Chapter 15

Cereals Taxonomy: The Role of Domestication and Breeding on Gluten Intolerance

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Abstract

Storage proteins of wheat, rye, barley and, to a lesser extent, oats contain epitopes responsible for triggering the celiac disease (CD). In recent decades an increased frequency of CD has been observed, and though the reasons for this increase are unclear, modern plant breeding has attracted criticism attributing to the new varieties a part of the responsibility in worsening the data of prevalence. Wheat is one of the most important crops worldwide, presenting both high adaptability to different environments and yields. The domestication of wheat is the result of a previous natural interspecific hybridization first between diploid, and then between diploid and tetraploid species that resulted in hexaploid wheat. The old farmers began to select the traits that were better adapted to the use. In the 20th century the wheat breeding had its great advance and modern varieties were developed. The gliadin-related genes, responsible for triggering CD, have no adaptive value and therefore, if the toxicity of wheat was increased during the process of domestication and breeding this would have been made unconsciously. During the process of natural hybridization the number of gliadin-related genes was increased. Bread wheat, rye, and *Ae. tauschii* have the highest number of CD epitopes per gene, and it seems that in bread wheat, this high number of epitopes is explained by the D genome from *Ae. tauschii*. During the process of domestication and breeding, the number of CD epitopes per gene did not increase and even decreased in some cases.

Keywords

Cereal domestication, wheat breeding, prolamins, gluten, immunotoxicity.

1. Introduction

The change in the human diet during the Neolithic Revolution has been associated with a general decline in health in some areas[1]. Celiac disease (CD) is one of diseases that emerged in that period[2,3] but despite of being known since ancient times, its history is relatively recent. The first references concerning the intake of certain foods may be harmful do not appear until the late nineteenth century, and the first breakthrough came after World War II, with the demonstration of the role of gluten as the agent responsible for triggering the disease[4]. In the past 60 years, the knowledge about CD has improved significantly, resulting in a better understanding of the disease pathogenesis, diagnosis, and therapy[5]. Progress in the understanding of the disease includes the recognition of its autoimmune nature, its genetic basis, and the identification of immunogenic gluten fragments causing CD in many patients[6].

The definition of the disease and the diagnostic criteria, have undergone changes as all questions concerning the CD have become clearer. Thus, the guidelines for the diagnosis of CD recommended by the European Society for Paediatric Gastroenterology Hepatology and Nutrition (ESPGHAN), first published in 1970, have been revised twice. As defined in the last guidelines of 2012[7], "CD is an immune-mediated systemic disorder elicited by gluten and related prolamins in genetically susceptible individuals and characterized by the presence of a variable combination of gluten-dependent clinical manifestations, CD-specific antibodies, HLA-DQ2 or HLA-DQ8 haplotypes, and enteropathy. CD-specific antibodies comprise autoantibodies against TG2, including endomysial antibodies (EMA), and antibodies against deamidated forms of gliadin peptides (DGP)".

The prevalence of CD is 1% of the general population in western countries but varies from country to country. However, an increased frequency of CD in recent decades has been observed, which can be partly attributed to the advent of serological testing and increased public awareness in some countries[8]. The reasons for this increase are unclear, but several hypotheses as

hygiene[9] and the rising consumption of cereals, especially wheat (or its derivatives)[10], have been proposed among others. The possible roles attributed to modern plant breeding in worsening figures of prevalence of CD are discussed in this chapter.

2. Taxonomy and Domestication of Cereals

The transition process from hunter-gathering to a sedentary, agriculture-based human society started around 12,000 years ago[11]. Cereals cultivation and their elaborated products have played an essential role in the development of human societies, and nowadays represent an important element in most of the different cultures. Archaeological evidences show that humans harvested the wild forms of cereals from natural stands before they started to deliberate, cultivate and domesticate cereals, which involved the selection and modification of important traits such as seed size and rachis stiffness in the first cultivated fields. The easy carry and storage, together with the high content in carbohydrates and proteins, are some of the characteristics associated to the first plants that were cultivated and domesticated. Cereals are the domestic variants of the species from the *Gramineae* family (*Poaceae* Barnhart). The paleobotanical records suggest that this family was generated about 50-70 million years ago (MYA). Within this family, the main species of agronomical interest are found in the three subfamilies: *Ehrhartoideae* Link (rice), *Panicoideae* Link (maize, sugar cane, and sorghum) and *Pooideae* Bentham (oat, wheat, rye, and barley), this last subfamily is formed by 15 tribes, being the tribes *Avenae* (oat) and *Triticeae* (barley, rye, and wheat) the most important (Figure 1). Although now, species of both tribes are named as cereals, phylogenetic relationships suggest that the separation between both groups began about 20 MYA[12]. The separation within the *Triticeae* tribe is more recent and has been established around two MYA; although a recent study suggests that these speciation events might have occurred along to the last ten million years[13].

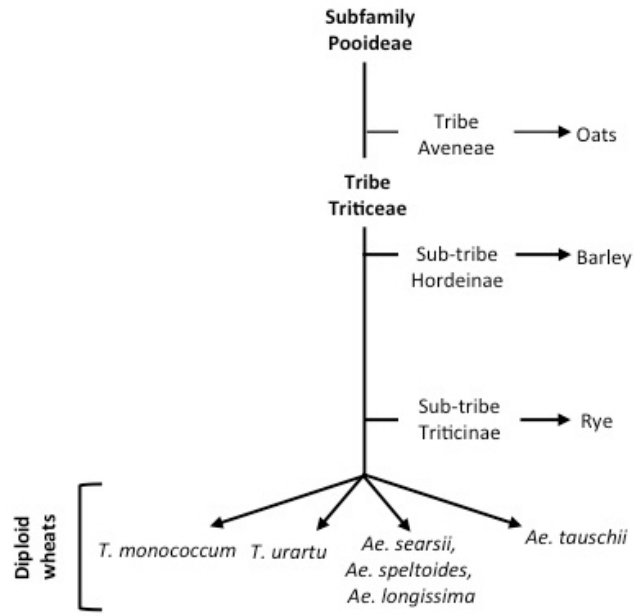

Figure 1. Origin and relationship of major cereals. Subfamily Pooideae *Bentham (oat, wheat, rye, and barley), comprised 15 tribes, being the tribes* Avenae *(oat) and* Triticeae *(barley, rye, and wheat) the most important.*

2.1. Oat

The genus *Avena* includes cultivated species with different ploidy level. Three cytogenetically independent stocks of *Avena* L. occurred during domestication (*A. sativa*, *A. strigosa*, and *A. abyssinica*), although only *A. sativa* became a principal cereal. Oats were probably evolved from weedy types that infected wheat and barley fields, and not under the domestication as a crop[14]. The hexaploid *A. sativa*, common oat (2n = 6× = 42), is widely cultivated, and its main uses are for human consumption (oat meals and rolled oats) and livestock feed. The wild relative of *A. sativa* is the hexaploid wild oat *A. sterilis*, whose first evidences of cultivation date ~11,400 years before present (BP), in which is thought to be a predomestication cultivation practice[15]. Besides, the first evidences of domesticated *A. sativa* cultivation have been found in Sacarovca, Moldavia (~7,600-7,400 BP).

2.2. Barley

Cultivated barley (*Hordeum vulgare*) (2n = 2× = 14; HH) constitutes one of the first domesticated crops, and one of the most important world crops. It evolved from its wild progenitor *H. spontaneum*, which was originated 5.5 MYA. Wild barley started to be harvested by humans as early as 50,000 years ago[16], and the main characters associated with its domestication were: non-brittle rachis, increased seed weight, six-rowed ears and naked seeds. The domestication of wild barley has been described in the literature to occur in several geographical locations, with at least three main centers of domestication: Fertile Crescent, Central Asia, and Tibet (reviewed in Greco et al[17]). However, molecular data obtained from wide collections of wild and cultivated barleys indicate a single origin for all modern varieties and landraces[18,19] consistent with a single domestication event, which probably took place in the Israel-Jordan area[17]. Compared to wheat, barley has an inferior staple and bread-making quality. However, it withstands drier conditions, poorer soils, and some salinity[14], which makes it an important crop in several areas. It is used for animal feed and for human consumption in soups, stews and barley bread, although its main use is for the production of beers (malt) and distilled beverages.

2.3. Rye

Domesticated rye belongs to the small genus *Secale* L. The domestication of this cereal has brought less attention than other cereals in the literature on the origin of agriculture since rye was not among the cereals that promoted the Agricultural Revolution. Some archaeological evidences found in the Euphrates valley in modern Syria indicate that the domestication of rye occurred around 11,500 BC[20], although as other cereals wild rye was cultivated long before its domestication. The wild progenitor of rye is thought to be *S. vavilovii*[14,21], which is fully inter-fertile in crosses with *S. cereale* and has been found in primary habitats[22]. Rye is particularly cultivated in Northern and Eastern Europe. It adapts well to acidic and sandy soils, and is

resistant to cold and dry conditions. The grain has a high content of proteins, and most of present world production is consumed in the form of bread[14].

2.4. Wheat

Wheat is one of the most important crops worldwide, and its extended cultivation is in part due to its high adaptability to different environments and its high yields, but also to the unique viscoelastic properties of wheat dough, which allow the entrapment of CO_2 during fermentation, enabling the preparation of leavened breads and other baked products. The domestication of wheat began around 10,000 years ago as part of the Agricultural Revolution, and it has been placed in the Near East, in the zone known as Fertile Crescent[23]. Wheat is a polyploidy complex formed by multiple species of different ploidy level, consequence of the merge of genomes from different species of the *Triticeae* tribe (Figure 2). Thus, diploid (2n = 2× = 14, AA), tetraploid (2n = 4× = 28, AABB), and hexaploid species (2n = 6× = 42, AABBDD) of wheat can be found.

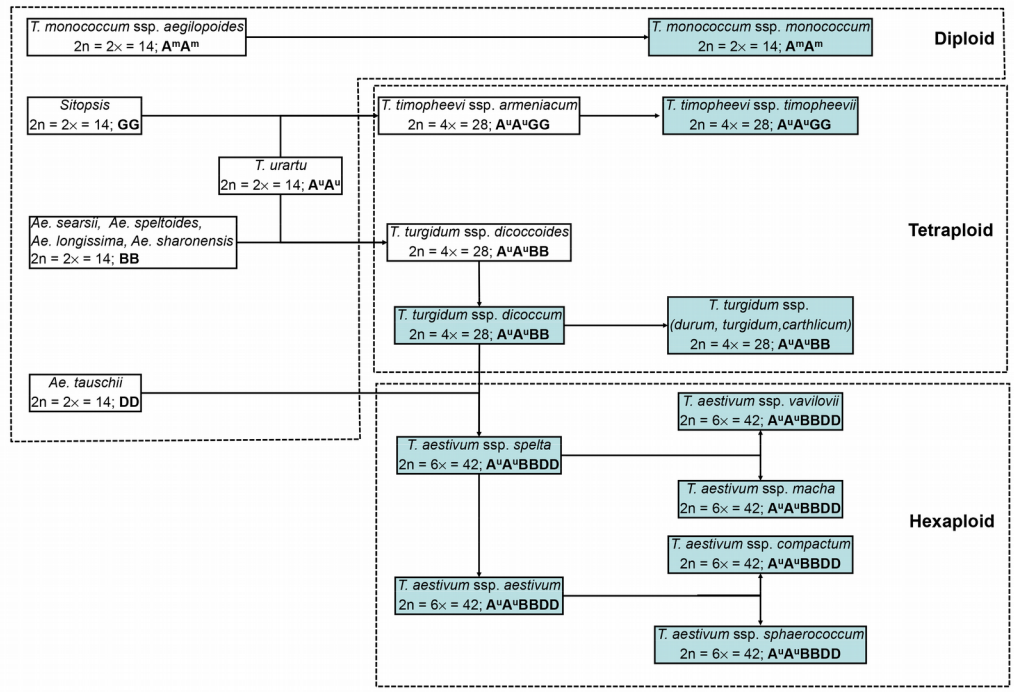

Figure 2. Wheat and its ancestral relatives. Hybridization events leading to tetraploid and hexaploid wheats. The species in gray are or have been cultivated.

The origin of the A and D genomes is well known. *T. urartu* Thum ex. Gandil (2n = 2× = 14, A^uA^u), a wild diploid specie, has been proposed as the donor of the A genome in polyploid species of wheat[24]. However, the origin of the B genome presents certain controversy. The currently accepted hypothesis suggests that *T. urartu* could have generated the wild tetraploid wheats mainly in two different events. On the one hand, by crossing with an *Aegilops* species (section *Sitopsis*), probably *Ae. speltoides* Tausch. (2n = 2× = 14, putative BB), and subsequent chromosome doubling it was generated wild emmer (*T. turgidum* ssp. *dicoccoides* Korn. ex Asch. & Graebner em. Thell., 2n = 4× = 28, A^uA^uBB), from which cultivated emmer (*T. turgidum* ssp. *dicoccum* Schrank Thell., 2n = 4× = 28, A^uA^uBB) was domesticated. The rest of the tetraploid wheats, including durum wheat (*T. turgidum* ssp. *durum* Desf. em. Husn.), as well as the hexaploid wheats derive from this species[25]. On the other hand, the crossing with some other species of the section

Sitopsis with *T. urartu* originated *T. timophevii* ssp. *armeniacum* Jakubz. em. Slageren (2n = 4× = 28, A^uA^uGG), which domesticated form (*T. timophevii* ssp. *timophevii*) is restricted to western Georgia. With respect to the D genome, several studies suggest that the donor of this genome is *Aegilops tauschii* Coss. (2n = 2× = 14, DD)[26,27], which after crossing with cultivated emmer (*Triticum turgidum* ssp. *dicoccum* Schrank em. Thell., 2n = 4× = 28, A^uA^uBB) and subsequent chromosome doubling, led to spelt (*T. aestivum* ssp. *spelta* L. em. Thell., 2n = 6× = 42, A^uA^uBBDD), putative ancestor of bread wheat (*T. aestivum* ssp. *aestivum* L. em. Thell.), the most important species of the genus *Triticum* today.

Despite the relative recent origin, wheat shows an enormous genetic variability that has allowed the development of around 25,000 different types[28]. Hexaploid bread wheat (AABBDD) represents ~95% of today's cultivated wheat, whereas durum wheat represents almost the other 5%. Cultivation of diploid wheats has been reduced to marginal lands.

3. Grain Storage Proteins of Cereals

Cereal grains contain relatively little protein compared to legume seeds, with about 10-12% dry weight. Storage proteins form approximately half of this protein, which can be included in four different fractions (albumins, globulins, prolamins and glutelins) according with their solubility. The gluten proteins of wheat classically fall into two of these fractions, with the alcohol-soluble gliadins being defined as prolamins and the alcohol-insoluble glutenins as glutelins. With exception of oats and rice, the main endosperm storage proteins in cereal grains are prolamins, which are so named because they present high content of proline and glutamine. On the contrary, in oats and rice the storage proteins are mainly globulins 11-12S, although the rice storage proteins have been classically classified as glutelins since they are not readily soluble in salt solutions[29].

In wheat, the prolamins are divided in two groups: gliadins and glutenins. The former are monomeric while the latter are polymeric. For this reason,

although both fractions are soluble in alcohol, the glutenins were originally classified as glutelin (alcohol-insoluble) because they have to be denatured by reducing agents (β–mercaptoethanol or dithiothreitol) to make them soluble in alcoholic solutions. Both protein groups are the major components of the gluten, which has been defined as "*the viscoelastic mass that remains after thoroughly washing out the starch from a dough*"[30]. This structure is the main responsible of the properties of the wheat flour that permit technological processes as bread making.

These proteins make up a complex mixture that can range between 50 components in hexaploid wheat and about 20 in diploid species[31]. Glutenins are classified into high molecular weight subunits (HMWGs) and low molecular weight subunits (LMWGs)[32]. The HMWGs, with molecular weights ranging between 80-140 kDa, are encoded by the *Glu-1* complex loci located on the long arm of each chromosome of group 1, called *Glu-A1*, *Glu-B1* and *Glu-D1*, respectively[33]. The LMWGs, meanwhile, have molecular weights between 30-50 kDa, and are encoded by the *Glu-A3*, *Glu-B3* and *Glu-D3* loci located on the short arm of group 1 chromosomes[34]. Gliadins are classified in *α/β*-, *γ*-and *ω*-gliadins, being synthesized also by genes on the short arm of group 1 chromosomes (*Gli-A1*, *Gli-B1* and *Gli-D1* loci that encode the *γ*- and *ω*-gliadins,) and group 6 chromosomes (*Gli-A2*, *Gli-B2* and *Gli-D2* loci that codes the *α*- and *β*-gliadins)[35]. Other minor loci of gliadins and glutenins have been also detected in the short arm of the group 1 chromosomes[36]. In a single bread wheat cultivar, the gluten proteins might be comprised of up to 45 different gliadins, 7 to 16 LMW-GS, and 3 to 6 HMW-GS. All those gluten proteins are synthesized and deposited in the starchy endosperm during grain development. Wieser[37] determined by reversed-phase HPLC, using a range of cultivated wheat species, that α-gliadins were predominant in most cases, followed by *γ*-gliadins and LMW-GS; *ω*-gliadins and HMW-GS were generally minor components.

The increased availability of detailed information on the molecular structures and genetics of the proteins present in glutenin and gliadin fractions has allowed them to be redefined into three groups, or families,

called sulfur-rich (S-rich), S-poor, and high molecular weight (HMW) prolamins. In wheat, the HMW prolamins are present only in glutenin polymers, while the S-rich prolamins are present as monomers (gliadins) and polymers (glutenins), and the S-poor predominantly (but not solely) as monomers[38].

Barley is a diploid species and therefore the genetics of storage proteins is much simpler than in hexaploid wheat. The prolamins of cultivated barley consist of S-rich B- and γ-hordein, S-poor C-hordein, and HMW D-hordein[39]. In cultivated barley all the hordein genes are linked in the short and long arms of chromosome 5, where they are organized in complex loci[40]. The C-hordeins are encoded in the *Hor 1* locus of the short arm of chromosome 5, whereas the D-hordeins are found in the *Hor 3* locus of the same chromosome[41].

All prolamin genes are intronless, and consist of several domains, being one of them a long repetitive domain formed by motifs with high content in proline and glutamine. The other domains present in these genes show high conservation of their nucleotide and amino acid sequences, suggesting that all these genes could derive from a unique ancestral gene[42]. However, gliadins and glutenins are not at the same amounts in the grain of cereals, their proportions can vary within a broad range and depends on both genotype (variety) and growing conditions (soil, climate, fertilization, etc.). The ratio gliadins to glutenins was examined in a range of cereals[43], and hexaploid common wheat showed the lowest ratio (1.5–3.1), followed by oats and spelt (1.7–3.3), barley (1.4–5.0), durum wheat and kamut (3.1–3.4), emmer wheat (3.5–7.6), rye (6.3–8.2), and einkorn wheat (4.0–13.9).

4. Wheat Breeding

In the Mediterranean region, wheat cultivation is linked to its flour transformation and consumption. The original consumption of the flour was in the form of porridge, since it does not require special conditions for its elaboration[44]. A more elaborated use is in the form of bread, whose first

written references go back to 4.6 thousand years ago (TYA); although, the archaeological findings indicate the possibility that it was already known in Babylon six TYA. However, the baking process was developed in the Ancient Egypt where the beer yeast (*Sacharomyces cerevesiae* L.) began to be used to ferment the dough[45].

Along the History, this process has suffered scarce changes. Until the Industrial Revolution, all baking processes were carried out by hand; this permitted the use of wheat varieties with rheological properties very different to those of the current wheat varieties. When the use of machinery in the baking processes started, producers were forced to look for varieties with very specific qualities[46]. The dough made with these flours must have a certain tolerance to mechanical mixing and over-mixing, a process that is very different from the manual process. Consequently, many traditional wheat varieties were neglected, mainly due to their smaller yields and in many cases to their difficult mechanization. Along with this, part of the allelic prolamin variation present in these ancient materials was lost, mainly in those regions where the replacement of landraces with improved varieties was indiscriminate, which has been particularly intense in the last century[47,48]. Fortunately, part of this variation missed in the fields was stored in Germplasm Banks, and now can be used to enlarge the genetic pool of the modern cultivars.

4.1. The Role of the Old Farmers in Wheat Breeding

The artificial selection of the plants is as old as the Agriculture. The old farmers began to select the traits that were better adapted to the use of each crop. This empirical process has made possible the generation of different materials for a same crop, for example, the classically mentioned case of *Brassica oleraceae*, one species that in hands of these farmers gave rise to such different crops as cabbage, cauliflower, kohlrabi, Brussels sprouts, broccoli and kale. If we think of wheat, it is possible to find peoples that throughout their history used hulled wheats as emmer or spelt, while others readily associated the naked wheat (durum or common wheat) to their diet.

Equally, the use or not of yeast originated wheat with different breadmaking characteristics. All these processes entailed the selection of the different allelic prolamin variants implicated in the technological processes, which joined to the physiological role that these proteins have in the wheat plant (source of amino acids during the germination) might have allowed the fixation of mutations in the repetitive domain of these proteins where the reactive peptides in relation to CD are located.

The dispersion of wheat cultivation from the Fertile Crescent has been documented around the 5th millennium B.C. both for the tetra- and hexaploid species[23]. This expansion was linked to the human migrations and the commercial exchanges between the Near-East peoples and other peoples of Asia, Europe and North of Africa. In this context, numerous events of genetic drift, due to serial founder effects and subsequent expansions, might have taken place. Furthermore, the adaptation to the climatic and edaphic conditions, together with the diversification of end uses, should have generated a large diversity within the crop. For this reason, the possibility that old farmers unconsciously selected the most toxic prolamins is scarce and without any scientific base.

4.2. The Scientific Wheat Breeding.

Although along of the 18th and 19th centuries, the effort of wheat breeders was notable, it was in the 20th century when the wheat breeding was significantly improved. At this respect, the introduction of the reduced height gene (*Rht8*), together with daylight-insensitive gene, had great importance in the development of wheat cultivars during the first decades of the past century. These genes, in combination with the increased availability of nitrogen fertilizer due to the Haber-Bosch process, substantially increased wheat yields around the World[49].

A central figure in the introduction of these traits into European germplasm was Nazareno Strampelli, an Italian wheat breeder that in 1913 developed varieties with shorter straw, lodging resistance and earlier maturity by the use of the Japanese variety Akakomugi. The Strampelli wheats were

lately used to develop wheat varieties worldwide. In 1952, other dwarfing genes (*Rht1* and *Rht2*) from other Japanese variety (Norin-10) were incorporated into the modern wheat varieties by US Department of Agriculture (USDA) breeders[49]. Norin-10 and its derivatives were transferred to the International Maize and Wheat Improvement Center (CIMMYT), in Mexico, and used by Norman Borlaug as part of the key varieties in the Green Revolution, which permitted increase yields worldwide and, in particular, some developing nations as India or Pakistan to greatly improve their food security.

As commented above, in some countries the substitution of the old landraces by these new wheat varieties was indiscriminate, and generated the loss of local genetic diversity. However, although some traits of these improved varieties come from a little number of landraces or old varieties, the genotypes used in the breeding programs of the Global Wheat Program of CGIAR (Consultative Group on International Agricultural Research) represent an important part of the worldwide genetic diversity of wheat.

5. Cereal Species and CD

From all the aforementioned cereals, wheat has been the most widely studied and discussed in relation with the development of the CD. Wheat gluten proteins are composed by the monomeric gliadins and the polymeric glutenins. The majority of CD reactive epitopes have been found in the gliadin fraction. Moreover, the immunotoxicity of many gluten peptides has been assessed by activation of gluten-specific T cells isolated from duodenal biopsies of CD sufferers, and α and γ-gliadins have been found to contain the vast majority of the epitopes triggering the CD[50]. These assays also showed that the number of immunotoxic epitopes identified in wheat gluten proteins and other grasses has significantly increased in the last years. Although wheats with different ploidy levels have been tested for differences in the content of immunoreactive peptides, there is very little information on the genetic diversity in cultivated wheat germplasm. Most studies have included

few genotypes from each species (Table 1) with the exception of two works (one of durum wheat and other of bread wheat), which included more than 30 accessions. On the other hand, the methodology used to assess toxicity was not the same for all studies (Table 1) and therefore, comparisons between species and ploidy levels are complicated.

Nevertheless, wheats with different ploidy levels have shown differences in the content of immunoreactive peptides. Some authors have identified diploid and tetraploid wheats, and even some old hexaploid wheat varieties, as a potential source of variability for the introduction of low CD toxic as a new breeding trait[51-53]. Molberg et al.[54] and Spaenij-Dekking et al.[51] found a large variation in the amount of CD4 T cell stimulatory peptides present in α-, γ-gliadins, and glutenins among diploid, tetraploid, and hexaploid wheat accessions. Similarly, variation for immunotoxicity in *Ae. tauschii*, as determined by epitope screening in expressed proteins, was found to be broader than for *T. aestivum* in a study that included 43 genotypes[55]. In that work, some *Ae. tauschii* genotypes expressed relatively less amounts of CD toxic epitopes. However, this variability does not mean lower toxicity as efficient recognition by α and γ-gliadin specific T-cell clones of the gluten digests of all the accessions was reported in another study[56].

Table 1. Revision of studies analyzing immunotoxicity of cultivated wheats of different level of ploidy: number of genotypes included in the study, type of protein and detection method.

Species	Number of accessions	Protein	Detection method	Reference
T. monococcum	15	Alpha and gamma gliadins	T-cell	[54]
T. monococcum	2	Alpha and gamma gliadins HMW and LMW glutenins	mAbs IFN-γ T-cell	[51]
T. monococcum	1	Alpha gliadins	Epitope screening	[69]
T. monococcum	1	NS[(a)]	IFN-γ	[70]

Species	Number of accessions	Protein	Detection method	Reference
T. monococcum	1	All[(b)]	Epitope screening	[71]
T. monococcum	3	Alpha gliadins	Epitope screening mAbs IFN-γ	[72]
T. monococcum	1	Gamma gliadins	Epitope screening T-cell	[73]
T. monococcum	2	NS	IFN-γ T-cell IL-15	[74]
T. monococcum	1	NS	T-cell	[75]
T. durum	10	Alpha and gamma gliadins	T-cell	[54]
T. durum	4	NS	T-cell	[75]
T. durum	6	Alpha gliadins	mAbs	[76]
T. durum	7	Alpha gliadins	Epitope screening	[77]
T. durum	51	Alpha gliadins	mAbs	[52]
T. aestivum	1	Alpha and gamma gliadins	T-cell	[54]
T. aestivum	5	Alpha and gamma gliadins HMW and LMW glutenins	mAbs IFN-γ T-cell	[51]
T. aestivum	1	NS	T-cell	[75]
T. aestivum	8	Alpha gliadins	Epitope screening	[77]
T. aestivum	2	Alpha gliadins	Epitope screening	[78]
T. aestivum	3	Alpha gliadins	mAbs	[52]

Species	Number of accessions	Protein	Detection method	Reference
T. aestivum	86	Alpha and gamma gliadins HMW and LMW glutenins	mAbs	[53]
T. aestivum	2	NS	IFN-γ anti-tTG	[79]

[(a)]Not specified; [(b)]All prolamin fractions.

In barley, all prolamins fractions are immunotoxic, but D- and C-hordeins have been reported as the most active in triggering the CD[57]. Efforts have been made to identify new varieties of barley with a reduced immunotoxicity. In this line, Tanner and colleagues[57] reported that barley lines lacking B- and C-hordeins had 20-fold reduced immunotoxicity compared with wild-type barley.

Rye is also among the "forbidden" cereals for CD patients and T-cell stimulatory epitopes have been detected in it[58]. However, little is known about the variability in the toxicity of different varieties of rye, including those used for wheat breeding in the Green Revolution.

6. Has Domestication and Breeding Increased the Immunotoxicity of Wheat?

Two types of selection operate (and complement each other) under domestication and breeding: (a) conscious or intentional selection applied by breeders for the traits of interest; (b) the unconscious or automatic selection caused by the fact that these plants were taken out of their original habitat and placed in the new (and usually very different) human-managed environments[59]. The genes for storage proteins have no adaptive value, they are neutral genes, and none of the major genes that regulate the main qualitative traits subjected to strong selection pressure in the domestication of wheat (i.e., loss of seed shattering and threshability) are located in chromosomal regions encoding prolamin genes, nor do most of

the quantitative trait loci (QTL) with small effects on the domestication syndrome[60]. Agronomic traits and adaptation to biotic and abiotic stresses have been the targets of this local selection. A meta-QTL analysis carried out to identify the major and consistent QTLs for the yield and its components has shown that among the prolamin loci only *Glu-A1* and *Glu-B1* for HMWGs were located in the vicinity of two of these meta-QTLs[61]. However selection for resistance to diseases and pests, major constraints of yield in crop plants, could have implied the selection of particular alleles of prolamins, as genes for disease resistance are distributed in gene rich regions all over the wheat genome, including those in group 1 and group 6 chromosomes where gliadin loci are encoded[62,63]. So, if the process of domestication increased the number of toxic peptides or favored gliadin genes with greater toxicity this would have been made unconsciously, and due to the small size of the selected population. Among the multiple and groundless criticisms attracted by the wheat varieties of the Green Revolution, is the little number of parents used by the breeders in the first phases, which imply low variation for prolamins, in particular for gliadins.

Proteomic and genomic data available from bread wheat and its diploid and tetraploid ancestors provide valuable information about prolamin proteins, which include the content of proline and glutamine and the abundance and frequency of CD related epitopes. This information is highly relevant as gliadin genes are rich in the amino acids proline and glutamine and the highly antigenic gluten epitopes are mainly located in the proline-rich regions[50]. So, if post-domestication mutation events have affected immunotoxic regions of gliadin genes, that would be reflected in differences in prolamin proteins among wheat species, and in particular between diploid and hexaploid wheats.

Full gliadin-related protein sequences for organisms indicated in Table 2 were analyzed for parameters such as protein length, the content of proline and glutamine, and the number of CD epitopes per sequence. In this analysis,

relevant gluten T-cell epitopes restricted by HLA-DQ molecules were considered[6].

Protein sequences of oats and barley, which separated first during cereal evolution (Figure 1), are shorter than those of rye and wheat and its ancestors (except *T. turgidum* ssp. *turgidum).* Two diploid ancestors of bread wheat; *Ae. speltoides* (BB) and *Ae. tauschii* (DD) show gliadin lengths of 308 and 298 amino acids, respectively, longer than that of bread wheat (Table 2). *T. turgidum* ssp. *Dicoccoides*, the ancestor of cultivated tetraploid wheats has gliadin proteins of 306 amino acids average length, significantly longer than that of *T. turgidum* ssp. *durum* and *T. turgidum* ssp. *turgidum*, with 287 and 259 amino acids, respectively. It seems that the process of domestication and breeding has not increased the length of gliadin-related proteins in cultivated bread or durum wheat. With respect to the content of proline (P) and glutamine (Q), there is a good correlation between protein length and the content of glutamine ($r^2 = 0.8328$), which indicates that the longer the sequence the higher the glutamine content.

Table 2. Proteomic analysis of gliadin-related proteins in cereals. Only complete protein sequences were considered.

Organism	Common name	Genome	Average length (1)	Proline (%)	Glutamine (%)	Epitopes /seq (2)
A. sativa	Oat	AACCDD	207 d	8.9	26.9	0.4 e
H. vulgare	Barley	HH	265 c	16.9	29.2	1.3 de
S. cereale	Rye	RR	296 ab	18.2	32.9	5.2 a
T. urartu	Wild form	A^uA^u	283 abc	15.5	31.6	3.2 bcd
Ae. speltoides	BB genome donor	BB	308 a	14.9	34.3	3.0 bcd
T. monococcum ssp. *aegilopoides*	Wild einkorn	A^mA^m	288 abc	15.4	31.0	3.4 bcd
T. monococcum ssp. *monococcum*	Cultivated einkorn	A^uA^u	281 bc	15.0	31.8	2.7 bcd
Ae. tauschii	DD genome donor	DD	298 a	15.7	33.5	5,1 a
T. turgidum ssp. *dicoccoides*	Wild emmer	A^uA^uBB	306 a	14.7	33.7	4,4 ab
T. turgidum ssp. *durum*	Macaroni wheat	A^uA^uBB	287 abc	14.7	32.8	2.2 cd
T. turgidum ssp. *turgidum*	Cone, rivet wheat	A^uA^uBB	259 c	16.7	31.9	4,1 ab
T. aestivum ssp. *aestivum*	Bread wheat	A^uA^uBBDD	291 ab	15.7	32.2	5,1 a

(1) Number of amino acids in mature peptides.
(2) Relevant gluten T-cell epitopes restricted by HLA-DQ molecules were considered[6].
Means within a column followed by the same letter are not significantly different at $p<0.05$, as determined by the LSD all-pairwise comparisons test.

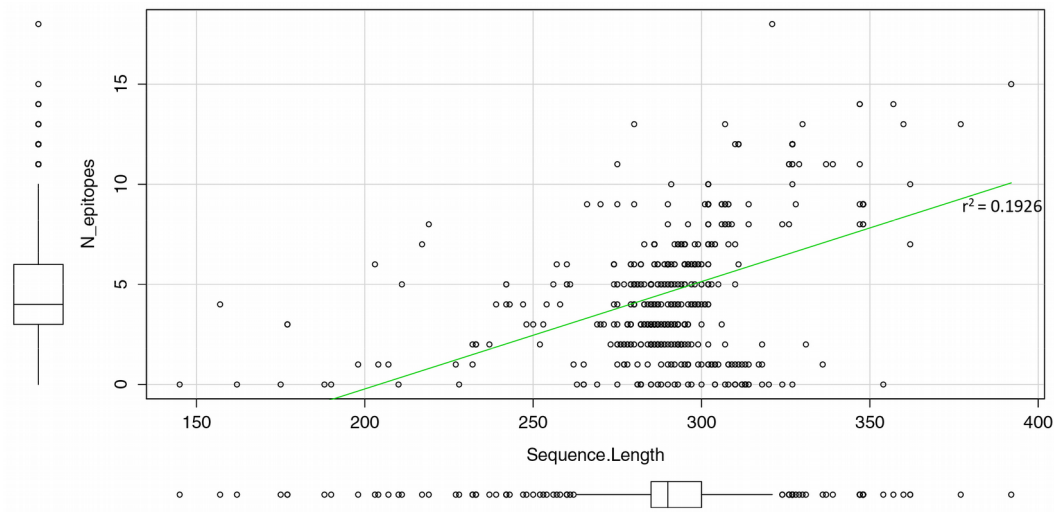

Figure 3. Distribution of number of CD epitopes vs gliadin protein lenght for species indicated in Table 2.

The number of CD related epitopes per sequence is also indicated in Table 2 and plotted in Figure 3. As showed, the lowest number of epitopes per sequence corresponds to oats and barley (Table 2). Rye, *Ae. tauschii*, and bread wheat have the highest number of CD related epitopes per sequence, 5.2, 5.1, and 5.1, respectively. *T. urartu* and *Ae. speltoides*, the donors of the AA and BB genomes, respectively, have a comparable number of epitopes per sequence, and significantly lower than that of *Ae. tauschii,* the donor of the DD genome in bread wheat. The natural hybridization between *T. urartu* and *Ae. speltoides* provided *T. turgidum* ssp. *diccocoides* (Figure 2), wild emmer. According with the gliadin protein data available, this hybridization process seems to have increased the number of CD epitopes per sequence to 4.4 (Table 2). Surprisingly, the development of modern durum wheat varieties (macaroni wheat) led to a significant decrease in the number of epitopes per sequence for this variety (*T. turgidum* ssp. *durum*) but nor for cone or rivet wheat (*T. turgidum* ssp. *turgidum),* which keeps a number of epitopes per sequence comparable to that of its wild ancestor *T. dicoccoides*. *Ae. tauschii* provided the DD genome to bread wheat and hence to modern cultivated hexaploid varieties by the natural hybridization with cultivated emmer

(*T. turgidum* ssp. *dicoccum*) (Figure 2). The number of CD epitopes per sequence was increased in bread wheat (5.1) with respect to wild emmer (4.4). Again, it seems that the hybridization process had increased the number of epitopes per sequence, and this is ascribable to *Ae. tauschii* in the hybridization process. If we look at Figure 3, there are a high number of sequences that contain more than 10 epitopes per sequence. Those sequences are present at a frequency comparable in *Ae. tauschii* and bread wheat (Figure 4A). On the contrary, sequences with low number of CD epitopes per sequence are present at high frequency in *Ae. speltoides*, *T. monococcum* ssp. *monococcum* and *T. turgidum* ssp. *durum* (Figure 4B).

It is clear that the natural hybridization processes described above resulted in genome duplication, and consequently the number of gliadin-related genes should have increased from diploids to tetraploids, and from tetraploids to hexaploids. So, not only the number of epitopes per sequence is relevant but also the number of genes containing those epitopes. There is little information about copy number determination of gliadin genes. Anderson et al.[64] estimated the copy number of α-gliadin genes both in bread and durum wheat. They reported 60 and 150 copies of α-gliadin genes in bread wheat (cv Chinese Spring and Cheyenne, respectively) and 90 for the durum line. However, Lafiandra et al.[65] resolved at least 16 major α-gliadin spots by 2-D PAGE of protein extracts from cv Cheyenne seed. This number is considerably less than the estimated 150 genes for the same cultivar. Among the possible explanations for this discrepancy are that many of the family members are pseudogenes and/or that single protein bands/spots could originate from multiple genes.

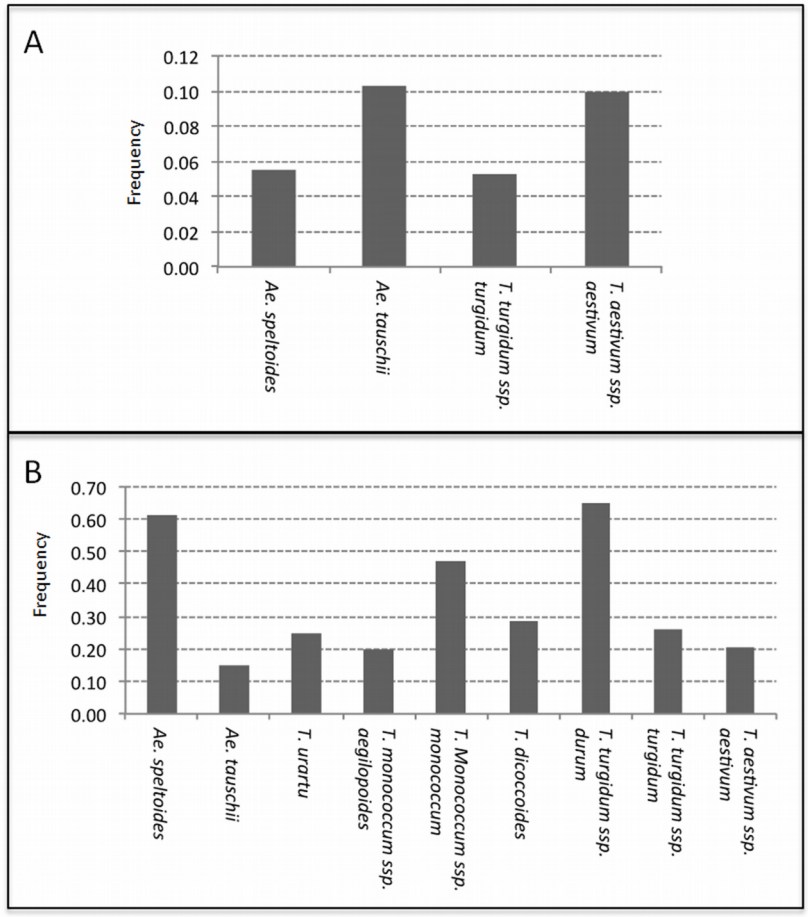

Figure 4. Frequency of sequences containing higher than 10 CD epitopes per sequence (A) or lower than 2 CD epitopes per sequence (B).

Anderson et al.[66] reported the complete set of unique γ-gliadin genes for the wheat cultivar Chinese Spring using a combination of expressed sequence tags (ESTs) and Roche 454 DNA sequences. They reported 11 active genes and two pseudogenes. Four of these genes were assigned to *Ae. tauschii* (the donor of the D genome of bread wheat) while the other γ-gliadins genes were assumed as being encoded in any of the A or B genomes[66].

Regarding the ω-gliadins, the precise number of ω-gliadin proteins and genes in wheat has not been determined. Sabelli and Shewry[67] used Southern blotting to suggest that bread wheat contained about 15-18 ω-gliadin genes. Anderson et al.[68] analyzed all available ω-gliadin DNA sequences and ESTs identified from the large wheat EST collection. They found three groupings of ω-gliadin active gene sequences assigned to each of the three hexaploid wheat genomes, and a fourth group consisting of pseudogenes assigned to the A genome. This is very interesting as active genes reported for each genome were as low as two, and most of ω-gliadin sequences were pseudogenes[68].

7. Conclusions

Wheat is one of the most important crops worldwide, and its extended cultivation is in part due to its high adaptability to different environments and its high yields. Bread wheat accounts for about 95% of cultivated wheat while durum wheat (macaroni wheat) represents only about 5%. The domestication of wheat is the result of a previous natural interspecific hybridization first between diploid, and then between diploid and tetraploid species that resulted in hexaploid wheat. The old farmers began to select the traits that were better adapted to the use. In the 20th century the wheat breeding had its great advance and modern varieties were developed. The gliadin-related genes, responsible for triggering CD, have no adaptive value and therefore, if the toxicity of wheat was increased during the process of domestication and breeding this would have been made unconsciously. During the process of natural hybridization, apparently as a consequence of genome

duplication, the number of gliadin-related genes was increased. Bread wheat, rye, and *Ae. tauschii* have the highest number of CD epitopes per gene, and it seems that in bread wheat, this high number of epitopes is explained by the D genome from *Ae. tauschii*. During the process of domestication and breeding, the number of CD epitopes per gene did not increase and even decreased in some cases. This large variation in the amount of CD4 T cell stimulatory peptides among diploid, tetraploid, and hexaploid wheat accessions is a valuable potential source of variability for the introduction of low CD toxic as a new breeding trait.

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