to provide a guide to the relative profitability of similar enterprises and an indication of management operations involved in different enterprises. Gross margins are used to assess the technical and economic efficiency of conventional farm businesses.

(farm accountancy data network) for the period 2016–2018. The results reported in the sections above have not indicated crop yield differences or differences in the use of fertilisers and plant protection between conventional and low-gluten, celiac-safe wheat. No significant additional costs have either been pointed out from agronomic management or to segregate and identity-preserve the GE low-gluten, celiac-safe wheat compared to those of standard wheat at field level. Therefore, the impact parameters for the adoption of GE low-gluten, celiac-safe wheat were set up according to those elicited by Jones et al. (2017) on the changes in prices (outputs) and costs (seeds).

The impacts of GE low-gluten, celiac-safe wheat adoption were matched with their counterparts' categories in the FADN annual farm budget (i.e. outputs and seed costs). The gross margin impact is then calculated as the difference between the gross margin of conventional wheat and the gross margin once the effects of GE low-gluten celiac-safe wheat adoption are accounted. Our results show that the adoption of GE low-gluten wheat could increase the gross margin per hectare by, on average, 30% compared to the conventional wheat for this specific niche market product.

Table 10. Impact of the adoption of gene-edited (GE) low-gluten, celiac-safe wheat on the gross margin in the EU.

Farm budget parameters (€/ha)	**Annual EU farm budget for common wheat (€/ha)***			**Elicited impact of GE low-gluten, celiac-safe wheat (%)****	**Impact of adoption of GE low-gluten, celiac-safe wheat (€/ha)**		
	Min	Avg	Max		Min	Avg	Max
Outputs (O)	878	975	1 066	+ 9.5% (via price premium)	961	1067	1 167
Production costs (PC)	660	681	705		664	685	709
Specific costs							
Seeds	71.1	73.3	76.3	+ 5.47%	75.0	77.3	80.5
Fertilisers	163.4	157.0	154.4	No effect	-	-	-
Crop protection	122.7	124.6	126.4	No effect	-	-	-
Water	0.9	1.1	1.2	No effect	-	-	-
Other specific costs	14.7	15.8	16.5	No effect	-	-	-
Non-specific costs							
Motor fuels and lubricants	70.4	77.5	88.0	No effect	-	-	-
Machines, building upkeep	62.6	65.9	69.1	No effect	-	-	-
Contract work	59.1	62.7	65.2	No effect	-	-	-
Energy	10.8	11.6	12.6	No effect	-	-	-
Other direct costs	84.7	91.0	95.0	No effect	-	-	-
Gross margin (O-PC)	**218**	**294**	**361**		**297**	**383**	**458**

*Data (2016–2018) from FADN – EU cereal farms report based on 2017 data EU-UK;
**Data collected from Jones et al. (2017).

Source: Own elaboration.

8.5 Labelling and information availability

Low-gluten food products currently require separate labelling. Gluten-free products are labelled as such when they meet the requirement of containing less than 20 mg/kg gluten threshold and labelled as very low gluten when the content is less than 100 mg/kg according to Regulation (EU) No 828/2014. Gluten in foods can be determined by legally approved tests (the R5 ELISA assay [of R-Biopharm]) for the detection of contaminating gluten (Valdés et al., 2003). Other tests, such as the G12 assay (Morón et al., 2008), can also reliably quantify the amount of contaminating gluten, but the G12 cannot, e.g. distinguish oat avenins from wheat gliadins in contrast to newer monoclonal antibody tests (Sajic et al., 2017).

Bustamante et al. (2017) carried out a study to explore the presence of gluten contamination in cereal-based products labelled as gluten-free for the above-20 mg/kg threshold. During the period 1998–2002, over 13% of products labelled as gluten-free contained gluten above the 20 mg/kg threshold; the situation improved in the period 2013–2016 with only 3% products, these results proving that testing remains a requirement.

Many products on the market contain 'hidden' gluten or are incorrectly labelled or not quantified regarding their gluten content (Gilissen and Smulders, 2021a). For instance, the declaration on the product label of the origin, of e.g. the glucose syrup, is not always adequate. The presence of such additives or ingredients is no issue to healthy individuals, but it hinders the compliance of diagnosed CD people with a GFD. Non-compliance has health-impairing effects on susceptible persons suffering from wheat-related diseases (Freeman, 2017).

In the case of GE low-gluten, celiac-safe wheat, the R5 ELISA assay will not distinguish between gluten with and without CD epitopes, and low-gluten, celiac-safe wheat will therefore likely contain more than 20 mg/kg and 100 mg/kg gluten, even when it is safe for CD patients. The immunoreactivity of the GE low-gluten, celiac–safe wheat is reduced by 85%, but the gluten content is above the two gluten thresholds 20 mg/kg and 100 mg/kg as set in the regulation on labelling gluten-free and very low gluten, respectively. Under the current legislation, GE low-gluten, celiac-safe wheat will face challenges in being labelled as such and re-evaluating the current regulations may be a lengthy process. It will also require the development of a quantitative detection method for CD epitopes. New health claims (hypoimmunogenic, CD-safe, etc.) must be scientifically assessed by the European Food Safety Authority (EFSA) and authorised by the European Commission and Member States before a clear and unambiguously defined 'safe gluten' label can be used on the EU market.

8.6 Competitiveness of the EU agri-food system

The introduction of the GE low-gluten, celiac-safe wheat on the market may bring beneficial outcomes in terms of competitiveness of the EU agri-food system for the different stakeholders of the wheat value chain by producing high value-added products which can be safely consumed by CD patients (Maaß et al., 2019). The potential innovation opportunities for the different stakeholders of the wheat value chain are listed in **Table 11**.

Table 11. Innovation opportunities from the adoption of gene-edited (GE) low-gluten, celiac-safe wheat for the different stakeholders of the wheat value chain.

Wheat chain target	Product	Outcomes
Farmers	Seeds for sowing	Expand the catalogue of varieties with hypoallergenic wheat with high added value. Profitability is guaranteed under contracts to maintain the GE identity.
	Crop production	The increase in direct wheat sales from farmers to processors (e.g. millers) due to contract farming that also guarantee the purchase of their production at fixed and high prices by the contractual partners.
Millers and starch producers	Grain to produce hypoallergenic flour	The flour is a product not only for bakers but also on the shelves of supermarkets and specialised stores. Do it yourself. The possibility to yield a higher market price. The possibility of saving the costs of applying special techniques to produce gluten-free wheat starch.
Bakers	Flour for bread-based products	New specialised bakeries. Tasteful hypoallergenic bread wheat and other baker-derived products. The possibility to yield a higher market price.
Other industries	Flour or semolina for other products and commodities	New products such as pizzas, pasta, biscuits, etc., with a real wheat taste. The possibility to yield a higher market price.
End consumers	End consumers will benefit from all new products, with a particular impact on the domestic economy and health. In particular, CD or NCWS patients and relatives who will have more alternatives for low immunogenic food products that may be more affordable and nutritious when following a GFD.	

Source: own elaboration based on Maaß et al. (2019).

Potential end-consumers will be those who want to reduce gluten-wheat consumption. To date, a GFD is the only treatment for people affected by CD, and it is also recommended for other GRDs such as NCWS or those affected by irritable bowel syndrome (IBS). However, in recent years, people not suffering from any GRDs are also embracing the GFD. These people can be categorised in two groups: (i) family members of people with CD who follow a GFD to avoid food contamination at home and (ii) other people who do not have any specific symptoms but follow a GFD for whatever reasons.

In 2015, the Nielsen report on healthy eating reported that 21% of participants rated gluten-free as a very important health attribute, ranging from 16% in the EU to 32% in Latin America (Nielsen, 2015). Moreover, 23% of participants were very willing to pay a premium for gluten-free products, ranging from 15% in the EU to 31% in Latin America (Nielsen, 2015). In Italy, approximately 6 million people follow a GFD voluntarily (Xhakollari et al., 2021), which is 10 times more than the 1% of the population affected by CD. Similar results were reported in a survey of French adults without CD: 10.31% of the participants declared avoiding gluten (Perrin et al., 2019). In contrast, a recent survey of dietary attitudes towards gluten in the UK showed that 3.7% regularly follow a GFD, although one third of the general population perceived that they have symptoms related to the ingestion of gluten (Croall et al., 2019).

Therefore, different trade scenarios have to be taken into account. An initial scenario considering the incidence of pathologies related to gluten consumption. Second, a scenario including the family units that follow the GFD to avoid cross-contamination. Lastly, a third scenario for the general population expressing their predisposition to reducing gluten consumption because they consider it unhealthy, who value the GFD positively and would even pay extra for it. On the other hand, the current wheat market would not be affected since people following a GFD do not consume wheat. In other words, the impact of the introduction of GE low-gluten, celiac-safe wheat on exports and imports would mainly depend on whether it is grown in the EU (with an increase in exports) or in other non-EU countries (with an increase in imports) (**Table 12.**). Assuming a prevalence of 2.2–12.4% of CD, NCWS and WA patients willing to purchase the new product with a price premium 5–30% more expensive than standard wheat (see Section 8.3), two potential trade impact scenarios could be expected: i) GE low-gluten, celiac-safe wheat is cultivated in the EU and exports volumes could therefore be expected to increase in the range of EUR 0.5–2.6 billion; ii) GE low-gluten, celiac-safe wheat is cultivated in non-EU countries but not in the EU and import volumes could therefore be expected to increase in the range of EUR 0.1–0.5 billion.

Table 12. The impact of GE low-gluten, celiac-safe wheat on exports and imports depending on three levels of adoption considering the prevalence of gluten-related pathologies (GRPs). The prevalence of GRPs was as per Sharma et al., 2020.

Adoption of GE low-gluten, celiac-safe wheat (%)		EU wheat exports [a] in EUR 1 000		EU wheat imports [b] in EUR 1 000	
		Min. price premium (+ 5%)	Max. price premium (+ 30%)	Min. price premium (+ 5%)	Max. price premium (+ 30%)
Low [1]	+ 2.2%	6 558 306	8 087 761	1 387 898	1 711 568
Medium [2]	+ 8.4%	6 937 611	8 467 066	1 468 168	1 791 839
High [3]	+ 12.4%	7 182 324	8 711 779	1 519 956	1 843 626

[1], Considering only the prevalence of celiac disease (CD) of 1.4%, the lower range of non-celiac wheat sensitivity (NCWS) of 0.6% and the lower range of wheat allergy (WA) of 0.2%.

[2], Estimating a cumulative CD of 1.4%, the upper range of NCWS of 6%, the upper range of WA of 1%.

[3], All medium adoption plus 4% non-suffering gluten-related pathologies (GRPs) but following a gluten-free diet (GFD).

[a], GE low-gluten, celiac-safe wheat is regulated and produced in the EU. Average EU export value (2012–2022) of conventional wheat is EUR 6.1 billion.

[b], GE low-gluten, celiac-safe wheat is regulated in non-EU countries. Average EU import value (2012–2022) of conventional wheat is EUR 1.3 billion.

Source: own elaboration.

Takeaway points from Section 8 – Economic impacts

i) There would be no differences in terms of crop yields, fertiliser and plant-protection-product use and agronomic management (i.e. new machinery, extra labour or fuel consumption) between low-gluten, celiac-safe wheat and standard wheat.

ii) There is a low risk of cross-contamination, since wheat self-pollinates, and hence a small cost will be expected for farmers to segregate and preserve the gene-edited low-gluten, celiac-safe wheat at field level. Minor costs are expected for cleaning and inspection of farm equipment and facilities. Higher segregation costs will be incurred by grain handlers and processors and those, in turn, translated into consumer prices from 5–30% more expensive than standard wheat. These consumer costs are still lower than the current costs of gluten-free products (on average, 200% more expensive).

iii) Considering higher prices for purchasing seeds of gene-edited low-gluten wheat of about 5% and a higher producer price of 10% received by the farmer for this product, the farm gross margin could increase on average by 30% per hectare compared to the conventional wheat for this specific niche market product.

iv) The immunoreactivity of the gene-edited low-gluten, celiac–safe wheat is reduced by 85%, but the gluten content is above the two gluten thresholds 20 mg/kg and 100 mg/kg as set in the regulation on labelling gluten-free and very low gluten, respectively (Regulation (EU) No 828/2014). Under the current legislation, gene-edited low-gluten, celiac-safe wheat will face challenges in being labelled as such.

v) The cultivation of GE low-gluten, celiac-safe wheat in the EU may increase the competitiveness of the agri-food system by increasing export volumes in the range of EUR 0.5–2.6 billion, and avoid high import volumes of this product if eventually cultivated in other parts of the world but not in the EU (in the range EUR 0.1–0.5 billion).

9 Environmental impacts

With regard to the environment, no negative or positive impacts are expected of GE low-gluten, celiac-safe wheat in comparison to regular wheat.

As explained in Section 8.2, although the composition of the gliadin proteins in low-gluten, celiac-safe wheat is modified, and possibly the amount of gliadin proteins is reduced, it is not expected that the total amount of protein in the grain will reduce significantly since compensation will occur during grain development through increased synthesis of mostly non-gluten proteins (Altenbach et al., 2019; Bose et al., 2020). Therefore, the N requirements of the wheat crop for protein production in its seeds will remain the same, and thus there is no effect on fertilisation practices during cultivation. No differences in the use of fertiliser in comparison to standard wheat were observed at different trials with wheat plants modified with RNAi technology, which has a similar protein-silencing profile to the GE low-gluten, celiac-safe wheat (García-Molina and Barro, 2017; Marín-Sanz et al., 2020)

Takeaway points from Section 9 – Environmental impacts

i) No negative or positive impacts are expected of gene-edited low-gluten, celiac-safe wheat in comparison to regular wheat, since the N requirements of the wheat crop for protein production in its seeds will remain the same with no effect on fertilisation practices during cultivation.

10 Conclusions

Wheat is one of the main crops grown throughout the world. It covers 240 million hectares and represents a quarter of the total cereal production, with about 760 million tonnes produced in 2020. Soft wheat represents roughly 90–95% of the total production, and durum wheat only 5–10%, but the Mediterranean Basin is the largest production area in the world. For human consumption, the main uses for which wheat is currently destined are the manufacture of pasta in the case of durum wheat, and baking, cakes and breakfast cereals in the case of common wheat. However, wheat consumption is associated with several pathologies, which have been increasing extensively in recent years and could affect up to 12% of the European population.

Low-gluten soft wheat (*Triticum aestivum ssp. aestivum*) that is safe for celiac individuals can be developed through gene editing. GE low-gluten, celiac-safe wheat is characterised by the inactivation or elimination of the protein fragments (epitopes) that trigger CD in genetically predisposed individuals. The editing concerns the genes for gliadin-type gluten proteins which carry the most important (dominant) CD epitopes, leaving the genes for glutenin-type gluten proteins that are responsible for the food technological and dough-making qualities of wheat largely undisturbed. Due to the structural complexity of the gene coding for gliadins, the high copy number of these genes, segregating in blocks, it is extremely difficult to apply conventional breeding techniques to obtain comparable varieties of low-gluten wheat safe for celiac people.

The GE low-gluten, celiac-safe wheat is a new product for which there is no equivalent on the market. This hypoallergenic wheat has very low or no ability to cause the adverse reactions in human health that common wheat or other gluten-containing cereals cause, but it retains the proteins responsible for the viscoelastic properties of dough. The introduction of such GE low-gluten, celiac-safe wheat varieties on the market will have an economic and social impact on the entire wheat value chain, but if the technology can also be easily applied to other gluten-containing cereals such as rye and/or barley, impacts will be expected on their value chain.

GE low-gluten wheat is thus designed to be safe for CD patients, about 1% of the world population, so food produced with GE low-gluten, celiac-safe wheat would be an alternative as well as an addition to the gluten-free products which currently are the only treatment for this disease. The large group of NCWS people, who prefer not to consume wheat and gluten for presumed reasons of health improvement, may be interested in GE low-gluten, celiac-safe wheat products as well. It should be considered that approximately 85% of CD patients are not diagnosed or misdiagnosed because of the complexity of the disease.

Clinical trials carried out with an equivalent wheat product developed by RNAi have previously shown that it is well tolerated by NCWS patients, causing no different symptoms from the GFD. On the contrary, it reverses the negative effects observed in the intestinal microbiota when following a GFD, favouring a less inflammatory phenotype that could be related to the increase in *Faecalibacterium*, an anti-inflammatory genus, and the reduction in *Bacteroides,* a proinflammatory genus, thereby improving the gut inflammation characteristics of NCWS patients. In addition, oral consumption of bread from this RNAi wheat line with strongly silenced gliadins elicited no immunogenic response in a pilot study with CD patients.

Ensuring food security, nutrition and public health, making sure that everyone has access to sufficient, safe, nutritious and sustainable food is the main aspect to which GE low-gluten, celiac-safe wheat can contribute. On average, gluten-free products have a price 200% higher than their gluten-containing counterparts and little financial support is offered to cover these costs for CD patients and relatives. Even if GE low-gluten, celiac-safe wheat were to be marketed at a significant price premium for consumers (5–30% higher than standard wheat products), the savings compared to the costs of gluten-free diets nowadays would still be substantial. The increasing prevalence rate of gluten-related pathologies implies a higher cost to the EU health system and therefore for the economy and society. Other aspects difficult to quantify are the indirect costs related to time lost from work, school or other activities that also place a heavy burden on citizens and employers. The mainstream adoption of the gene-edited low-gluten, celiac-safe wheat, in combination with full compliance of a balanced GFD, could reduce the need for medical care post-diagnosis and the lost days at work or school as it has very low or no ability to cause adverse reactions to gluten, but still provides comparable fibre, protein and energy to standard wheat. Data are scarce and inconsistent on the use and costs of healthcare in CD patients, and this assumption is therefore difficult to quantify in terms of potential cost savings for the EU.

In terms of crop yield, GE soft and durum wheat varieties yielded amounts comparable to that of standard wheat in GMO greenhouse trials. Therefore, it is expected that these varieties will yield the same as the wheat from which they are derived under field conditions. As gene editing was carried out on gliadin genes that are only expressed in the wheat endosperm during grain filling, no changes or additional costs in the use of

fertilisers or plant-protection products are expected. Agronomic management will be the same as for standard wheat and no additional costs for new machinery, labour or fuel consumption are expected. IP costs are expected to be minimum at field level due to the self-pollinating nature of wheat, reducing measures to avoid cross-contamination. The likely effect of adopting GE low-gluten, celiac-safe wheat on seed prices is an increase of 5.47%, and an expected price premium of 9.5% more than conventional wheat. Therefore, an increase is expected in the gross margin for farmers of approximately 30% more per hectare than conventional wheat for this specific niche market product. Other segregation costs incurred by grain handlers or processors are likely to be reflected in consumer prices in a range 5–30% more expensive than for processing standard wheat products. The cultivation of GE low-gluten, celiac-safe wheat in the EU may increase the competitiveness of the agri-food system by increasing export volumes in the range of EUR 0.5–2.6 billion, and avoid high import volumes for this product if eventually adopted in other parts of the world but not in the EU (in the range of EUR 0.1–0.5 billion).

With regard to labelling, under the current EU regulation (Regulation (EU) No 828/2014), gene-edited low-gluten, celiac-safe wheat may not be labelled as gluten-free or very low gluten, even if it is eventually displayed as safe for CD patients. Immunoreactivity is reduced by 85% but the gluten content is above the two gluten thresholds 20 mg/kg and 100 mg/kg, as set in the regulation on labelling gluten-free and very low gluten, respectively. These new wheat lines would require the development of a quantitative detection method for CD epitopes. New health claims (hypoimmunogenic, CD-safe, etc.) must be scientifically assessed by the European Food Safety Authority (EFSA) and authorised by the European Commission and Member States before a clear and unambiguously defined 'safe gluten' label can be used on the EU market.

No negative or positive environmental impacts are expected of low-gluten, celiac-safe wheat in comparison to regular wheat because the nitrogen requirements of the GE low-gluten, celiac-safe wheat crop will remain the same, and there will thus not be any effect on fertilisation practices during cultivation.

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List of abbreviations and definitions

EU	European Union
EC	European Commission
JRC	Joint Research Centre
DG SANTE	Directorate-General for Health and Food Safety
EFSA	European Food Safety Authority
NGTs	New genomic techniques
GFD	Gluten-free diet
CD	Celiac disease
WA	Wheat allergies
WDEIA	Wheat-dependent exercise-induced anaphylaxis
NCWS	Non-celiac wheat sensitivity
PCR	Polymerase chain reaction
GE	Gene-edited
GMO	Genetically modified organism
SDN	Site-directed nuclease
CRISPR	Clustered regularly interspaced short palindromic repeats
HMW	High molecular weight
LMW	Low molecular weight
EMA	Endomysial antibody
tTG	Tissue transglutaminase
Ab	Antibodies
AEA	Anti-endomysial antibodies
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgE	Immunoglobulin E
DGP	Deamidated gliadin peptide
AGA	Anti-gliadin antibodies
HLA	Human leukocyte antigen

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