Reduced-Immunogenicity Wheat Now Coming to Age

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Abstract This chapter focuses on the gluten-induced dietary disorders, conceived therapies, and hysteria associated with wheat/gluten consumption. Gluten proteins are one of the most widely consumed dietary proteins in the world and also the sole source of nutrition to many, especially those dwelling in developing countries. Prevalence of these disorders has compounded in the last couple of decades due to change in lifestyle, which includes an adaptation of the gluten-laden diet and excessive use of antibiotics in childhood with a suppressive effect on the development of the immune system and the improvements in diagnostics. Several therapies have been sought, but none of them has proven perfect. The issues associated with gluten-induced disorders and existing and possible therapies and prospects will be discussed under the following headings and subheadings.

Keywords Wheat · Celiac disease · Wheat allergy · Non-celiac wheat sensitivity · Reduced-immunogenicity wheat · Epitopes

1 Introduction

Wheat is a global staple and the second most-produced crop in the world after corn. In terms of calorific and nutritional output, wheat stands even before corn (Langridge 2017). It is the primary source of plant proteins in the most resource-deprived and

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populated parts of the world (Langridge 2017). The common wheat is an outcome of the human selection of a natural hybrid of domesticated tetraploid wheat (emmer) and a wild diploid goat grass relative (Shewry 2019). Hence it is relatively a young species (Venske et al. 2019), which is evolving slowly under the intense selection pressure for enhanced yield and end-use quality. The intensive breeding as a consequence has narrowed the genetic base of elite wheat germplasm and also reduced the possibility to select for specific traits.

Gluten is a complex of seed storage proteins with unique structural and compositional properties (Rustgi et al. 2019). These proteins consist of repetitive tracts of proline and glutamine residues, which confer them unusual resistant to digestion. However, this unique composition of gluten proteins is inherently beneficial to the plant, as it allows dense packing of nitrogen in grains for use during germination and by making the grain less attractive to insect pest due to poor digestibility (Shewry 2019).

In the last few decades, a significant increase in the number of cases with gluten-associated disorders was reported. This increase in the number of cases with gluten-associated disorders could be attributed to many factors: (i) a dramatic change in the eating habits, which could be witnessed by the spread of celiac disease to areas where wheat is not grown or consumed historically; (ii) increasing adaptation of the plant-based diets and also fast foods enriched in gluten, due to affordability, convenience, durability in transport, etc.; (iii) better diagnostics and increasing public awareness; and (iv) however controversial, the underdeveloped immune system due to excessive use of antibiotics (Rustgi et al. 2019).

Since the gluten-associated disorders affect about 7–10% of the world population, and this number is increasing, a permanent and more affordable solution should be sought. Therefore, to promote research in this area, an effort has been made to summarize the current knowledge in this field of research.

2 Wheat Gluten Proteins

Gluten proteins contain two major fractions, the monomeric gliadins (30–80 kDa) and the polymeric glutenins (up to 20 MDa) (Delcour et al. 2012). Gliadins are monomeric and have a more or less globular shape (Veraverbeke and Delcour 2002). They are soluble in aqueous ethanol and thus classified as prolamins (Osborne 1907). An important difference between glutenins and gliadins is that the latter have no free sulfhydryl (SH) groups (Shewry et al. 1986).

Gliadins can be further subdivided in three groups: α-, γ-, and ω-gliadins (Shewry et al. 1986; Balakireva and Zamyatnin 2016; Shewry 2019). Only the first two types have intramolecular disulfide (SS) bonds (respectively, 6 and 8 in α- and γ-gliadins). The intramolecular SS bonds are found in highly conserved regions, which makes them unaccessible for SH/SS exchange reactions at room temperature (e.g., during dough mixing) (Muller and Wieser 1995, 1997). However, during heat treatments, they can become involved in intermolecular SS bonds (see below). Omega-gliadins

have no cysteine amino acid residues and are believed to have a stiff coil structure (Shewry et al. 2009). In general, gliadins are rich in glutamine, proline, asparagine, and arginine (Muller and Wieser 1997).

Glutenins, due to their large size, are not soluble in mild media. They consist of different glutenin subunits (GS), the structures and solubility of which are comparable to those of gliadins, but they do contain free SH groups. With their SH groups, the GS form intermolecular SS bonds which are at the basis of the polymeric glutenin structure in mature wheat. There are two types of GS: low molecular weight-GS (LMW-GS) and high molecular weight-GS (HMW-GS).The LMW-GS show high similarities with α- and γ-gliadins (but as stated above, they do have free SH groups) and can be further subdivided in different subcategories (Delcour et al. 2012).

HMW-GS also have free SH groups. They are important contributors to the elasticity of gluten networks, even if they only occur in small numbers (Gianibelli et al. 2001). These subunits are rich in glutamine, proline, and glycine (Shewry et al. 1992).

The huge variation in both the amount and the occurrence of the different types of gliadins and of GSs is an important element at the base of the distinction between good and poor bread making quality wheat (Veraverbeke and Delcour 2002).

A wide range of proteins with similarities to gluten proteins at sequence or structure levels were identified. These proteins are collectively grouped under the "prolamin-superfamily". These proteins generally show homology to gluten proteins in the non-repetitive cysteine-rich N- and C-terminal domains and perform diverse metabolic or structural roles in grains or other plant parts. Small but some effect of these proteins on the processing quality was also reported (Shewry 2019). Among these proteins, amylase trypsin inhibitors (ATIs) and lipid transfer proteins (LTPs) were shown to be involved in gluten-associated disorders (Juhász et al. 2018).

3 Gluten-Associated Disorders

A large number of epitopes belonging to all families of gluten proteins have been shown to elicit various reactions in different individuals, which correspond with their genetic constitutions. In other words, different celiac patients are sensitive to different gluten proteins (Koning 2012). Despite extensive efforts, the repertoire of epitopes is still incomplete. So far, 356 genes with known epitopes and an additional 472 potential allergen genes were assigned to the wheat genome. Of these 356 genes, 226 belong to the prolamin gene superfamily (Juhász et al. 2018). Of all the epitopes with a known immune response (determined based on the IFNγ-ELISpot assay), 25 mapped to the HMW glutenin subunits, and only 1 of these 25 epitopes was shown to trigger a medium immune response (SFU value between 10 and 20). The rest of the epitopes were reported to have weak immune reactions (SFU values of less than ten) (Juhász et al. 2018).

Similarly, all epitopes that mapped to sequences of the LMW glutenin subunits were known to have a weak immune response. It suggests that all families of gliadins (α-, γ-, and ω-gliadins) are highly immunoreactive and especially the one

mapping to D- and A-subgenomes of wheat and related species. The epitopes that map to the repetitive domain in the gliadin sequences were more immunoreactive than the one mapping to the C-terminal non-repetitive domain. The epitopes rarely mapped to the N-terminal non-repetitive domain of prolamin sequences (Tye-Din et al. 2010; Juhász et al. 2018).

As mentioned earlier, gluten intake in sensitive individuals could manifest diverse symptoms – cutaneous, gastrointestinal, or neurological – and these reactions could be from mild to fatal (Brouns et al. 2019). The symptoms can be widely classified into celiac disease, wheat allergy, and wheat sensitivity (Sapone et al. 2012) (Fig. 1). The manifestation of celiac disease in an individual depends primarily on the three factors: (i) the environmental trigger, which is exposure to gluten and related proteins of the prolamin superfamily (Rustgi et al. 2019; Shewry 2019); (ii) gut abnormalities, i.e., leaky intestine (Fasano 2009); and iii) genetic predisposition, i.e., the presence of susceptibility alleles (Fig. 2) (Brouns et al. 2019).

The adaptive immune system mediates celiac disease (gluten intolerance). If left untreated, it induces the production of antibodies against the indigestible gluten peptides and also against a housekeeping enzyme, tissue transglutaminase 2 (tTG2) (Brouns et al. 2019). The tTG2 is also responsible for chemical modification of gluten peptides, which facilitates their recognition as foreign entities by the immune system. But the faulty immune system in genetically predisposed individuals recognizes tTG2 as an enemy and triggers an autoimmune response (Osorio et al. 2012).

Given the parallelism between the gluten peptides and living (bacteria) or non-living (prions and viruses) pathogens, Dr. Chaitan Khosla of Stanford University, a pioneer in the oral enzyme therapy for celiac disease considered gluten peptides as the non-replicating pathogens. Since the gluten peptides like pathogens evade

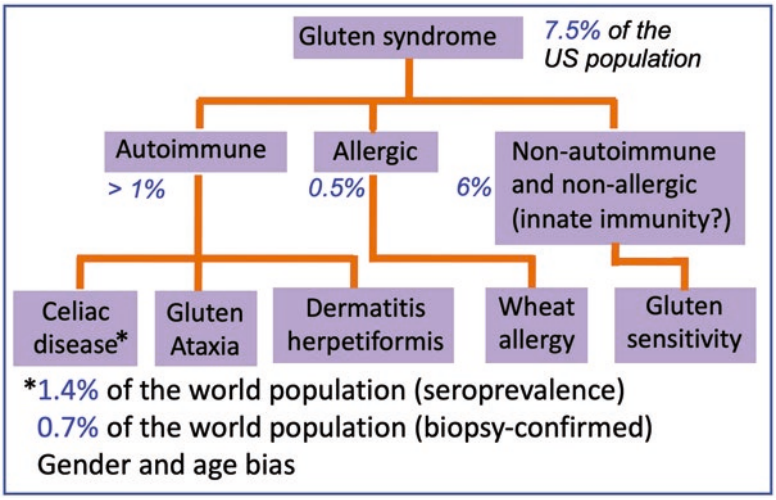

Fig. 1 Gluten-associated dietary disorders and the present US population affected by these disorders. (Modified from Sapone et al. 2012)

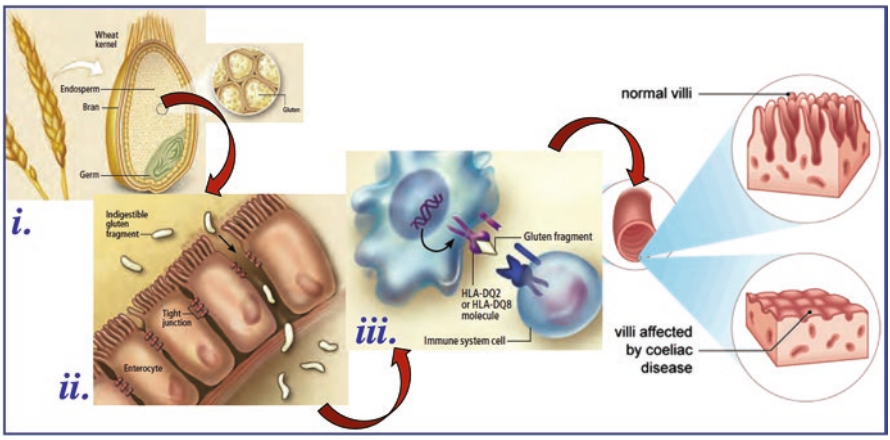

Fig. 2 A trio of factors responsible for celiac disease. (Modified from Fasano 2009)

"host" defenses by escaping digestion through gastrointestinal enzymes, invade intestinal epithelium, take a more aggressive form after chemical modification by tTG2, and trigger the cascade of reaction leading to the intestinal and extraintestinal symptoms (Bethune and Khosla 2008). As stated above, the first reaction initiated by gluten peptides gets amplified to take a more aggressive form of an autoimmune disorder upon recognition of tTG2 by the immune system as antigen to develop autoantibodies against it, which cause damage to the intestine and other tissues. The second kind of reaction is a wheat allergy, which involves both innate and adaptive immune systems. It is a quick reaction against the external allergen within a couple of minutes to hours after ingestion, which results in various symptoms including dermatitis, anaphylaxis, and various other symptoms (Tatham and Shewry 2008). The third kind of reaction known as gluten sensitivity is very complex and least understood. It involves the innate immune system, and the symptoms associated with this reaction are quite diverse, ranging from fatigue, distress, depression, migraines to gastrointestinal symptoms (Sapone et al. 2012). The trigger to the latter reaction is yet unknown and has been recently suggested to be fermentable oligo-, di-, monosaccharides, and polyols (FODMAPs) that coexist with gluten in wheat grains (Skodje et al. 2018; Verbeke 2018; Brouns et al. 2019).

To sum up, wheat and derived products elicit many diet-induced health issues in more than 7.5–10% of the population in some countries (Rosella et al. 2014; Aziz et al. 2015; Golley et al. 2015). In particular, the celiac disease alone affects more than 71 million individuals around the globe (i.e., ~1% of the world population), which makes it one of the most devastating disorders of the gastrointestinal tract (Bai et al. 2013). There is no known therapy for these disorders other than the strict lifelong adherence to wheat (gluten)-exclusion diet, which has associated side effects (Rustgi et al. 2019). Given the high prevalence of gluten-induced disorders in all studied populations throughout the globe, a large number of studies have been dedicated to finding more effective therapies for these disorders.

4 Gluten Threshold

A gluten-free diet does not necessarily signify "zero gluten" as low levels of gluten are generally tolerated by gluten intolerant and sensitive individuals. Establishing a threshold for gluten intake is of high interest to regulatory bodies of different countries around the globe and also to develop methods of precise quantification of gluten from various commodities. After a large number of studies conducted globally and the meta-analysis of the credible studies, a daily intake of less 50 mg gluten for an extended period was found to be generally tolerated by celiac patients. Therefore, a threshold of 20 ppm (20 mg in a kg), which restricts the daily intake of gluten from "gluten-free" food far below 50 mg, was considered safe. This decision on the threshold depended not only on the maximum tolerable dose of gluten in food but also on the amount of "gluten-free" product(s) consumed daily in different parts of the world. In this respect, the current limit of 20 ppm allows a safety margin for variation in the gluten sensitivities and dietary habits of different patients. Therefore, now, most of the countries around the world have adopted the ≤20 ppm limit recommended by the Codex Alimentarius Commission (Brouns et al. 2019).

5 Gluten Detection Methods

Over the years, several gluten detection and quantification methods have been developed and tested using the gluten-containing and/or spiked samples. These methods can be grossly partitioned into immunological and non-immunological methods. The non-immunological methods rely on the physical and biochemical properties of the gluten proteins and involve several different methods including the Kjeldahl and the Dumas combustion method, which are very restrictive and can only be applied to test the wheat starches used in the preparation of the gluten-free products. These methods rely on the determination of nitrogen content, which should stay below 0.05% on the dry matter basis. Other assays include the polymerase chain reaction (PCR), which relies on the determination of specific DNA and is more sensitive by several orders of magnitude in comparison with protein assays. Some research groups suggested that PCR shows 10–30 times more sensitivity than ELISA (Koppel et al. 1998; Henterich et al. 2003). Albeit PCR-based assays are a highly sensitive tool for gluten analysis in comparison with ELISA and/or Western blotting, these cannot be applied to the hydrolyzed products such as beer, syrup, and malt extracts for determination of their gluten content.

The relatively more direct and precise method for gluten detection and quantification is matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), which can simultaneously measure protein and protein hydrolysate ranging in size from 1000 to 100,000 Daltons without a need of chromatographic purification. In addition, this technique allows reliable determination of protein levels as low as 0.01 mg/ml in the food samples (Camafeita et al. 1997,

1998; Iametti et al. 2005, 2006). Although MALDI-TOF MS is a highly sensitive non-immunological approach for detection and quantification of gluten contamination in food samples, its routine application is constrained by the considerable sample processing cost and the requirement of the specialized equipment.

Another approach that has extensively been used for characterization, separation, and quantification of the cereal protein fractions is column chromatography. Among chromatographic methods, gel permeation (GP) chromatography, which separates proteins based on their molecular weights, and reversed-phase (RP) chromatography that separates proteins according to their hydrophobicities have been used often. These procedures have advantages in terms of speed (usually 30 min) and detection capacity, which is as low as 1–2 μg for gluten (Weiser and Seilmeier 2003). Although this method can be used to determine gluten contamination reliably, it has the disadvantage of being unable to differentiate between gluten and non-gluten proteins in the analysis of complex food products.

The more versatile and commonly accepted assays are immunological assays in particular ELISA. Owing to the sensitivity and speed of detection, the Codex Committee on Methods of Analysis and Sampling has endorsed these methods. Several variations of these methods have been developed over the years (extensively reviewed in Scherf and Poms 2016). A number of antibodies (monoclonal and polyclonal) and a variety of commercial kits are available in the market to perform these assays. The commonly used ELISA systems can be grossly divided into two categories: the sandwich ELISA and the competitive ELISA. The sandwich ELISA is only suitable for large antigens because the antigen should have at least two spatially separated epitopes to bind both of the antibodies. Thus, this ELISA system is not an appropriate choice when working with partially hydrolyzed gluten samples like in the sourdough products, malt, and beer, whereas the competitive ELISA is suitable for the detection of small-sized antigens with a single epitope. The major problem associated with both of the ELISA systems is the determination of gluten contamination in heat-processed food samples, which cause conformational changes to the antigen masking or modifying the antibody recognition site(s). It has been documented that the α/β- and γ-gliadins by the heat treatment lose 49–67% of the original reactivity, while the ω-gliadins remain mostly unaffected, i.e., they only lose reactivity by 7% (Ellis et al. 1994; Rumbo et al. 2001). A detailed list of commercially available prolamin detection kits and specifications can be found in Scherf and Poms (2016) and Osorio et al. (2019a).

Recently, aptamers have emerged as an alternative to antibodies because these molecules can overcome the limitations of using antibodies for the detection, identification, and quantification of specific targets (Song et al. 2012). The aptamers are "single-stranded oligonucleotides that can bind proteins, small-molecules, and living cells with high affinity and specificity" (Berezovski et al. 2006). In the later years, aptamers against the immunodominant 33-mer peptide of α2-gliadin have been developed and successfully used in a variety of assays for gluten quantification. Specifically, the 33-mer peptide-specific aptamers dubbed "Gli4" showed a gluten detection limit of 0.5 ppm, but it failed to detect gluten in heat-treated and hydrolyzed food samples, whereas "Gli1" worked better on such samples, but

exhibited a detection limit of 5 ppm (Amaya-Gonzalez et al. 2014, 2015; Pinto et al. 2014; López-López et al. 2017; Malvano et al. 2017).

6 Approaches to Reduce Gluten-Exposure in Sensitive Individuals

So far, the only approved prescription for the gluten-associated disorders is adherence to a gluten-free diet. However, following a gluten-free lifestyle is challenging. And as mentioned earlier, it is not without penalties. For instance, (i) strict adherence to a diet devoid of gluten-containing grains deteriorates gut health by its negative influence on the gut microbiota, and (ii) long-term adherence to carbohydrate-rich gluten-free diet results in multiple deficiencies and change in the patient's body mass index (BMI). Therefore, a significant effort has been put in developing therapies for these disorders. The treatments in development for gluten-associated disorders can be grossly divided into dietary and non-dietary approaches, which are discussed below.

Dietary Procedures

The approaches which are preventive or prophylactic are grouped under this category. These approaches include the use of reduced-gluten wheat genotypes or gluten detoxification methods. And each of these approaches is elaborated in the following headings.

Screening of Wheat Germplasm

A body of research has suggested that any gluten peptide larger than nine amino acids can elicit an immune reaction in the susceptible individuals (Osorio et al. 2012). Therefore, no wheat genotype either new or old wheat varieties, landraces, or diploid/tetraploid wheat progenitors could be considered safe for celiac patients (Mitea et al. 2010; Goryunova et al. 2012; Brouns et al. 2013; Shewry 2018). The wide genetic screens performed on wheat and related species using immunological and non-immunological methods to study allergenicity and antigenicity of these genotypes supported this conclusion. The immunological methods used were ELISA and the T-cell assays, whereas the non-immunological methods used were based on sequence analysis, gene/transcript sequencing, and gluten profiling (cf. Rosella et al. 2014; Gilissen et al. 2014).

These studies conclusively revealed that gliadins are ubiquitously present in all wheat lines and related wild species. Also, seeds of certain ancient tetraploid wheat

types like Graziella Ra, Khorasan, or Kamut have shown to have even higher amounts of total gliadin than modern accessions (Colomba and Gregorini 2012; Brouns et al. 2013), therefore deemed unsuitable for celiac patients (Gregorini et al. 2009; Shewry 2018). However, based on limited data, Pizzuti et al. (2006) proposed that the diploid Einkorn wheat (*Triticum monococcum*) is non-toxic for celiac patients, but later studies revealed its unsuitability for consumption by celiac patients (Kasarda 2007; Vaccino et al. 2009; Gianfrani et al. 2012). Similarly, none of the tetraploid durum wheat (van den Broeck et al. 2010a; Salentijn et al. 2013) and hexaploid wheat genotypes (Molberg et al. 2005; van Herpen et al. 2006; van den Broeck et al. 2010b; Gilissen et al. 2014) were found suitable for general use by celiac patients. To sum up, after careful scrutiny of the facts, it would be safe to say that all wheat and related species such as barley, rye, triticale, tritordeum, and their hybrids are immunogenic and should be avoided by celiac patients (Rustgi et al. 2019).

Screening of the Genetic Stocks of Wheat and Related Cereals

Wheat cultivar 'Chinese Spring'-derived nulli-tetrasomic and deletion lines lacking a specific chromosome or chromosome segment were screened for their immunogenic potential. As expected, these genotypes showed low toxicity with gliadin-specific antibodies and under the T-cell-based assays, due to the lack of particular gliadin loci (Ciclitira et al. 1980a, b; Frisoni et al. 1995; van den Broeck et al. 2009, 2011). However, concerning the technological properties of these lines, mixed results were obtained. The results showed that deleting the α-gliadin locus from the short arm of chromosome 6 of the D genome leads to substantial loss in dough mixing and rheological properties. However, deleting the ω-gliadin, γ-gliadin, and LMW glutenin subunit loci from the short arm of chromosome 1D showed little to no effect on the technological properties (van den Broeck et al. 2009).

A large number of wheat genotypes in both winter and spring backgrounds and different market classes (hard, soft, red, and white) were bred to carry a reciprocal chromosome translocation involving wheat chromosome arm 1BS [with loci for ω- and γ-gliadins (*Gli1*) and LMW glutenin subunits (*Glu3*)] and rye chromosome arm 1RS (Lukaszewski 2015). The rye chromosome arm carries genes for resistance to three major rust diseases of wheat, grain yield, and the *Sec1* locus that encodes ω-secalins. This translocation was primarily bred in wheat for the agronomical advantage, but it was later realized to damage the technological properties (Lukaszewski 2015). Specifically, the dough made from some 1BL/1RS hard wheat lines was found unacceptable for breadmaking purposes because of excessive stickiness and mixing intolerance (Schwarzlaff et al. 2001). The inheritance of secalin proteins from rye and absence of glutenin subunits in these genotypes was suggested as a possible explanation for the sticky dough phenotype (Barbeau et al. 2003). However, higher amounts and/or differences in the composition of cell wall polysaccharides, β-glucans, and pentosans and/or the presence of a ferulic acid ester were later suggested as other possible explanations (Barbeau et al. 2003). Besides

the sticky dough phenotype in hard wheat lines, the 1BL/1RS translocation has been shown to reduce cookie diameter in soft wheat lines.

Upon 2D-PAGE gel analysis of 1BL/1RS translocation lines, eight protein spots were explicitly found in these genotypes; at the same time, 16 other spots were found missing. And another 12 protein spots, which were present in both regular wheat and the translocation lines showed either up- or downregulation. Out of these 12 spots, a highly overexpressed spot in translocated genotypes was identified as a γ-gliadin. It suggested that overexpression of a γ-gliadin compensates for the lack of LMW subunits in translocation lines. Also, a spot that was absent from the translocation line was identified as an α-amylase inhibitor, which was also proposed as a candidate for the sticky dough phenotype observed in the translocation lines (Gobaa et al. 2007).

Recent studies have revealed that all ω-secalins are enriched in tetrapeptide, PQQP, commonly present in celiac disease-associated epitopes. It suggested that ω-secalins can potential have celiac toxicity. A more recent study suggested that besides immunodominant and toxic epitopes, ω-secalin encodes a decapeptide QQPQRPQQPF that prevents K562(S) cell agglutination and celiac mucosa immune activation induced by toxic gliadins (De Vita et al. 2012). Therefore, identification of this immunomodulatory gliadin sequence, naturally occurring in wheat cultivars toxic for celiac patients, might offer new therapeutic strategies for celiac disease.

Wheat mutants lacking α/β-, γ-, and/or ω-gliadins and/or showing reduced accumulation to complete elimination of specific gliadins and/or LMW glutenin subunits were identified (Rustgi et al. 2019). Among these genotypes, the ω-gliadins-free genotype "3xN" (*Gli-B1*, *Gli-A1*, and *Gli-D1* null) developed by intercrossing of mutant lines lacking particular ω-gliadin groups and a genotype lacking almost all gliadins "TeM1" (*Gli-B1*, *Gli-D1, Gli-A2*, and *Gli-D2* null) deserve specific mention. These genotypes are not glute-free, albeit when 3xN was tested with the sera derived from the patents with wheat allergy showed a significant reduction in allergenicity (Waga and Skoczowski 2014; Skoczowski et al. 2017). Similarly, when peptic-tryptic digest of prolamins from TeM1 was tested for toxicity in celiac disease via monitoring the agglutinating activity against human myelogenous leukemia K562(s) cells, 3.5-fold more (572.5 mg/L) prolamin digest in comparison to the single mutants (161.5 mg/L) was tolerated (Pogna et al. 1998). Albeit the observed reductions in allergenicity and antigenicity of these genotypes are remarkable, these genotypes are still unsafe for consumption by celiac patients (Rustgi et al. 2019).

Similar reduced-gluten (hordein) mutants were also identified in barley. However, these mutants were initially selected for their high lysine content, which is an important trait in feed barley (Rustgi et al. 2019). One such low hordein barley mutant is *Risø 1508*; it is also known as *sex3c* (shrunken endosperm xenia) due to its shrunken endosperm and altered carbohydrate profile (Munck 1992). The mutant *Risø 1508* completely lacks class C hordeins and accumulates considerably reduced amount of class B hordeins (200 ppm hordein, 100-fold less than control). When this mutant was fed to gluten-sensitive rhesus macaques (*Macaca mulatta*), remission of the anti-gliadin antibody serum responses and improvement of clinical diarrhea were observed. However, the subjects never showed complete recovery. Hence the authors of the study concluded that "the reduced gluten barley diet might be used for the

partial improvement of gluten-induced disease, but its therapeutic value still requires upgrading" (Sestak et al. 2015). Recently, Tanner and co-workers developed ultra-low gluten (ULG) barley genotype using this mutant in a cross-breeding approach with another reduced-gluten barley mutant (Tanner et al. 2016) and achieved almost zero gluten status. However, given the large number and complexity of the gliadin genes in wheat and their inheritance in blocks, the possibility of pyramiding all low toxicity gliadin genes in a single wheat variety seems remote (Koning 2012).

Other Cereals and Non-cereals as an Alternative

Other than wheat, some individuals show sensitivity to oat gluten proteins (avenins) and, in rare cases, to even maize gluten proteins dubbed zeins (Comino et al. 2013; Rosella et al. 2014; Ortiz-Sánchez et al. 2013). However, all oat varieties are not immunogenic. So far, two cereals, which are unequivocally accepted for celiac patients' consumption, are rice and sorghum (Rosella et al. 2014; Pontieri et al. 2013). But, the rice kernels have low protein and fiber content and are highly enriched in easily digestible carbohydrates that may contribute to the high glycemic index. The rice kernels also tend to sequester large quantities of arsenic (Rosella et al. 2014; Da Sacco et al. 2013; Munera-Picazo et al. 2014), and its grain storage proteins (other than prolamins and glutelins) are reported to trigger a variety of allergic reactions (asthma, atopic dermatitis, diarrhea, and anaphylaxis) in different individuals (Matsuda et al. 2006; Nambu 2006; Trcka et al. 2012; Gilissen et al. 2014). Therefore, rice is not the best choice for consumption by celiac patients. Sorghum is primarily used as animal feed in the Western countries, albeit in many parts of Africa and Asia, it is used for human food. Therefore, the main issue hampering its acceptance in the West is the lack of research into the end-uses of sorghum. There are some alternatives available for celiac patients, in particular, the minor cereals like fonio, tef, millet, teosinte, and Job's tears. However, these cereals are less common and have been cultivated regionally; for instance, tef is a crop in Ethiopia. All tef varieties examined so far are free of stimulatory epitopes (Hopman et al. 2008; Spaenij-Dekking et al. 2005), but the primary concern about its use is the possible cross-contamination with other gluten-containing grains like wheat (Saturni et al. 2010). There are other crops that are processed similarly to cereals and hence called pseudocereals. The most popular of these is the nutritionally dense quinoa, which unfortunately is controversial due to the immunotoxicity of some varieties (Zevallos et al. 2012, 2014). Similarly, for buckwheat, there have been reports of allergies (Panda et al. 2010; Stember 2006).

Engineered Celiac-Safe Wheat Genotypes

In the wake of the difficulties associated with breeding "celiac-safe" genotypes, many research groups adapted to the genetic engineering procedures. Two kinds of wheat genotypes were developed, one where gluten proteins were eliminated, and

the other where the gluten-detoxification enzymes were expressed. Following the former lead, Becker and co-workers produced a series of transgenic lines where α-gliadin genes were downregulated using RNA interference (RNAi). In these lines, α-gliadins were reduced by over 60% compared to the control cultivar (Becker et al. 2006, 2012; Becker and Folck 2006; Wieser et al. 2006). Using a similar approach silencing of the ω5-gliadins (Altenbach and Allen 2011; Altenbach et al. 2014) and ω1,2-gliadins was achieved by Altenbach and co-workers (Altenbach et al. 2019). However, in the attempt to silence the ω1,2-gliadins, the authors identified a transgenic line almost completely lacking gliadins and LMW glutenin subunits. When tested, the flour proteins from this genotype showed a stark decline in reactivity with serum IgG and IgA antibodies from a cohort of celiac disease patients (Altenbach et al. 2019). But the line suffered from the diminished mixing properties (Altenbach et al. 2019). Similarly, downregulation of γ-gliadins was also achieved using RNAi, and genotypes showing 65–97% reduction in the target proteins were identified (Gil-Humanes et al. 2008; Piston et al. 2011). More recently, following this lead, Smulders and co-workers developed CRISPR-Cas9-based constructs to specifically induce mutations in the genes encoding α- and γ-gliadin genes (Jouanin et al. 2018, 2019) and Barro and co-workers in the α2-gliadin genes (Sánchez-León et al. 2018).

The studies mentioned in the paragraph above were focused on the elimination of the specific gluten proteins using an RNA interference approach or genome editing. On the other hand, the studies mentioned below have either utilized a chimeric hairpin construct to target all gliadin (α/β-, γ-, and ω-) genes together (Gil-Humanes et al. 2010, 2011, 2012a, b, 2014a, b) or used RNAi to silence the master regulator (*DEMETER*) of the prolamin transcription (Wen et al. 2012; Rustgi et al. 2014). The lines showing 60–88% reductions in the gliadin content were identified using the chimeric hairpin construct. Tests of these genotypes with the intestinal T-cell clones derived from the biopsy samples of celiac patients showed almost complete suppression of disease-related T-cell epitopes (Gil-Humanes et al. 2010). When tested, these lines also showed reasonable baking characteristics and organoleptic properties as well as exhibited increased lysine content (Gil-Humanes et al. 2012a, b, 2014a, b). Two kinds of transgenic lines were produced to achieve DME suppression, one with DME-specific hairpin RNA and the other with DME-specific artificial micro RNA (amiRNA). The lines expressing DME-specific hairpin construct showed 45–76% reductions in the content of immunogenic prolamins (Wen et al. 2012; Rustgi et al. 2014) (Fig. 3). And the lines expressing one of the three amiRNAs exhibited 54–88% reductions in their respective prolamin contents (Rustgi et al. 2014).

Following the latter (gluten detoxification) approach, the Rustgi and co-workers expressed "glutenases" in wheat endosperm. Based on the parameters like target specificity, substrate length, optimum pH, and site of action, two prolyl endopeptidases one from *Flavobacterium meningosepticum* and the other from a thermophilic bacterium, *Pyrococcus furiosus*, and a glutamine-specific endoprotease from barley (EP-B2) were selected for expression in wheat endosperm (Osorio et al. 2012, 2019b). Wheat transformants expressing a FmPEP-EPB2 combination with up to

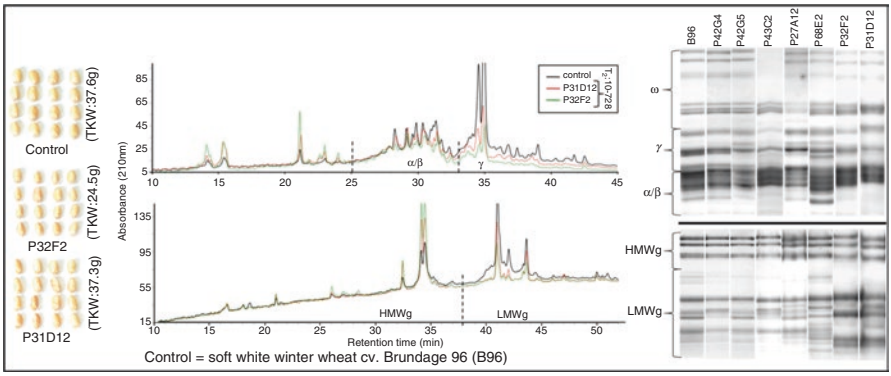

Fig. 3 Liquid chromatography (middle) and polyacrylamide gel electrophoresis (right) of gliadins (top) and glutenin (bottom) fractions extracted from the grains of the two progeny plants (P31D12 and P32F2) of a genetically engineered wheat line. A random sample of grains from the selected lines with their respective thousand kernel weights (TKWs) is shown on the left. (Modified from Wen et al. 2012)

58% reduction and a PfuPEP-EP-B2 combination with up to 68% reduction in the content of the immunogenic gluten peptides were obtained (Osorio et al. 2019b) (Fig. 4).

This latter approach has specific advantages: (1) Some celiac patients show sensitivity to the HMW-GSs peptides (Dewar et al. 2006). Therefore, the formerly discussed transformants, which lack specific gliadins and/or LMW-GSs, are unsuitable for such patients. (2) The combination of enzymes used in this approach prevents degradation of gluten proteins within grains; therefore avoid the distraction of the end-use quality. The glutamine-specific endoprotease used in this study is encoded as a proenzyme, where the propeptide serves as both inhibitor and chaperone to respectively facilitate spatiotemporal regulation of the proteolytic activity and proper folding of the proteases (Bethune et al. 2006; Cappetta et al. 2002; Schilling et al. 2009; Cambra et al. 2012). These properties are of immense importance, as it avoids degradation of the prolamins in the protein bodies within grains and also in flour during the dough-making process. In addition, the prolyl endopeptidase due to its strict preference for substrates with ≤33 amino acids in size can only degrade peptides generated by the endoprotease (Gass and Khosla 2007), therefore permitting both of these enzymes to accumulate within the protein bodies containing the gluten proteins without degrading them and affecting the baking properties of the flour. (3) Intake of foods prepared from wheat engineered to express glutenases in grains does not require consumers to intake dietary supplements (none of these supplements are yet available in the market) before or with each meal. (4) The proposed therapy is expected to reach the general public without specific efforts and/or adding to the daily expenses of the consumers, as the remedy to wheat allergy and gluten intolerance is packed in the grain. (5) Contamination of regular wheat in the glutenases expressing wheat, at any level from farm to shelf, is less likely to make

Parameter	EP-B2	Fm-PEP
Substrate specificity	Glutamine-specific endoprotease	Post-proline cleaving endopeptidase
Substrate length	Protein	Peptide (~30 aa)
pH	4-7	7
Site of action	Stomach	Small intestine

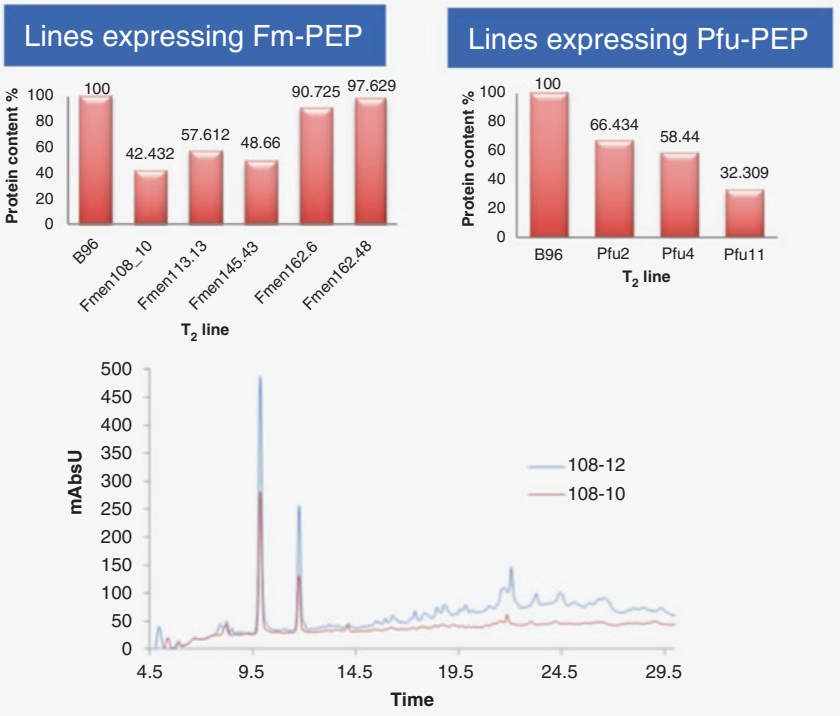

Fig. 4 List of parameters used to select the two peptidases, one from barley (barley endoprotease B2; EP-B2) and another from *Flavobacterium* (*F. meningosepticum* prolyl endopeptidase; Fm-PEP). The total reduction in the content of the immunogenic gluten peptides in wheat transformants expressing a FmPEP-EPB2 combination (left) or a PfuPEP (*Pyrococcus furiosus* prolyl endopeptidase)-EP-B2 combination. Total protein was extracted from the grains of genetically engineered lines, treated with gastric enzymes and quantified by liquid chromatography (an example profile shown below, where 108–12 = control and 108–10 = a transgenic line). Proteins were measured in μg/mL and expressed as percent amount of immunogenic peptides remaining in each line in relation to the control line (B96). (Modified from Osorio et al. 2019b)

it unsuitable for celiac patients, as the glutenases expressing in the grains will degrade the contaminating gluten protein.

Management Practices and Processing Procedures

Other than using genetic alterations, the reduced-immunogenicity wheat can be achieved by modulating growth conditions of wild-type wheat genotypes or by changing the processing parameters of the whole grains or the wheat flour. In fact, a correspondence was observed between nitrogen and sulfur dose and the amount as well as the composition of proteins accumulated in wheat grains (Godfrey et al. 2010; Shewry 2011) (see Table 1 for examples). An increase in nitrogen supply results in a significant increase in the content of gliadins and glutenins, but not of albumins and globulins (Johansson et al. 2001). Specifically, the effect on gliadins was more pronounced than on glutenins. High levels of nitrogen increased the proportions of hydrophilic proteins (ω-gliadins and HMW subunits), and those of hydrophobic proteins (γ-gliadins and LMW subunits) were decreased (Wieser and Seilmeier 1998). In a separate study, the majority of HMW subunits and ω-gliadins and some α-gliadins showed increased accumulation, while two LMW subunits and a minor γ-gliadin exhibited decreased accumulation in response to fertilizer or high temperature, whereas fertilizer did not influence gluten protein accumulation under high-temperature conditions (Hurkman et al. 2013). More recently, two commercial spelt wheat varieties evaluated through seven nitrogen fertilization modalities did not influence the epitope expression of the first variety, whereas it had a slight effect on the epitope expression of the second variety (Dubois et al. 2018). Similar effects of nitrogen fertilizer on hordein, specifically C-hordein biosynthesis during early stages of grain development, were reported in barley (Giese and Hopp 1984; Müller and Knudsen 1993).

Much like nitrogen fertilizers, sulfur fertilization showed influence on the amount of total gluten as well as the crude protein content of flour. In the case of sulfur deficiency, the amount of S-free ω-gliadins increased drastically and that of S-poor HMW subunits increased moderately. In contrast, the amounts of S-rich γ-gliadins and LMW subunits decreased significantly, whereas the amount of α-gliadins was reduced only slightly. Sulfur deficiency results in a remarkable shift in protein proportions, such that the gliadin to glutenin ratio increases distinctly, and among gliadins, the ω-gliadins become significant components and γ-gliadins minor elements (Wieser et al. 2004).

Other than nutrient status, temperature regime during grain development was reported to have a significant influence on the amount and type of proteins accumulation. For instance, the low-temperature conditions during grain development were shown to decrease the level of protein fractions primarily associated with celiac disease but increase the content of protein families related to WDEIA or barker's asthma, such as LTPs, hydrolases, peroxidases, and ATIs. On the other hand, under the high temperature conditions, the changes in seed storage protein accumulation were shown to result in slightly increased accumulation of ω-gliadins (3–26%) and

Table 1 Effect of different environmental factors and management regimens on the gluten content and composition

Type	Genotype	Experiment duration (years)	Locations	Factor(s)	Comment	References
Winter wheat	Ana Morava, Toplica, Vizija, Takovcanka, and Lazarica	3	3	Genotype x environment effect on gluten content	Analysis of variance suggested highly significant differences among genotypes, years, and locations for gluten content	Zecevic et al. (2009)
Durum wheat	Canadian varieties – Kyle, AC Avonlea, AC Navigator, AC Pathfinder, and AC Morse; breeding lines, DT674, DT 672, DT 675, and DT 666; U.S. cultivar, Durex	3	2	Effect of nitrogen on gluten content	Increase in nitrogen fertilizer resulted in increased protein content in all cultivars across environments	Ames et al. (2003)
Bread wheat	Eagle, Oxley, Egret, Halberd, and Cook	11	3	Effect of heat stress on gluten	Gliadin synthesis continues at a greater rate than glutenin synthesis during a period of heat stress. Consequently, the mature grains show a higher ratio of gliadins to glutenins and produce weaker dough. These results now provide a basis for formulating strategies to minimize variations in dough properties due to growing conditions	Blumenthal et al. (1993)
Bread wheat	Pandas, Spada, Maestra, and Oderzo	–	4	Effect of heat stress on gluten	During heat stress, there is a continuous synthesis of both gliadin and LMW glutenin subunits, but synthesis of HMW glutenin subunits is suppressed	Ciaffi et al. (1996)
Semi-hard and soft common wheat	40 wheat genotypes	2	3	Genotype x environment effect on gluten content	Significant effects of both genotype and environment were onserved on the gluten content	Jing et al. (2003)

Type	Genotype	Experiment duration (years)	Locations	Factor(s)	Comment	References
Bread wheat	140 cultivars and experimental lines	4	–	Genotype x environment effect on gluten content	Both cultivar and cultivar by environment interaction had significant effects on all quality traits. Variances of quality traits associated with genetic factors (cultivar) were generally larger than those for cultivar by environmental interaction effects	Denčić et al. (2011)
Winter wheat	Lars	4	2	Effect of sulfur on gluten proteins	Applied sulfur positively affected the quality of wheat flour by increasing gluten and protein concentration. Furthermore, sulfur positively affected the development, stability, softening, and quality of dough as well as bread volume	Järvan et al. (2008)
Bread wheat	Ningmai9	–	2	Effect of nitrogen and potassium on gluten content	N and K fertilization significantly increased grain yield, protein content, and wet gluten content	Zou et al. (2006)
Spring weak-gluten wheat	Yangmai 9 (YM9), Ningmai 9 (NM9), and Yangmai 13 (YM13)	2	–	Effect of phosphorus fertilizer on grain proteins	Optimum P fertilizer increased grain yield and improved grain quality of weak-gluten wheat	Zhu et al. (2012)
Winter wheat	Erzurum (Turkey)	–	–	Effect of water stress at various growth stages on quality	Late water stress caused an increase of 8.3% in grain protein content, 8.7% in sedimentation volume, 10.8% in wet gluten content, and a reduction in 1000-kernel weight	Ozturk and Aydin (2004)

(continued)

Table 1 (continued)

Type	Genotype	Experiment duration (years)	Locations	Factor(s)	Comment	References
Winter wheat	S + D23ulamit (E), Samanta (A), apache (B), Meritto (B), Mladka (C), and Rapsodia (C)	2	2	The effect of organic and conventional growing systems on gluten	Conventionally grown wheat varieties showed more HMW glutenins than organically grown wheat varieties. However, organically grown varieties showed more albumin and globulin content	Krejčířová et al. (2008)
Bread wheat	PBW 343 and C306	2	1	Effect of sowing time and drought on gluten content	Late sown conditions lead to high grain protein content by increasing albumins/globulins but decreasing glutenins. Rain-fed conditions decreased trypsin inhibitors' activity, and exposure to heat and drought together led to a decline in the glutenin-to-gliadin ratio.	Singh et al. (2012)
Weak- or medium-gluten cultivar	Yangmai 9 (weak-gluten) and Yangmai 12 (medium-gluten)	–	–	Effect of planting density on the protein content	Increasing planting density from 105×10^4 to 240×10^4 plants/ha increased the protein content	Liu et al. (2006)
Spring wheat	–	21	–	Effect of temperature on the gluten content	The gluten content decreased when daily minimum and maximum temperatures exceeded 11–12 °C and 21–22 °C, respectively	Peltonen et al. (1990)
Winter wheat	Spark, rialto, Soissons, and beaver	1	1	Effect of temperature and moisture on gluten	Good processing quality varieties showed significant differences related to environmental conditions than the variety with poor processing qualities	Georget et al. (2008)

Type	Genotype	Experiment duration (years)	Locations	Factor(s)	Comment	References
Red spring wheat	Butte 86	1	1	Effect of temperature, water and fertilizer on gluten content	Under high-temperature regimen, transcripts from all gene families appeared slightly earlier, but the time frame of accumulation was shorter as compared to plants under moderate daytime temperature with optimum water and fertilizer	Altenbach et al. (2002)
Spring wheat	Star	–	2	Effect of sulfur on gluten properties	Overall, gluten content was affected little by different doses of the sulfur fertilizer, but the individual single protein sub-units were influenced strongly. With a sulfur deficiency, the gliadins: glutenins ratio increased, whereas the ratio of HMW glutenins to LMW glutenins stayed in balance	Wieser et al. (2004)
Spring wheat	Sport, Dacke, Dragon, Thasos	3	3	Effect of nitrogen application rate on gluten strength	High nitrogen fertilizer rate increased protein concentration, decreased gluten strength, and increased the total amount of glutenins and gliadins. The timing of fertilization (early application) increases gluten strength	Johansson et al. (2004)
Red spring wheat	Butte 86	1	1	Effect of temperature and fertilizer on gluten composition	At high temperature, HMW glutenins, omega-gliadins, and some alpha gliadins increased, while LMW glutenins and gamma gliadins decreased. Fertilizer did not influence gluten protein accumulation under high-temperature conditions	Hurkman et al. (2013)

(continued)

α-gliadins (25–33%), more specifically in the content of 33-mer containing α-gliadins (Juhász et al. 2018). Collectively, these studies suggest that it is possible to alter the amount and type of proteins accumulated in wheat grains by modulating with the growing condition of wheat plants.

Besides, it is possible to obtain reduced-immunogenicity flour from regular wheat genotypes by applying specific processing procedures such as milling techniques or twin-screw extrusion techniques. More recently, the use of the microwaves to remove antigenic properties of the wheat gluten proteins was proposed (Landriscina et al. 2017). Additionally, the use of wheat, barley, and rye sprouts (germinated grains) as a safe food for celiac patients or to be used as an ingredient for other products was proposed. Although cereal endopeptidases synthesized during sprouting can efficiently hydrolyze gluten (Hartmann et al. 2006), other research showed that using peptidases from sprouted wheat to digest gliadin did not result in food safe for celiac patients (Stenman et al. 2009). Another proposed method was the use of sourdough fermentation to produce bakery products suitable for celiac patients (Zannini et al. 2012). However, no conclusive data exist on the use of any of the methods mentioned above [for details the readers are recommended to consult Rustgi et al. (2019) and references cited therein].

Non-dietary Procedures

In parallel to the efforts to develop dietary therapies for the celiac disease, extensive research was performed to developing non-dietary therapies. These therapies can be largely classified into (1) luminal therapies which are based on the detoxification of gluten proteins and can be further classified into enzyme therapy, probiotic therapy, flour/dough pretreatment, and gluten inactivation by polymeric binding; (2) intestinal barrier enhancing therapies, which focus on reducing the permeability of intestinal epithelial barrier; and (3) immune-targeted therapies, which target either celiac disease-specific pathways or inflammatory mediators common in gastrointestinal inflammation. These non-dietary therapies to treat the celiac disease has been extensively reviewed in the past by Schuppan et al. (2009), Sollid and Khosla (2011), Osorio et al. (2012), Rashtak and Murray (2012), McCarville et al. (2015), and Ribeiro et al. (2018) and, therefore, have not been discussed here.

7 Conclusion

Outstanding genetic resources, such as conventionally produced reduced-gluten mutations in each one of the gliadin and glutenin loci and the cytogenetically, as well as genetically engineered reduced-gluten lines, are available to researchers to breed wheat genotypes for celiac patients. Specifically, a vast collection of well-characterized chromosome substitution and alien introgression lines developed in

the background of elite hexaploid and tetraploid wheat genotypes exist today, which could be screened for their gluten composition, antigenicity, and allergenicity as well as technological properties. These lines carry alien introgression spanning almost all parts of the wheat genome, exist in the elite background, and carry many desirable exotic attributes such as insect pest, fungal, or abiotic stress tolerance. Besides, remarkable genomic resources and approaches such as genomic selection are available, which could facilitate the selection process of desirable lines from the interbreeding program. These new genomic prediction methods also reduce the dependence on the expensive phenotyping for technological properties, allergenicity, and antigenicity tests. Therefore, we believe that desired resources are available to the breeders today to make sturdy progress in the direction of developing "celiac-safe" wheat genotypes.

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References

Altenbach SB, Allen PV (2011) Transformation of the US bread wheat "Butte 86" and silencing of omega-5 gliadin genes. GM Crops 2:66–73

Altenbach SB, Kothari KM, Dao L (2002) Environmental conditions during wheat grain development alter temporal regulation of major gluten protein genes. J Cereal Chem 79:279–285

Altenbach SB, Tanaka CK, Seabourn BW (2014) Silencing of omega-5 gliadins in transgenic wheat eliminates a major source of environmental variability and improves dough mixing properties of flour. BMC Plant Biol 14:1

Altenbach SB, Chang H-C, Yu XB, Seabourn BW, Green PH, Alaedini A (2019) Elimination of omega-1,2 gliadins from bread wheat (*Triticum aestivum*) flour: effects on immunogenic potential and end-use quality. Front Plant Sci 10:580

Amaya-Gonzalez S, De-Los-Santos-Alvarez N, Miranda-Ordieres AJ, Lobo-Castanon MJ (2014) Aptamer binding to celiac disease-triggering hydrophobic proteins: a sensitive gluten detection approach. Anal Chem 86:2733–2739

Amaya-Gonzalez S, De-Los-Santos-Alvarez N, Miranda-Ordieres AJ, Lobo-Castanon MJ (2015) Sensitive gluten determination in gluten-free foods by an electrochemical aptamer-based assay. Anal Bioanal Chem 407:6021–6029

Ames NP, Clarke JM, Dexter JE, Woods SM, Selles F, Marchylo B (2003) Effects of nitrogen fertilizer on protein quantity and gluten strength parameters in durum wheat (Triticum turgidum L. var.durum) cultivars of variable gluten strength. J Cereal Chem 80:203–211

Aziz I, Hadjivassiliou M, Sanders DS (2015) The spectrum of noncoeliac gluten sensitivity. Nat Rev Gastroenterol Hepatol 12:516–526

Bai JC, Fried M, Corazza GR, Schuppan D, Farthing M et al (2013) World gastroenterology organisation global guidelines on celiac disease. J Clin Gastroenterol 47:121–126

Balakireva AV, Zamyatnin AA (2016) Properties of gluten intolerance: gluten structure, evolution, pathogenicity and detoxification capabilities. Nutrients 8:E644

Barbeau WE, Schwarzlaff SS, Uriyo MG, Johnson JM, Harris CH, Griffey CA (2003) Origin and practical significance of the sticky dough factor in 1BL/1RS wheats. J Sci Food Agric 83:29–38

Becker D, Folck A (2006) Inhibierung der α-Gliadingeneexpression in hexaploiden Brotweizen. Getreidetechnologie 30:153–156

Becker D, Folck A, Knies P, Lörz H, Wieser H (2006) Silencing the a-gliadins in hexaploid bread wheat. In: Lookhart LG, Ng WPK (eds) Gluten proteins. AACC International, St Paul, pp 86–89
Becker D, Wieser H, Koehler P, Folck A, Mühling KH, Zörb C (2012) Protein composition and techno-functional properties of transgenic wheat with reduced α-gliadin content obtained by RNA interference. J Appl Bot Food Qual 85:23–33
Berezovski M, Musheev M, Drabovich A, Jitkova J, Krylov S (2006) Non-SELEX: selection of aptamers without intermediate amplification of candidate oligonucleotides. Nat Protoc 1:1359–1369
Bethune MT, Khosla C (2008) Parallels between pathogens and gluten peptides in celiac sprue. PLoS Pathog 4:e34
Bethune MT, Strop P, Tang Y, Sollid LM, Khosla C (2006) Heterologous expression, purification, refolding, and structural-functional characterization of EP-B2, a self-activating barley cysteine endoprotease. Chem Biol 13:637–647
Blumenthal CS, Barlow EWR, Wrigley CW (1993) Growth environment and wheat quality: the effect of heat stress on dough properties and gluten proteins. J Cereal Sci 18:3–21
Brouns F, van Buul VJ, Shewry PR (2013) Does wheat make us fat and sick? J Cereal Sci 58:209–215
Brouns F, Van Rooy G, Shewry P, Rustgi S, Jonkers D (2019) Adverse reactions to wheat or wheat components. Compr Rev Food Sci Food Saf. https://doi.org/10.1111/1541-4337.12475
Camafeita E, Alfonso P, Lopez JA, Sorell L, Mendez E (1997) Sample preparation optimization for the analysis of gliadins in food by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. J Mass Spectrom 32:444–449
Camafeita E, Solis J, Alfonso P, Lopez JA, Sorell L, Mendez E (1998) Selective identification by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry of different types of gluten in foods made with cereal mixtures. J Chromatogr A 823:299–306
Cambra I, Hernández D, Diaz I, Martinez M (2012) Structural basis for specificity of propeptide-enzyme interaction in barley C1A cysteine peptidases. PLoS One 7:e37234
Cappetta M, Roth I, Díaz A, Tort J, Roche L (2002) Role of the prosegment of Fasciola hepatica cathepsin L1 in folding of the catalytic domain. Biol Chem 383:1215–1221
Ciaffi M, Tozzi L, Borghi B, Corbellini M, Lafiandra D (1996) Effect of heat shock during grain filling on the gluten protein composition of bread wheat. J Cereal Sci 24:91–100
Ciclitira PJ, Lennox ES, Hunter JO (1980a) Clinical testing of bread made from nullisomic-6A wheats in celiac patients. Lancet 2:234–236
Ciclitira PJ, Hunter JO, Lennox ES (1980b) Clinical testing in celiac patients of bread made from wheats deficient in some alpha-gliadins. Clin Sci 59:25
Colomba MS, Gregorini A (2012) Are ancient durum wheats less toxic to celiac patients? A study of α-gliadin from Graziella Ra and Kamut. Sci World J 2012:837416
Comino I, Moreno M, Real A, Rodríguez-Herrera A, Barro F, Sousa C (2013) The gluten-free diet: testing alternative cereals tolerated by celiac patients. Nutrients 5:4250–4268
Da Sacco L, Baldassarre A, Masotti A (2013) Diet's role in the toxicity of inorganic arsenic (iAs): a journey from soil to children's mouth. J Geochem Explor 131:45–51
De Vita P, Ficco DBM, Luciani A, Vincentini O, Pettoello-Mantovani M, Silano M, Maiuri L, Cattivelli L (2012) A ω-secalin contained decamer shows a celiac disease prevention activity. J Cereal Sci 55:234–242
Delcour JA, Joye IJ, Pareyt B, Wilderjans E, Brijs K, Lagrain B (2012) Wheat gluten functionality as a quality determinant in cereal-based food products. Annu Rev Food Sci Technol 3:469–492
Denčić S, Mladenov N, Kobiljski B (2011) Effects of genotype and environment on breadmaking quality in wheat. Int J Plant Prod 5:71–82
Dewar DH, Amato M, Ellis HJ, Pollock EL, Gonzalez-Cinca N, Wieser H, Ciclitira PJ (2006) The toxicity of high molecular weight glutenin subunits of wheat to patients with coeliac disease. Eur J Gastroenterol Hepatol 18:483–491
Dubois B, Bertin P, Hautier L, Muhovski Y, Escarnot E, Mingeot D (2018) Genetic and environmental factors affecting the expression of α-gliadin canonical epitopes involved in celiac

disease in a wide collection of spelt (*Triticum aestivum* ssp. *spelta*) cultivars and landraces. BMC Plant Biol 18:262

Ellis HJ, Doyle AP, Wieser H, Sturgress RP, Day P, Ciclitira PJ (1994) Measurement of gluten using a monoclonal antibody to a sequenced peptide of α-gladian from the coeliac-activating domain I. J Biochem Biophys Methods 28:77–82

Fasano A (2009) Surprises from celiac disease. Sci Am 301:54–61

Frisoni M, Corazza GR, Lafiandra D, De Ambroggio E, Filipponi C et al (1995) Wheat deficient in gliadins: promising tool for coeliac disease. Gut 36:375–378

Gass J, Khosla C (2007) Prolyl endopeptidases. Cell Mol Life Sci 64:345–355

Georget DMR, Underwood-Toscano C, Powers SJ, Shewry PR, Belton PS (2008) Effect of variety and environmental factors on gluten proteins: an analytical, spectroscopic and rheological study. J Agric Food Chem 56:1172–1179

Gianfrani C, Maglio M, Rotondi Aufiero V, Camarca A, Vocca I et al (2012) Immunogenicity of monococcum wheat in celiac patients. Am J Clin Nutr 96:1339–1345

Gianibelli MC, Larroque OR, MacRitchie F, Wrigley CW (2001) Biochemical, genetic, and molecular characterization of wheat glutenin and its component subunits. Cereal Chem 78:635–646

Giese H, Hopp HE (1984) Influence of nitrogen nutrition on the amount of hordein, protein z and α-amylase messenger RNA in developing endosperms of barley. Carlsberg Res Commun 49:365–383

Gil-Humanes J, Piston F, Hernando A, Alvarez JB, Shewry PR, Barro F (2008) Silencing of γ-gliadins by RNA interference (RNAi) in bread wheat. J Cereal Sci 48:565–568

Gil-Humanes J, Piston F, Tollefsen S, Sollid LM, Barro F (2010) Effective shutdown in the expression of celiac disease-related wheat gliadin T-cell epitopes by RNA interference. Proc Natl Acad Sci USA 107:17023–17028

Gil-Humanes J, Piston F, Shewry PR, Tosi P, Barro F (2011) Suppression of gliadins results in altered protein body morphology in wheat. J Exp Bot 62:4203–4213

Gil-Humanes J, Piston F, Gimenez MJ, Martin A, Barro F (2012a) The introgression of RNAi silencing of γ-gliadins into commercial lines of bread wheat changes the mixing and technological properties of the dough. PLoS One 9:e45937

Gil-Humanes J, Piston F, Rosell CM, Barro F (2012b) Significant down-regulation of γ-gliadins has minor effect on gluten and starch properties of bread wheat. J Cereal Sci 56:161–170

Gil-Humanes J, Piston F, Altamirano-Fortoul R, Real A, Comino I et al (2014a) Reduced-gliadin wheat bread: an alternative to the gluten-free diet for consumers suffering gluten-related pathologies. PLoS One 9:e90898

Gil-Humanes J, Piston F, Barro F, Rosell CM (2014b) The shutdown of celiac disease-related gliadin epitopes in bread wheat by RNAi provides flours with increased stability and better tolerance to over-mixing. PLoS One 9:e91931

Gilissen LJWJ, van der Meer IM, Smulders MJM (2014) Reducing the incidence of allergy and intolerance to cereals. J Cereal Sci 59:337–353

Gobaa S, Bancel E, Kleijer G, Stamp P, Branlard G (2007) Effect of the 1BL.1RS translocation on the wheat endosperm, as revealed by proteomic analysis. Proteomics 7:4349–4357

Godfrey D, Hawkesford MJ, Powers SJ, Millar S, Shewry PR (2010) Effects of crop nutrition on wheat grain composition and end use quality. J Agric Food Chem 58:3012–3021

Golley S, Corsini N, Topping D, Morell M, Mohr P (2015) Motivations for avoiding wheat consumption in Australia: results from a population survey. Public Health Nutr 18:490–499

Goryunova SV, Salentijn EM, Chikida NN, Kochieva EZ, van der Meer IM, Gilissen LJ, Smulders MJM (2012) Expansion of the gamma-gliadin family in *Aegilops* and *Triticum*. BMC Evol Biol 12:215

Gregorini A, Colomba M, Ellis HJ, Ciclitira PJ (2009) Immunogenicity characterization of two ancient wheat α-gliadin peptides related to coeliac disease. Nutrients 1:276–290

Hartmann G, Koehler P, Wieser H (2006) Rapid degradation of gliadin peptides toxic for coeliac disease patients by proteases from germinating cereals. J Cereal Sci 44:368–371

Henterich N, Osman AA, Mendez E, Mothes T (2003) Assay of gliadins by real time immunopolymerase chain reaction. Nahrung 47:345–348

Hopman E, Dekking L, Blokland ML, Wuisman M, Zuijderduin W, Koning F, Schweizer J (2008) Tef in the diet of celiac patients in The Netherlands. Scand J Gastroenterol 43:277–282

Hurkman WJ, Tanaka CK, Vensel WH, Thilmony R, Altenbach SB (2013) Comparative proteomic analysis of the effect of temperature and fertilizer on gliadin and glutenin accumulation in the developing endosperm and flour from Triticum aestivum L. cv. Butte 86. J Proteome Sci 11:8

Iametti S, Bonomi F, Ferranti P, Picariello G, Gabrovska D (2005) Characterization of gliadin content in beer by using different approaches. In: Stern M (ed) Proceedings of the 19th meeting of working group on Prolamin analysis and toxicity. Verlag Wissenschaftliche Scripten, Zwickau, pp 73–78.

Iametti S, Bonomi F, Ferranti P, de Martino A, Picariello G (2006) Characterization of peptides and proteins in beer by using different approaches. In Stern M (ed) Proceedings of the 20th meeting of working group on Prolamin analysis and toxicity. Verlag Wissenschaftliche Scripten, Zwickau, pp 47–52

Järvan M, Edesi L, Adamson A, Lukme L, Akk A (2008) The effect of Sulphur fertilization on yield, quality of protein and baking properties of winter wheat. Agronomy Res 6:459–469

Jing Q, Jiang D, Dai T, Cao W (2003) Effects of genotype and environment on wheat grain quality and protein components. J Appl Ecol 14:1649–1653

Johansson E, Prieto-Linde ML, Jönsson JÖ (2001) Effects of wheat cultivar and nitrogen application on storage protein composition and bread-making quality. Cereal Chem 78:19–25

Johansson E, Prieto-Linde ML, Svensson G (2004) Influence of nitrogen application rate and timing on grain protein composition and gluten strength in Swedish wheat cultivars. J Sci Food Agri 167:345–350

Jouanin A, Boyd L, Visser RGF, Smulders MJM (2018) Development of wheat with hypoimmunogenic gluten obstructed by the gene editing policy in Europe. Front Plant Sci 9:1523

Jouanin A, Schaart JG, Boyd LA, Cockram J, Leigh FJ, Bates R, Wallington EJ, Visser RGF, Smulders MJM (2019) Outlook for coeliac disease patients: towards bread wheat with hypoimmunogenic gluten by gene editing of α- and γ-gliadin gene families. BMC Plant Biol 19:333

Juhász A, Belova T, Florides CG, Maulis C, Fischer I et al (2018) Genome mapping of seed-borne allergens and immunoresponsive proteins in wheat. Sci Adv 4:eaar8602

Kasarda DD (2007) Letter to the editor: *Triticum monococcum* and celiac disease. Scand J Gastroenterol 42:1141–1142

Koning F (2012) Celiac disease: quantity matters. Seminars Immunopathol 34:541–549

Koppel E, Stadler M, Luthy J, Hubner P (1998) Detection of wheat contamination in oats by polymerase chain reaction (PCR) and enzyme-linked immumosorbent assay (ELISA). Z Lebensm Untersuch Forsch 206:399–403

Krejčířová L, Capouchová I, Petr J, Bicanová E, Faměra O (2008) The effect of organic and conventional growing systems on quality and storage protein composition of winter wheat. Plant Soil Environ 53:499–505

Landriscina L, D'Agnello P, Bevilacqua A, Corbo MR, Sinigaglia M, Lamacchia C (2017) Impact of gluten-friendly™ technology on wheat kernel endosperm and gluten protein structure in seeds by light and electron microscopy. Food Chem 221:1258–1268

Langridge P (2017) Achieving sustainable cultivation of wheat: breeding, quality traits, pests and diseases. Burleigh Dodds Science Publishing, Cambridge

Liu P, Guo WS, Xu YM, Feng CN, Zhu XK, Peng YX (2006) Effect of planting density on grain yield and quality of weak-gluten and medium-gluten. Wheat J Triticecae S512:1

López-López L, Miranda-Castro R, De-los-Santos-Alvarez N, Miranda-Ordieres AJ, Lobo-Castanon MJ (2017) Disposable electrochemical aptasensor for gluten determination in food. Sensors Actuators B Chem 241:522–527

Lukaszewski AJ (2015) Introgressions between wheat and rye. In: Molnár-Láng M, Ceoloni C, Doležel J (eds) Alien introgression in wheat. Springer, Cham, pp 163–190

Malvano F, Albanese D, Pilloton R, Matteo MD (2017) A new label-free impedimetric aptasensor for gluten detection. Food Control 79:200–206

Matsuda T, Nakase M, Alvarez AM, Izumi H, Kato T, Tada Y (2006) Rice-seed allergenic proteins and hypoallergenic rice. In: Mine Y, Shahidi F (eds) Nutraceutical proteins and peptides in health and disease. CRC Press, Hoboken, pp 493–511

McCarville JL, Caminero A, Verdu EF (2015) Celiac treatments, adjuvant therapies and alternatives to the gluten-free diet. In: Arranz E, Fernández-Bañares F, Rosell CM, Rodrigo L, Peña AS (eds) Advances in the understanding of gluten related pathology and the evolution of gluten-free foods. OmniaScience, Barcelona, pp 223–253

Mitea C, Salentijn EM, van Veelen P, Goryunova SV, van der Meer IM et al (2010) A universal approach to eliminate antigenic properties of alpha-gliadin peptides in celiac disease. PloS One 5:e15637

Molberg Ø, Uhlen AK, Jensen T, Flæte NS, Fleckenstein B et al (2005) Mapping of gluten T-cell epitopes in the bread wheat ancestors: implications for celiac disease. Gasteroenterology 128:393–401

Müller M, Knudsen S (1993) The nitrogen response of a barley C-hordein promoter is controlled by positive and negative regulation of the GCN4 and endosperm box. Plant J4:343–355

Muller S, Wieser H (1995) The location of disulfide bonds in alpha-type gliadins. J Cereal Sci 22:21–27

Muller S, Wieser H (1997) The location of disulphide bonds in monomeric gamma-type gliadins. J Cereal Sci 26:169–176

Munck L (1992) The case of high-lysine barley breeding. In: Shewry PR (ed) Barley: genetics, biochemistry, molecular biology and biotechnology. CAB International, Wallingford, pp 573–601

Munera-Picazo S, Ramirez-Gandolfo A, Burlo F, Carbonell-Barrachina AA (2014) Inorganic and total arsenic contents in rice-based foods for children with celiac disease. J Food Sci 79:T122–T128

Nambu M (2006) Rice allergy. Pediatrics 117:2331

Ortiz-Sánchez JP, Cabrera-Chávez F, de la Barca AM (2013) Maize prolamins could induce a gluten-like cellular immune response in some celiac disease patients. Nutrients 5:4174–4183

Osborne TB (1907) The proteins of the wheat kernel. Carnegie Institute, Washington

Osorio C, Wen N, Gemini R, Zemetra R, von Wettstein D, Rustgi S (2012) Targeted modification of wheat grain protein to reduce the content of coeliac causing epitopes. Funct Integr Genomics 12:417–438

Osorio CE, Mejías JH, Rustgi S (2019a) Gluten detection methods and their critical role in assuring safe diets for celiac patients. Nutrients 11:2920

Osorio C, Wen N, Mejias JH, Liu B, Reinbothe S, von Wettstein D, Rustgi S (2019b) Development of wheat genotypes expressing a glutamine-specific endoprotease from barley and a prolyl endopeptidase from *Flavobacterium meningosepticum* or *Pyrococcus furiosus* as a potential remedy to coeliac disease. Funct Integr Genomics 19:123–136

Ozturk A, Aydin F (2004) Effect of water stress at various growth stages on some quality characteristics of winter wheat. J Agron Crop Sci 190:93–99

Panda R, Taylor SL, Goodman RE (2010) Development of a sandwich enzyme-linked immunosorbent assay (ELISA) for detection of buckwheat residues in food. J Food Sci 75:T110–T117

Peltonen J, Karvonen T, Kivi E (1990) The effect of climatic factors on production of spring wheat quantity to quality ratio in southern Finland. Agric Food Sci 62:227–236

Pinto A, Polo PN, Henry O, Redondo MCB, Svobodova M, O'Sullivan CK (2014) Label-free detection of gliadin food allergen mediated by real-time apta-PCR. Anal Bioanal Chem 406:515–524

Piston F, Gil-Humanes J, Rodríguez-Quijano M, Barro F (2011) Down-regulating γ-gliadins in bread wheat leads to non-specific increases in other gluten proteins and has no major effect on dough gluten strength. PLoS One 6:e24754

Pizzuti D, Buda A, D'Odorico A, D'Incà R, Chiarelli S, Curioni A, Martines D (2006) Lack of intestinal mucosal toxicity of *Triticum monococcum* in coeliac disease patients. Scand J Gastroenterol 41:1305–1311

Pogna NE, Monari AM, Cacciatori P, Redaelli R, Ng PKW (1998) Development and characterization of bread wheat lines lacking chromosome 1B-, 1D-, 6A- and 6D-encoded prolamins. In: Proceedings of the IXth international wheat genetics symposium. Saskatoon, Saskatchewan, Canada, pp 265–268

Pontieri P, Mamone G, De Caro S, Tuinstra MR, Roemer E et al (2013) Sorghum, a healthy and gluten-free food for celiac patients as demonstrated by genome, biochemical, and immunochemical analyses. J Agric Food Chem 61:2565–2571

Rashtak S, Murray JA (2012) Review article: coeliac disease, new approaches to therapy. Aliment Pharmacol Ther 35:768–781

Ribeiro M, Nunes FM, Rodriguez-Quijano M, Carrillo JM, Branlard G, Igrejas G (2018) Next-generation therapies for celiac disease: the gluten-targeted approaches. Trends Food Sci Technol 75:56–71

Rosella CM, Barro F, Sousa C, Mena MC (2014) Cereals for developing gluten-free products and analytical tools for gluten detection. J Cereal Sci 59:354–364

Rumbo M, Chirdo FG, Fossati CA, Anon MC (2001) Analysis of the effect of heat treatment on gliadin immunochemical qualification using a panel of anti-prolamin antibodies. J Agric Food Chem 49:5719–5726

Rustgi S, Wen N, Osorio C, Brew-Appiah RAT, Wen S et al (2014) Natural dietary therapies for the 'gluten syndrome'. Scientia Danica Series B Biol 3:1–87

Rustgi S, Shewry P, Brouns F, Deleu LJ, Delcour JA (2019) Wheat seed proteins – factors influencing their content, composition, and technological properties, and strategies to reduce adverse reactions. Compr Rev Food Sci Food Saf 18:1751–1769

Salentijn EMJ, Esselink DG, Goryunova SV, van der Meer IM, Gilissen L, Smulders MJM (2013) Quantitative and qualitative differences in celiac disease epitopes among durum wheat varieties identified through deep RNA-amplicon sequencing. BMC Genomics 14:16

Sánchez-León S, Gil-Humanes J, Ozuna CV, Giménez MJ, Sousa C, Voytas DF, Barro F (2018) Low-gluten, nontransgenic wheat engineered with CRISPR/Cas9. Plant Biotechnol J 16:902–910

Sapone A, Bai JC, Ciacci C, Dolinsek J, Green PH, Hadjivassiliou M, Kaukinen K, Rostami K, Sanders DS, Schumann M, Ullrich R, Villalta D, Volta U, Catassi C, Fasano A (2012) Spectrum of gluten-related disorders: consensus on new nomenclature and classification. BMC Med 10:13

Saturni L, Ferretti G, Bacchetti T (2010) The gluten-free diet: safety and nutritional quality. Nutrients 2:16–34

Scherf KA, Poms RE (2016) Recent developments in analytical methods for tracing gluten. J Cereal Sci 67:112–122

Schilling K, Körner A, Sehmisch S, Kreusch A, Kleint R et al (2009) Selectivity of propeptide-enzyme interaction in cathepsin L-like cysteine proteases. Biol Chem 390:167–174

Schuppan D, Junker Y, Barisani D (2009) Celiac disease: from pathogenesis to novel therapies. Gastroenterology 137:1912–1933

Schwarzlaff SS, Uriyo MG, Johnson JM, Barbeau WE, Griffey CA (2001) Apparent dough stickiness of selected 1BL/1RS translocated soft wheat flours. Cereal Chem 78:93–96

Sestak K, Thwin H, Dufour J, Aye PP, Liu DX, Moehs CP (2015) The effects of reduced gluten barley diet on humoral and cell-mediated systemic immune responses of gluten-sensitive *Rhesus macaques*. Nutrients 7:1657–1671

Shewry PR (2011) Effects of nitrogen and sulfur nutrition on grain composition and properties of wheat and related cereals. In: Hawkesford MJ, Barraclough P (eds) The molecular and physiological basis of nutrient use efficiency in crops. Wiley-Blackwell, Chichester, pp 103–120

Shewry PR (2018) Do ancient types of wheat have health benefits compared with modern bread wheat? J Cereal Sci 79:469–476

Shewry PR (2019) What is gluten – why is it special? Front Nutr 6:101

Shewry PR, Tatham AS, Forde J, Kreis M, Miflin BJ (1986) The classification and nomenclature of wheat gluten proteins: a reassessment. J Cereal Sci 4:97–106
Shewry PR, Halford NG, Tatham AS (1992) High-molecular-weight subunits of wheat glutenin. J Cereal Sci 15:105–120
Shewry PR, D'Ovidio R, Lafiandra D, Jenkins JA, Mills ENC, Bekes F (2009) Wheat grain proteins. In: Khan K, Shewry PR (eds) Wheat: chemistry and technology, 4th edn. AACC International, St. Paul, pp 223–298
Singh S, Gupta A, Kaur N (2012) Influence of drought and sowing time on protein composition, antinutrients, and mineral contents of wheat. Sci World 2012:1–9
Skoczowski A, Obtułowicz K, Czarnobilska E, Dyga W, Mazur M, Stawoska I, Waga J (2017) Antibody reactivity in patients with IgE-mediated wheat allergy to various subunits and fractions of gluten and non-gluten proteins from ω-gliadin-free wheat genotypes. Ann Agric Environ Med 24:229–236
Skodje GI, Sarna VK, Minelle IH, Rolfsen KL, Muir JG et al (2018) Fructan, rather than gluten, induces symptoms in patients with self-reported non-celiac gluten sensitivity. Gastroenterology 154:529–539
Sollid LM, Khosla C (2011) Novel therapies for coeliac disease. J Intern Med 269:604–613
Song K, Lee S, Ban C (2012) Aptamers and their biological applications. Sensors 12:612–631
Spaenij-Dekking L, Kooy-Winkelaar Y, Koning F (2005) The Ethiopian cereal tef in celiac disease. N Engl J Med 353:1748–1749
Stember RH (2006) Buckwheat allergy. Allergy Asthma Proc 27:393–395
Stenman SM, Venalainen JI, Lindfors K, Auriola S, Mauriala T et al (2009) Enzymatic detoxification of gluten by germinating wheat proteases: implications for new treatment of celiac disease. Ann Med 41:390–400
Tanner GJ, Blundell MJ, Colgrave ML, Howitt CA (2016) Creation of the first ultra-low gluten barley (*Hordeum vulgare* L.) for coeliac and gluten-intolerant populations. Plant Biotechnol J 14:1139–1150
Tatham AS, Shewry PR (2008) Allergens to wheat and related cereals. Clin Exp Allergy 38:1712–1726
Trcka J, Schäd SG, Scheurer S, Conti A, Vieths S, Gross G, Trautmann A (2012) Rice-induced anaphylaxis: IgE-mediated allergy against a 56-kDa glycoprotein. Int Arch Allergy Immunol 158:9–17
Tye-Din JA, Stewart JA, Dromey JA, Beissbarth T, van Heel DA, Tatham A, Henderson K, Mannering SI, Gianfrani C, Jewell DP, Hill AV, McCluskey J, Rossjohn J, Anderson RP (2010) Comprehensive, quantitative mapping of T cell epitopes in gluten in celiac disease. Sci Transl Med 2:41ra51
Vaccino P, Becker HA, Brandolini A, Salamini F, Kilian B (2009) A catalogue of *Triticum monococcum* genes encoding toxic and immunogenic peptides for celiac disease patients. Mol Genet Genomics 281:289–300
van den Broeck HC, van Herpen TW, Schuit C, Salentijn EM, Dekking L et al (2009) Removing celiac disease-related gluten proteins from bread wheat while retaining technological properties: a study with Chinese spring deletion lines. BMC Plant Biol 9:41
van den Broeck HC, Hongbing C, Lacaze X, Dusautoir J-C, Gilissen L, Smulders M, van der Meer I (2010a) In search of tetraploid wheat accessions reduced in celiac disease-related gluten epitopes. Mol BioSyst 6:2206–2213
van den Broeck HC, de Jong HC, Salentijn EMJ, Dekking L, Bosch D et al (2010b) Presence of celiac disease epitopes in modern and old hexaploid wheat varieties: wheat breeding may have contributed to increased prevalence of celiac disease. Theor Appl Genet 121:527–1539
van den Broeck HC, Gilissen LJWJ, Smulders MJM, van der Meer IM, Hamer RJ (2011) Dough quality of bread wheat lacking alpha-gliadins with celiac disease epitopes and addition of celiac-safe avenins to improve dough quality. J Cereal Sci 53:206–216

Van Herpen TWJM, Goryunova S, van der Schoot J, Mitreva M, Salentijn EMJ et al (2006) Alpha-gliadin genes from the A, B, and D genomes of wheat contain different sets of celiac disease epitopes. BMC Genomics 7:1

Venske E, dos Santos RS, Busanello C, Gustafson P, de Oliveira AC (2019) Bread wheat: a role model for plant domestication and breeding. Hereditas 156:16

Veraverbeke WS, Delcour JA (2002) Wheat protein composition and properties of wheat glutenin in relation to breadmaking functionality. Crit Rev Food Sci Nutr 42:179–208

Verbeke K (2018) Non-celiac gluten sensitivity: What is the culprit? Gastroenterology 154:471–473

Waga J, Skoczowski A (2014) Development and characteristics of ω-gliadin-free wheat genotypes. Euphytica 195:105–116

Weiser H, Seilmeier W (2003) Determination of gliadin and gluten in wheat starch by means of alcohol extraction and gel permeation chromatography. In: Stern M (ed) Proceedings of the 17th meeting of working group on prolamin analysis and toxicity. Verlag Wissenschaftliche Scripten, Zwickau, pp 53–57

Wen S, Wen N, Pang J, Langen G, Brew-Appiah RAT et al (2012) Structural genes of wheat and barley 5-methylcytosine DNA glycosylases and their potential applications for human health. Proc Natl Acad Sci USA 109:20543–20548

Wieser H, Seilmeier S (1998) The influence of nitrogen fertilisation on quantities and proportions of different protein types in wheat flour. J Sci Food Agric 76:49–55

Wieser H, Gutser R, Tucher SV (2004) Influence of Sulphur fertilization on quantities and proportions of gluten protein types in wheat flour. J Cereal Sci 40:239–244

Wieser H, Koehler P, Folck A, Becker D (2006) Characterization of wheat with strongly reduced α-gliadin content. In: Lookhart GL (ed) Gluten proteins 2006. C.H.I.P.S., Weimar, pp 13–16

Zannini E, Pontonio E, Waters DM, Arendt EK (2012) Applications of microbial fermentations for production of gluten-free products and perspectives. Appl Microbiol Biotechnol 93:473–485

Zecevic V, Knezevic D, Boskovic J, Madic M (2009) Effect of genotype and environment on wheat quality. Genetika 41:247–253

Zevallos VF, Ellis HJ, Suligoj T, Herencia LI, Ciclitira PJ (2012) Variable activation of immune response by quinoa (*Chenopodium quinoa* Willd.) prolamins in celiac disease. Am J Clin Nutr 96:337–344

Zevallos VF, Herencia LI, Chang FJ, Donnelly S, Ellis HJ, Ciclitira PJ (2014) Gastrointestinal effects of eating quinoa (*Chenopodium quinoa* Willd.) in celiac patients. Am J Gastroenterol 109:270–278

Zhu X, Li C, Jiang Z, Huang L, Feng C, Guo W, Peng Y (2012) Responses of phosphorus use efficiency, grain yield, and quality to phosphorus application amount of weak-gluten wheat. J Integr Agric 11:1103–1110

Zou TX, Dai TB, Jiang D, Qi J, Cao WX (2006) Effects of nitrogen and potassium supply on grain yield and quality in weak gluten wheat. J Triticeae Crops S512:1