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Coeliac-safe wheat

A novel wheat to decrease the prevalence and symptoms of coeliac disease

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ABSTRACT: A significant increase has been observed in the prevalence of coeliac disease (CD) during the last decades. Increased consumption of wheat and in particular gluten is considered one of the major causes. CD is a food-related disease caused by certain gluten peptides from wheat, rye, and barley containing T-cell stimulating epitopes. The use of screening protocols in wheat breeding detecting the absence of CD-epitopes (e.g. Glia-α9 and Glia-α20) can result in the reduction of CD-epitopes in commercial wheat and wheat based products. This approach will lead to decreased exposure, especially in many CD-patients with silent or latent CD that have not yet been diagnosed. In the long-term, this may lead to a large-scale reduction of the incidence of CD and a significant improvement of the quality of life of CD-patients.

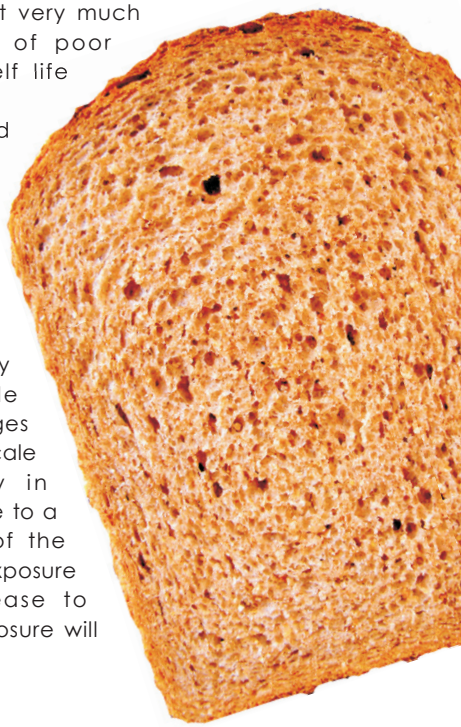
COELIAC DISEASE

Coeliac disease (CD) is a T-cell mediated gluten intolerance provoked by specific peptides (epitopes) in gluten proteins from wheat, rye and barley. Exposure to dietary gluten is essential to develop the disease together with genetic susceptibility of the consumer. CD is a worldwide public health problem with an occurrence of about 0.5-2 percent of the general population. Currently, 70-97 percent of the CD-patient population is still undiagnosed. Patients suffering from CD have a damaged small intestinal mucosa resulting in villous atrophy and crypt hyperplasia (1). The only cure for diagnosed CD-patients is the restriction to a lifelong gluten-free diet. This will prevent symptoms such as malnutrition, malabsorption, diarrhoea, abdominal pain, and, in the worst case, the development of lymphoma and cancer that can occur in refractory coeliac disease (RCD) in which patients no longer respond to a gluten-free diet (2-4). In children, CD can lead to growth retardation. CD can occur in a silent form without any clear symptoms and with or without villous atrophy and histological changes (5-7). CD can develop at any age but exposure to gluten at a young age in combination with breastfeeding seems to delay the development of CD (8, 9).

Adherence to a gluten-free diet is of high importance for CD-patients because it allows healing of the intestinal mucosa, thus removing symptoms and improving the quality of life. Maintaining a gluten-free diet, however, is very difficult because gluten proteins are increasingly used as ingredient in many processed foods (10-13). This increases the exposure to gluten in the many, still undiagnosed, CD-patients and induces the development of symptoms in CD-patients who were, so far, symptom-free. Wheat gluten and starch that could be contaminated with gluten, can also be present as a "hidden" and not expected ingredient in food products and even in medication. This, especially, may cause problems in very sensitive CD-patients. An additional problem is that gluten-free diets may not contain sufficient amounts of proteins, vitamins, minerals, and fibres. An even more emerging problem is that gluten-free food products often contain relatively high amounts of fat and sugar, which may cause overweight in CD-patients on a gluten-free diet (14) and increases the possibility to the development of diabetes type II.

THE NEED FOR COELIAC-SAFE WHEAT

Wheat flour is unique because of the presence of polymeric gluten proteins, which are essential for palatable, good quality bread. The gluten proteins from wheat (gliadins and glutenins), rye (secalins), and barley (hordeins) are characterized by high contents of the amino acids proline and glutamine and are also called prolamins. However, these amino acids are very important for T-cell binding of the CD-epitopes in these proteins. Gluten proteins from other cereals and pseudo-cereals are much lower in glutamine and proline content and lack CD-epitopes, but their flour is not suitable for bread making because of the lack of polymeric gluten proteins. Therefore, a lot of effort has already been put in the analysis of the nutritional quality of cereals such as oat, corn, rice, sorghum, and millet, or pseudo-cereals such as amaranth, buckwheat, and quinoa and how to optimize their process of bread baking. Many Western CD-patients, however, are raised with and are used to the consumption of wheat bread and other wheat products such as cookies, cake and pie. Gluten-free products prepared from different ingredients and (pseudo-)cereals are not very much appreciated because of poor texture, taste, and shelf life properties.



Since CD is a food-related disease, it allows a prevention strategy, which is the elimination of the CD-epitopes directly from the food. In addition, a substantial decrease in the amount of CD-epitopes in wheat may also have considerable health related advantages when applied on large scale in foods. Wheat low in CD-epitopes will contribute to a decreased incidence of the disease, as there is an exposure threshold for the disease to develop (15). Lower exposure will

also contribute to push back the number and severity of symptoms, especially in undiagnosed CD-patients who are unaware of having the disease and still consume wheat.
Many different gluten proteins exist and not all gluten proteins contain CD-epitopes (16). Small changes in amino acid composition can create or eliminate a bioactive CD-epitope. Thus, variation in gluten protein composition among varieties can be detected and exploited to search and select varieties low in CD-epitopes (17, 18). During many years of breeding up till now no attention has been paid to wheat properties related to the presence of CD-epitopes in gluten proteins. A requirement in the development of wheat varieties low in CD-epitopes is to maintain the technological properties of gluten to produce viscoelastic dough for preparation of food products, especially bread.

IN SEARCH OF COELIAC-SAFE WHEAT

Wheat can be divided in hard durum wheat (tetraploid, having the A and B-genome) and in hard and soft common (bread) wheat (hexaploid, having the A, B and D-genome). Hard durum wheat has relatively high protein content (13-15 percent) and is commonly used for the preparation of pasta. Common wheat has a lower (10-15 percent) protein content of which hard common wheat is mainly used for preparation of bread whereas soft common wheat is mainly used for preparation of biscuits and cakes. Thousands of years of cultivation, domestication, gene mutations and cross-fertilization resulted in genetic variability of tetraploid and hexaploid wheats. The introduction of the D-genome improved the bread-making properties (19, 20) and increased the ability of wheat to adjust to environmental changes. Many thousands of wheat varieties exist nowadays, both modern and old hexaploid and tetraploid varieties, landraces, cultivated and wild species, and ancestral diploid species. Also non-natural hexaploid wheats have been developed including e.g. synthetic hexaploid wheats and deletion lines in hexaploid wheat varieties, e.g. "Chinese Spring". Among wheat species, large variation exists in T-cell stimulatory immune responses. So far, the presence of CD-epitopes in all these wheat accessions has only been analysed to limited extent (21-29). Diploid and tetraploid wheats are commonly shown to contain less CD-epitopes compared with hexaploid wheats because of the absence of the D-genome. Screening wheat varieties for the presence of CD-epitopes in our studies was performed by using immunoblotting with specific mAbs against CD-epitopes by the strategy shown in Figure 1 (30). If screening of different hexaploid wheat varieties that are nowadays grown and used for breeding would result in the discovery of a variety that is reduced in CD-epitopes, this line would immediately be applicable for further breeding. However, when the genetic diversity among the modern hexaploid varieties would not be large enough and a variety low in CD-epitopes cannot be identified among modern hexaploid wheats, older hexaploid varieties, including landraces, can be analysed. Therefore, we analysed the occurrence and level of CD-epitopes in a set of modern wheat varieties from Europe and compared this with a set of older varieties and landraces obtained from all over the world (17). Results showed that genetic diversity is indeed limited in modern wheat varieties compared with the older varieties and landraces. Modern wheat varieties appear to contain an increased number of Glia-α9 epitopes and a reduced number of Glia-α20 epitopes. This may explain why currently patients respond more to the Glia-α9 epitope and less to the Glia-α20 epitope (31, 32). In addition, this may be the reason for a higher prevalence of CD, because the level of the immunodominant epitope Glia-α9 seems to have increased as a result from breeding during the last decades.
Wheat varieties used for breeding are mainly selected for high yield and good baking properties. So far, breeders did not include selection for absence of CD-epitopes. In our studies, older hexaploid varieties were identified that are reduced in CD-epitopes compared with modern varieties. In the future, wheat breeding using these low CD-epitope lines can aim at reduced levels of CD-epitopes as a new quality trait.
Another approach is to screen tetraploid wheat varieties low in CD-epitopes to be used for pasta preparation. Such a tetraploid (durum) wheat can be used directly for the production of CD-safe(r) foods such as pastas and pizzas, as well as for the development of a hexaploid bread wheat low in CD-epitopes by hybridisation with a diploid D-genome species low in CD-epitopes. The resulting synthetic hexaploid may, however, show different gluten protein patterns because of epigenetic regulation of gene expression and still needs to be tested for presence of CD-epitopes. Screening of tetraploid accessions including landraces, old, modern, and domesticated accessions of various tetraploid species and subspecies from many geographic origins has been performed (18). The resulting immunoblot banding patterns of these tetraploids were less complex compared with the immunoblot patterns of hexaploid accessions. This is most likely caused by the absence of the D-genome, which contains many α-gliadin epitopes (33, 34). However, from the tetraploid accessions nearly 25 percent was contaminated with hexaploid wheat. Durum wheat may legally contain up to 3 percent of common wheat (35), but for selection of accessions low in CD-epitopes this mixing is not acceptable. Tetraploid accession should be selected that are reduced in CD-epitopes when compared with hexaploid wheats, and need therefore to be uniform. We have selected two tetraploid accessions with very low CD-epitope profiles. From these accessions, single seed-derived lines are under development for further multiplication.

WHAT ABOUT TECHNOLOGICAL PROPERTIES?

Wheat gluten proteins are indispensable for the preparation of bread, pasta, pizza, noodles, and biscuits. The gluten composition determines the elasticity and viscosity of dough.

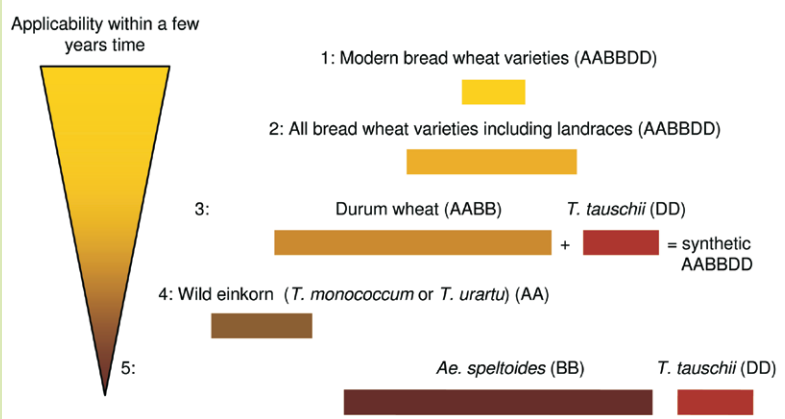


Figure 1. Hypothetical distribution of genetic variation within wheat.

During mixing, a three-dimensional structure is formed by the gluten proteins that is capable of retaining gas produced by the yeast during dough proofing. With the development of CD-safe wheat, the baking quality needs to be maintained to allow commercial, large-scale application. To maintain technological properties of wheat, certain gluten proteins cannot be removed, while other gluten proteins can, without affecting dough properties. Analysis of deletion lines of bread wheat variety Chinese Spring that are missing the gluten encoding loci *Glu-1*, *Glu-3*, *Gli-1*, *Gli-2* or *Gli-3* located on chromosomes 1 and 6, showed significantly reduced numbers of CD-epitopes, but also negatively affected dough properties (36, 37). Dough properties were analysed by small scale mixing experiments, stress relaxation testing, extensibility testing, and glutenin macropolymer (GMP) analysis, which reflects dough quality and bread making properties.

High molecular weight glutenin subunits (HMW-GS) and low molecular weight glutenin subunits (LMW-GS) are very important for the dough structure because of the formation of the gas-retaining gluten network. Deletion of the loci on the long arms of chromosome 1B and 1D encoding HMW-GS (no HMW-GS are expressed by chromosome 1A in "Chinese Spring") resulted in loss of dough structure, confirming that HMW-GS encoded by both loci are definitely needed in dough preparation. Deletions of loci from the short arms (S) of chromosome 1A and especially of chromosome 1D remove many CD-epitopes. In case of 1AS and 1DS deletions, LMW-GS and γ- and ω-gliadin encoding genes are removed. Apparently, absence of LMW-GS prevents the formation of a strong gluten network with the HMW-GS and results in weaker, more elastic dough.

Deletion of the locus from the short arm of chromosome 6D (6DS) leads to a reduction in α-gliadins containing important CD-epitopes (22). "Chinese Spring" is used as model wheat for research purposes, but has poor dough making quality: it produces wet and sticky dough. Because gliadins act as "plasticizers" in the gluten network (38, 39), removal of gliadins from "Chinese Spring" resulted in stiffer dough. Gliadins are therefore indispensible in high quality bread wheat with a strong gluten network. In this context, it has been analysed whether CD-safe gliadin-like proteins, such as the avenins from oat, can be used to replace the technological function of wheat gliadins. However, a few very sensitive CD-patients have been reported to respond to avenins from oat. Because this is a minority of the CD-population, avenins from oat are in general regarded as CD-safe (40 and references therein). Addition of purified avenins to flour of the 6DS deletion line showed improvement of dough quality by making the dough stiffer (37). These studies clearly showed the limitations of removing gluten proteins with regard to the technological properties. However, removal of α-gliadins, which also removes major CD-epitopes, is possible and may be compensated by addition of oat avenins. Crosses were performed between two lines having deletions on the short arms of chromosome 1D and 6D. Progeny plants that carry both deletions showed an increased reduction of a large amount of CD-epitopes as was detected by immunoblotting (Figure 2) (30). This new line with multiple deletions is currently being tested for dough quality. Furthermore, the "Chinese Spring" deletion lines are under investigation as breeding receptors for the introduction of high quality HMW-GS to improve baking properties.

In the frame of breeding bread wheat with reduced amounts of CD-epitopes by reduced numbers of gliadins containing these epitopes, a strategy could be to select for either lower quality HMW-GS or lower gluten protein content. Bread wheat containing lower quality HMW-GS or less gluten proteins will produce a less strong gluten network in which a high level of gliadins is not necessary for dilution of the network. In such wheat lines, reduction or absence of gliadins containing CD-epitopes will not negatively influence baking quality. Therefore, wheat breeding or the 'deletion line-approach' indicate possible strategies for wheat varieties that have a good baking quality and not necessarily need gliadins in their gluten network.

CONCLUSION

Diagnosis, treatment, and prevention of CD are of utmost importance, because CD affects a large part of the population and prevalence continues to increase. Since the majority (70-97 percent) of the CD-patients is undiagnosed, and because wheat and wheat constituents are increasingly applied in food products, reduction of CD-epitopes should become a major and general objective in wheat breeding. This has not been a point of interest in the past decades, where the focus has been on yield and quality improvement. Now, the availability of screening protocols for CD-epitopes offers a unique

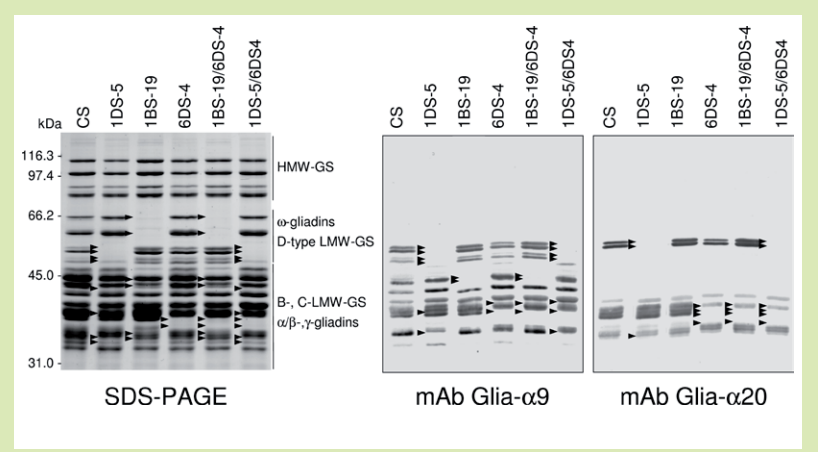


Figure 2. Immunoblotting of Chinese Spring deletion lines for presence of CD-epitopes.

opportunity for wheat breeders worldwide to start breeding for low CD-epitope wheats of good yield and quality.
Our studies underpin the feasibility of this approach. The long-term outcome will hopefully be a lower incidence of CD and a higher quality of life of diagnosed and undiagnosed CD-patients.

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