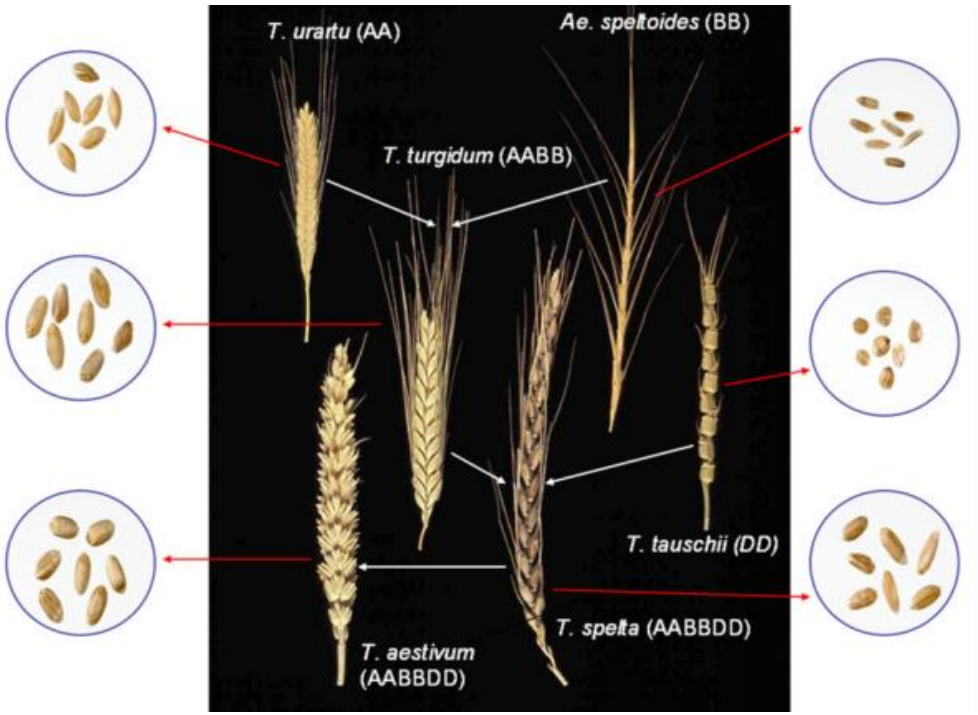
ALERGENICIDADE EM TRIGO

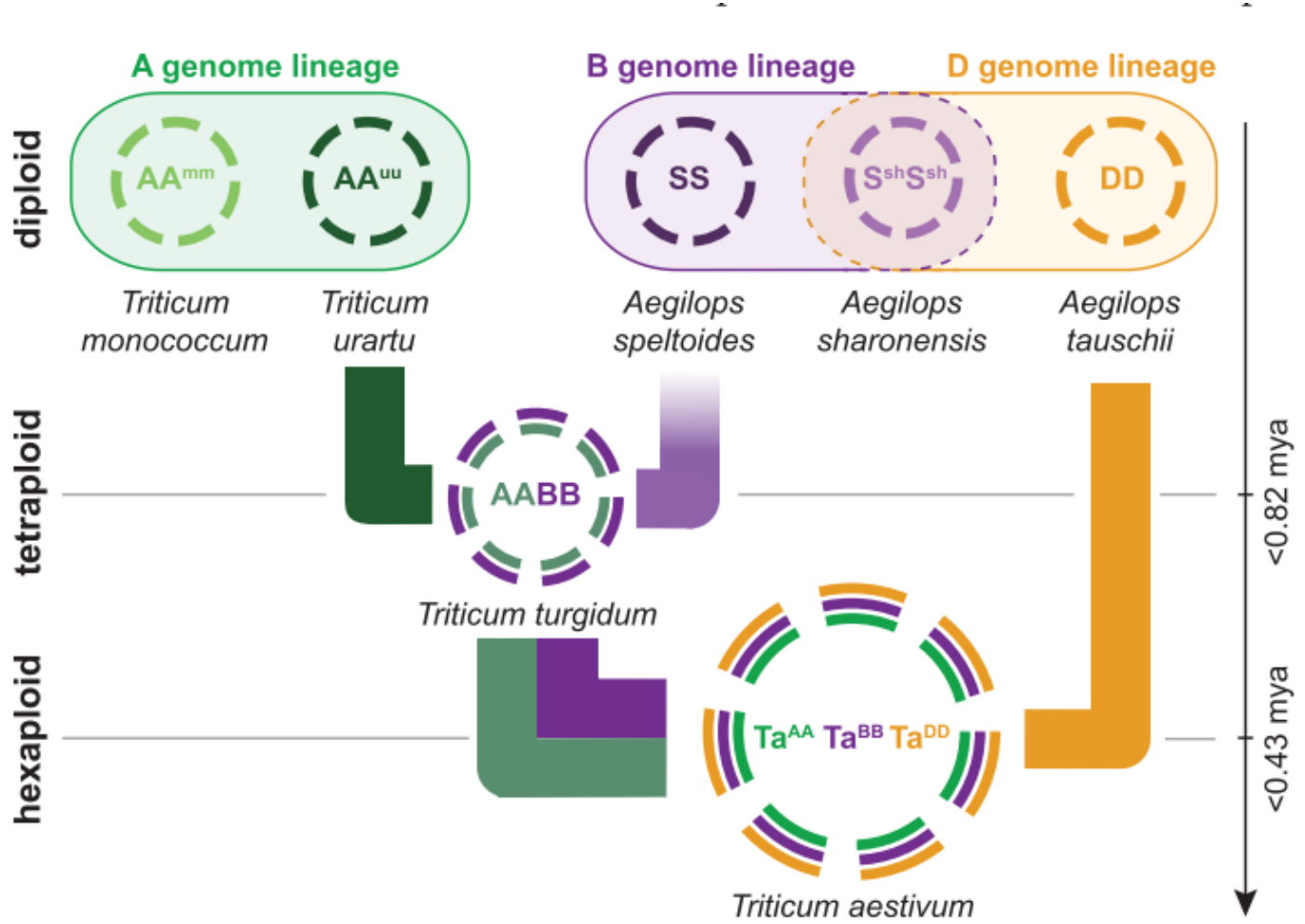
BRUNA MATTIONI
brunamattioni@gmail.com
UFSC

AGENDA

- Trigo
- Proteínas de cereais
- Glúten
- Digestibilidade
- Desordens relacionadas ao glúten
  - Imunomediadas
  - Alergias ao trigo
  - Sensibilidade ao Glúten Não Celíaca
- Proteínas envolvidas no desencadeamento de alergias ao trigo
- Considerações finais

TRIGO





Mayer et al., 2014

TRIGO

ARRANZ-OTAEGUI et al, 2018

TRIGO

Trigo panificável (*Triticum aestivum*)

Alimentos base para 30% da população mundial

EVERSOLE et al, 2016; ARRANZ-OTAEGUI et al, 2018

PROTEÍNAS DE CEREAIS

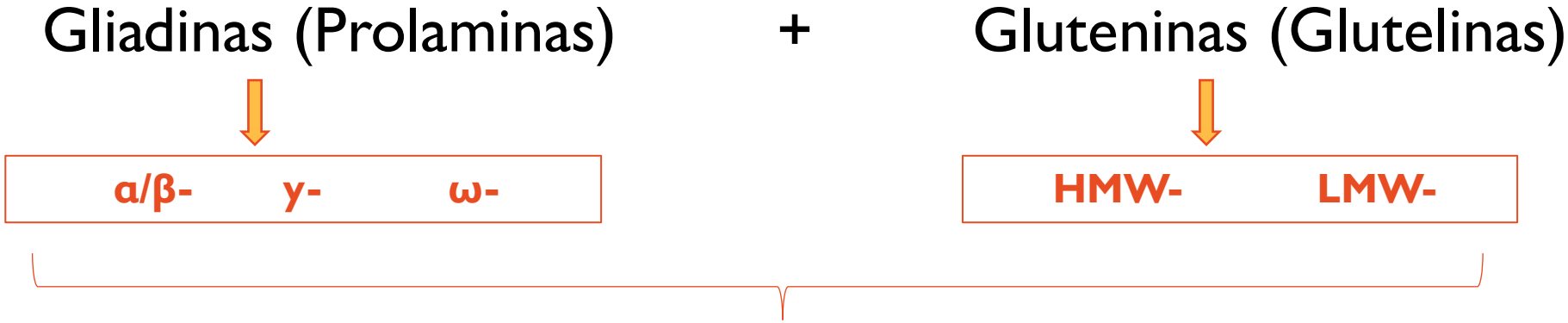
Classificação Osborne, de acordo com a solubilidade:

Albuminas	⇨	solúveis em água
Globulinas	⇨	insolúvel em água e solúvel em solução salina
Prolaminas	⇨	solúveis em solução alcoólica
Glutelinas	⇨	solúveis em ácidos diluído ou solução de NaOH

SMITH et al., 2010 Scherf et al., 2016

GLÚTEN

Glúten
(proteínas de reserva)



70-80% do total de proteínas do endosperma do trigo

O restante são globulinas e albuminas
(principalmente enzimas e inibidores, e proteínas estruturais insolúveis como lipoptoteínas de membrana)

SMITH et al., 2010; SCHERF et al., 2016

GLÚTEN

O número exato das proteínas do glúten não foi determinado

- 100 proteínas

Propriedade tecnológica do glúten

Gliadina (prolamina) e glutenina (glutelina)

SHEWRY, 2009; WIESER, 2007

DIGESTIBILIDADE

Proteínas do glúten são resistentes a completa digestão no trato gastrointestinal = longas cadeias peptídicas alcançam a mucosa intestinal

Os peptidios que alcançam a margem das vilosidades intestinais, e em indivíduos susceptíveis, passam a barreira do epitélio por via trans ou paracelular e chegam na lâmina própria.

Inicia-se as reações imune específicas de cada doença

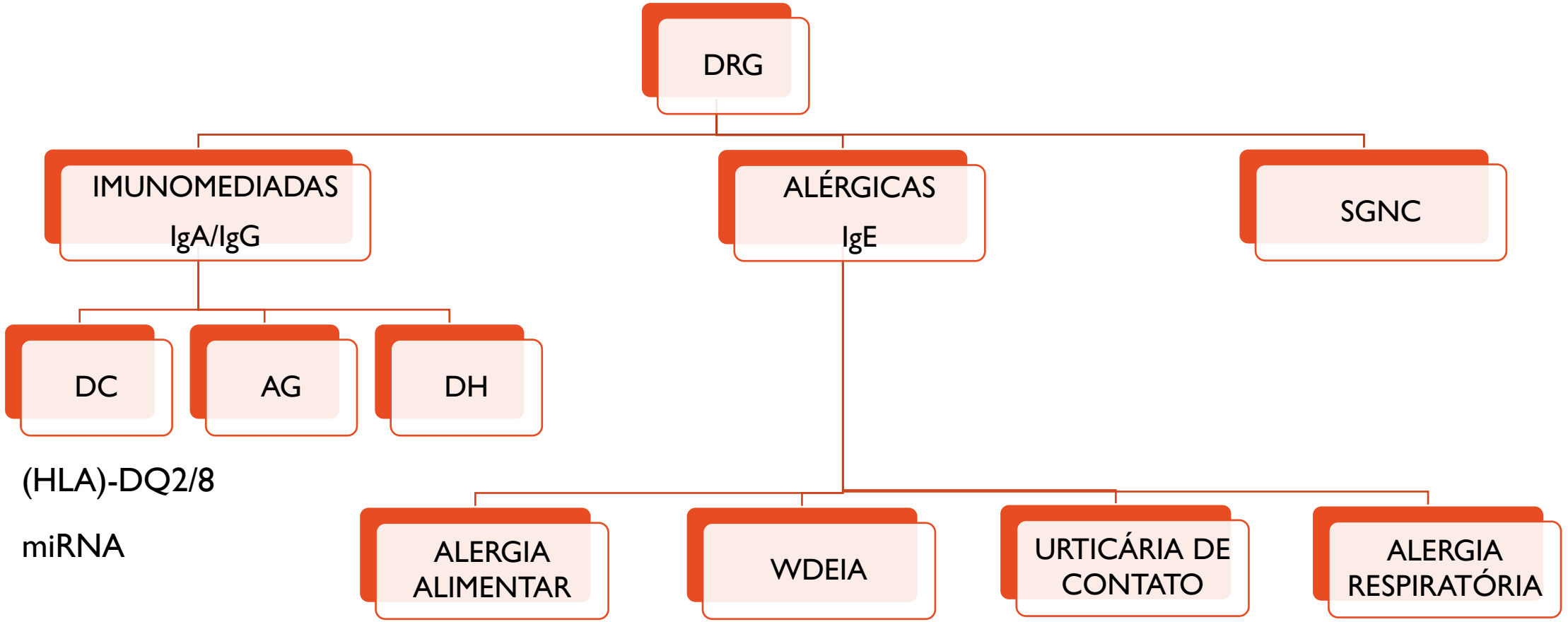
DC = resposta via células T e anticorpos IgA e IgG

AT = resposta de anticorpos IgE

SCHERF et al., 2016

DESORDENS RELACIONADAS AO GLÚTEN (DRG)

SCHERF et al., 2016



DC – DOENÇA CELÍACA; AG – ATAXIA PROVOCADA PELO GLÚTEN; DH – DERMATITE HERPETIFORME; SGNC – SENSIBILIDADE AO GLÚTEN NÃO CELÍACA;
WDEIA - ANAFILAXIA DEPENDENTE DE TRIGO INDUZIDA PELO EXERCÍCIO

DOENÇA CELÍACA

- Nem todo indivíduo que apresenta predisposição genética, desenvolve a doença

- Quem apresenta alguma imunomediada pelo glúten, normalmente apresenta outra doença autoimune

BROOKS et al., 2010; FASANO, 2011; SAPONE et al., 2012; WITHOFF et al., 2016

PROTEÍNAS ENVOLVIDAS NO DESENCADEAMENTO DE ALERGIAS AO TRIGO

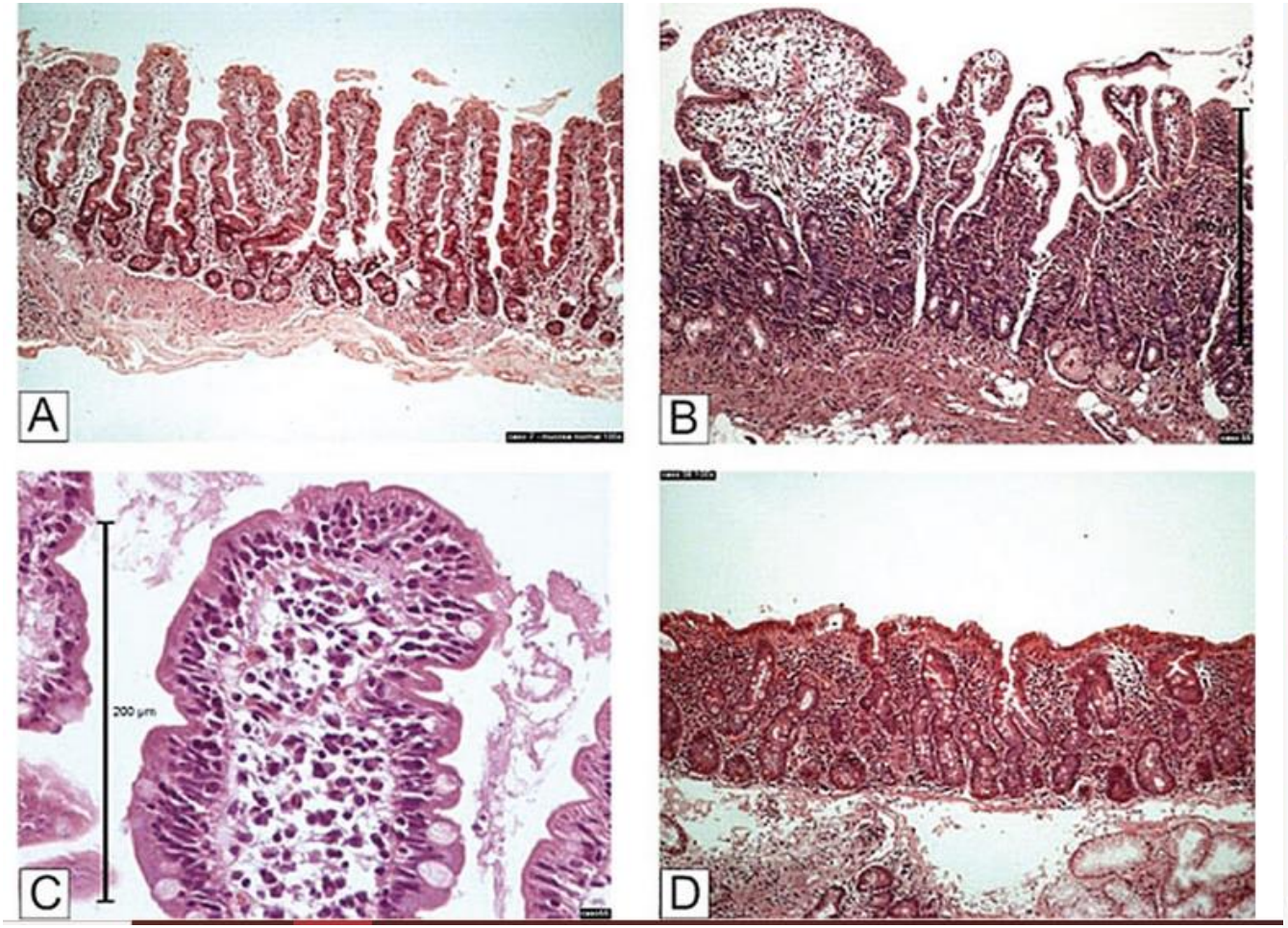
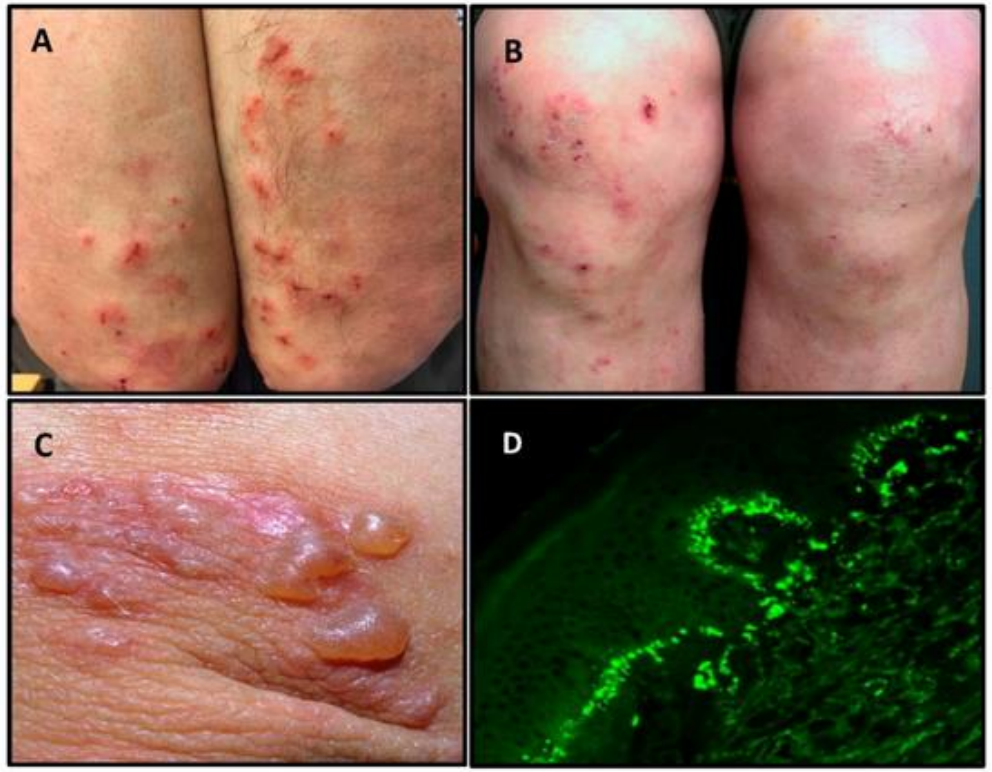


Figure 1. Dermatitis herpetiformis. Typical scratched papules and macules on the elbows (**A**), and on the knees (**B**). Fresh small blisters on the elbow (**C**). Direct immunofluorescence showing granular IgA deposits in the basal membrane zone between epidermis and dermis (**D**).



A prospective, double-blind, placebo-controlled trial to establish a safe gluten threshold for patients with celiac disease[1–3]

Carlo Catassi, Elisabetta Fabiani, Giuseppe Iacono, Cinzia D'Agate, Ruggiero Francavilla, Federico Biagi, Umberto Volta, Salvatore Accomando, Antonio Picarelli, Italo De Vitis, Giovanna Pianelli, Rosaria Gesuita, Flavia Carle, Alessandra Mandolesi, Italo Bearzi, and Alessio Fasano

ABSTRACT

Background: Treatment of celiac disease (CD) is based on the avoidance of gluten-containing food. However, it is not known whether trace amounts of gluten are harmful to treated patients.

Objective: The objective was to establish the safety threshold of prolonged exposure to trace amounts of gluten (ie, contaminating gluten).

Design: This was a multicenter, double-blind, placebo-controlled, randomized trial in 49 adults with biopsy-proven CD who were being treated with a gluten-free diet (GFD) for ≥2 y. The background daily gluten intake was maintained at <5 mg. After a baseline evaluation (t_0), patients were assigned to ingest daily for 90 d a capsule containing 0, 10, or 50 mg gluten. Clinical, serologic, and histologic evaluations of the small intestine were performed at t_0 and after the gluten microchallenge (t_1).

Results: At t_0, the median villous height/crypt depth (Vh/Cd) in the small-intestinal mucosa was significantly lower and the intraepithelial lymphocyte (IEL) count (× 100 enterocytes) significantly higher in the CD patients (Vh/Cd: 2.20; 95% CI: 2.11, 2.89; IEL: 27; 95% CI: 23, 34) than in 20 non-CD control subjects (Vh/Cd: 2.87; 95% CI: 2.50, 3.09; IEL: 22; 95% CI: 18, 24). One patient (challenged with 10 mg gluten) developed a clinical relapse. At t_1, the percentage change in Vh/Cd was 9% (95% CI: 3%, 15%) in the placebo group ($n = 13$), −1% (−18%, 68%) in the 10-mg group ($n = 13$), and −20% (−22%, −13%) in the 50-mg group ($n = 13$). No significant differences in the IEL count were found between the 3 groups.

Conclusions: The ingestion of contaminating gluten should be kept lower than 50 mg/d in the treatment of CD. *Am J Clin Nutr* 2007; 85:160–6.

KEY WORDS Gastroenterology, celiac disease, gluten toxicity

histologic remission (1). However, it is almost impossible to maintain a diet with a zero gluten content because gluten contamination is very common in food. "Hidden" gluten (used as a protein filler) may be found in commercially available products, such as sausages, soups, soy sauces, and ice cream. Even products specifically targeted to dietary treatment of CD may contain tiny amounts of gluten proteins, either because of the cross-contamination of originally gluten-free cereals during their milling, storage, and manipulation or because of the presence of wheat starch as a major ingredient.

The potential toxicity of trace amounts of gluten is still unclear. We previously showed in treated CD patients that the 4-wk ingestion of 100–500 mg gliadin/d (roughly equivalent to 200–1000 mg gluten) is able to cause measurable changes in the architecture of the small-intestinal mucosa (2). Only limited data are available on the toxicity of lower doses of gluten (3–6). This is an important issue because the daily ingestion of contaminating gluten in apparently well-treated CD patients is most likely to range from 5 to 50 mg.

Establishing a safe threshold of gluten consumption for CD patients is a matter of major public health importance, particularly in light of the recent reports concerning the high prevalence

[1] From the Center For Celiac Research, University of Maryland School of Medicine, Baltimore, MD (CC and AF); the Department of Pediatrics, Università Politecnica delle Marche, Ancona, Italy (CC, EF, and GP); the Department of Gastroenterology, Children Hospital, Palermo, Italy (GI); the University Department of Gastroenterology, Catania, Italy (CD); the University Department of Pediatrics, Bari, Italy (RF); the University Department of Gastroenterology, Pavia, Italy (FB); the University Department of Internal

RESEARCH ARTICLE

CELIAC DISEASE

Comprehensive, Quantitative Mapping of T Cell Epitopes in Gluten in Celiac Disease

Jason A. Tye-Din,[1,2,3]* Jessica A. Stewart,[1]* James A. Dromey,[1]* Tim Beissbarth,[1]*† David A. van Heel,[4] Arthur Tatham,[5] Kate Henderson,[6] Stuart I. Mannering,[1]‡ Carmen Gianfrani,[7] Derek P. Jewell,[8] Adrian V. S. Hill,[9] James McCluskey,[10] Jamie Rossjohn,[6] Robert P. Anderson[1,3]§

(Published 21 July 2010; Volume 2 Issue 41 41ra51)

Celiac disease is a genetic condition that results in a debilitating immune reaction in the gut to antigens in grain. The antigenic peptides recognized by the T cells that cause this disease are incompletely defined. Our understanding of the epitopes of pathogenic $CD4^+$ T cells is based primarily on responses shown by intestinal T cells in vitro to hydrolysates or polypeptides of gluten, the causative antigen. A protease-resistant 33–amino acid peptide from wheat α-gliadin is the immunodominant antigen, but little is known about the spectrum of T cell epitopes in rye and barley or the hierarchy of immunodominance and consistency of recognition of T cell

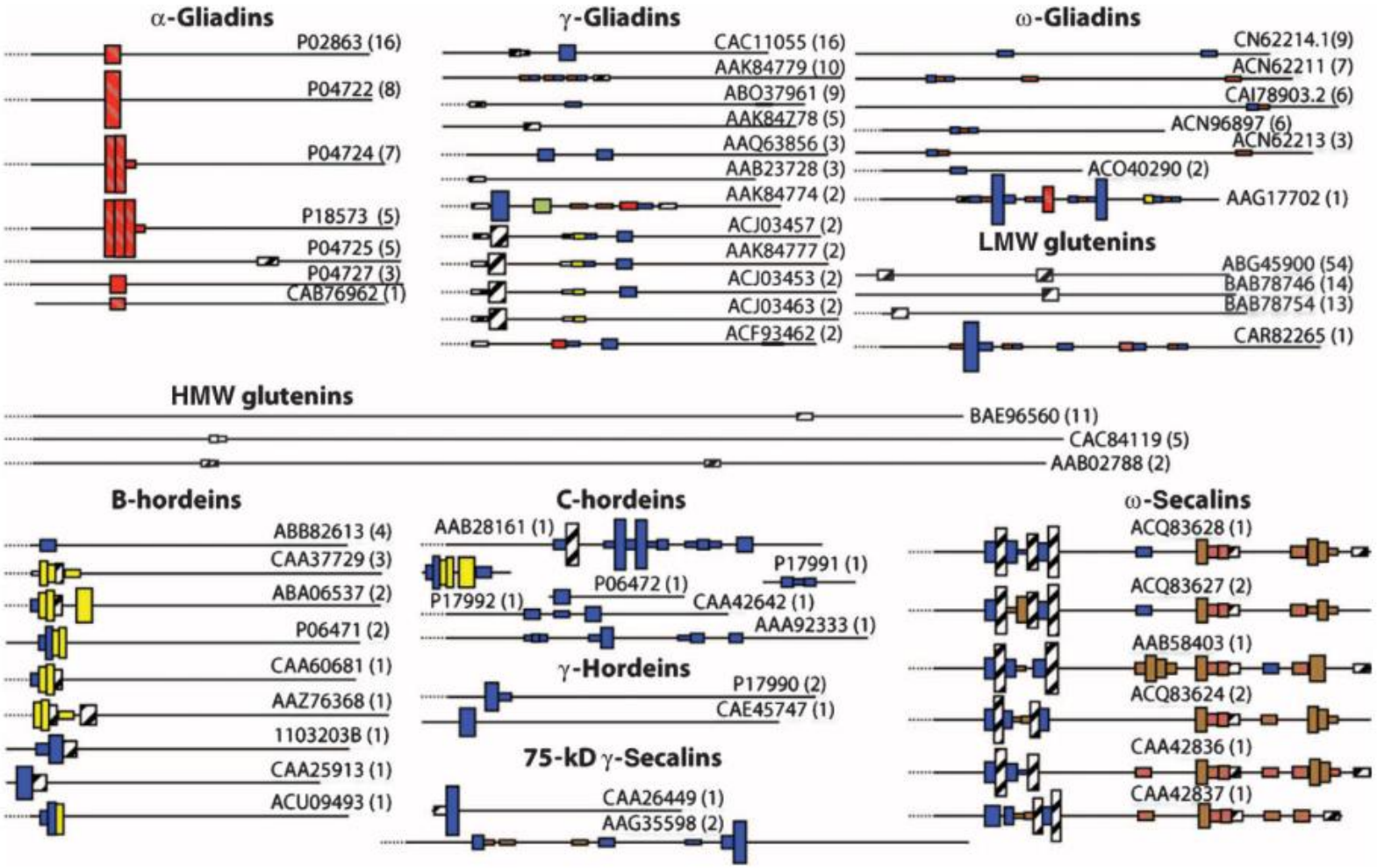


Fig. 5. The distribution of immunodominant sequences is restricted in α-gliadins but not in γ-gliadin, ω-gliadin, hordein, or secalin. Shaded boxes indicate the location and T cell stimulation score (vertical height) of peptide sequences derived from selected immunogenic gluten proteins in wheat, barley, and rye (dotted line indicates signal sequence). The box color indicates peptide recognition by TCCs specific for dominant wheat α-gliadin epitopes (DQ2-α-I or DQ2-α-II) (red), wheat ω-gliadin/barley hordein epitopes (DQ2-ω-I or DQ2-ω-II) (blue), B/C-hordein epitope (DQ2-Hor-I) (yellow), rye ω-secalin epitope (DQ2-Sec-I) (brown), and γ-gliadin epitope contained in W11-E7 (green). Colored hatched boxes indicate sequences recognized by TCCs specific for more than one of these peptides. Black/white hatched boxes indicate sequences not recognized by any of these clones. The number in brackets after GenBank accession numbers indicates the number of other polypeptides found in GenBank that share similar sequences and possess the same T cell–stimulatory peptides in the same relative positions.

T cell clones (TCCs)

Review

EXPERT REVIEWS

Proteomic analysis in allergy and intolerance to wheat products

Expert Rev. Proteomics 8(1), 95–115 (2011)

Gianfranco Mamone[1], Gianluca Picariello[1], Francesco Addeo[2] and Pasquale Ferranti[†2]

[1]*Istituto di Scienze dell'Alimentazione (ISA) – CNR, Via Roma 52 A/C, 83100 Avellino, Italy*
[2]*Dipartimento di Scienza degli Alimenti, University of Naples 'Federico II', Parco Gussone, Portici, I-80055, Italy*

Owing to its extensive use in the human diet, wheat is among the most common causes of food-related allergies and intolerances. Allergies to wheat are provoked by ingestion, inhalation or contact with either the soluble or the insoluble gluten proteins in wheat. Gluten proteins, and particularly the gliadin fraction, are also the main factor triggering celiac disease, a common enteropathy induced by ingestion of wheat gluten proteins and related prolamins from oat, rye and barley in genetically susceptible individuals. The role of gliadin and of its derived peptides in eliciting the adverse reactions in celiac disease are still far from being completely explained. Owing to its unique pathogenesis, celiac disease is widely investigated as a model immunogenetic disorder. The structural characterization of the injuring agents, the gluten proteins, assumes a particular significance in order to deepen the understanding of the events that trigger this and similar diseases at the molecular level. Recent developments in proteomics have provided an

Table 1. Main cloned wheat allergens, including original references and database accession number(s) in Protein Knowledgebase (UniProtKB)/TrEMBL at SwissProt.

Protein name	Database accession number(s) in UniProtKB/SwissProt (EMBL entry)	Entry name	Original ref.
Proteins involved in respiratory allergy			
Thioredoxin h1-type (Tri a 25. 13 kDa)	Q4W1F7 (AJ890020)	Q4W1F7_MAIZE	[11]
Thioredoxin H-type protein (Tri a 25. 13 kDa)	Q4W1F6 (AJ890021)	Q4W1F6_MAIZE	[11]
Thioredoxin h1-type protein (Tri a 25. 13 kDa)	64394 (X69915)	TRXH_WHEAT	[11,12]
D-type LMW glutenin subunits	B6ETR9 (FM212916)	B6ETR9_WHEAT	[13]
D-type LMW glutenin subunits	B6ETS1 (FM212928)	B6ETS1_WHEAT	[13]
LMW glutenin subunits	B6ETS0 (FM212927)	B6ETS0_WHEAT	[13]
LMW glutenin subunits	Q8H0J4 (AJ519838)	Q8H0J4_WHEAT	[13]
LMW glutenin subunits	Q8H0J5 (AJ519835)	Q8H0J5_WHEAT	[13]
LMW glutenin subunits	Q8GU18 (AJ519837)	Q8GU18_WHEAT	[13]
Putative LMW glutenin subunits	Q571Q5 (AJ937920)	Q571Q5_WHEAT	[13]
Thiol reductase (Tri a Bd. 27 kDa protein)	Q7Y1Z2 (AB085212)	Q7Y1Z2_WHEAT	[14]
ω5-gliadin	Q40215 (AB181300)	Q40215_WHEAT	[15]
ω5-gliadin (putative ω-gliadin)	Q571R2 (AJ937839)	Q571R2_WHEAT	[16]
α/β-gliadin A-III	Q41546 (X02540)	Q41546_WHEAT	[17]
Wheat serin protease inhibitor-like allergen	B3FHM6 (EU051824)	B3FHM6_WHEAT	[18]
Lipid transfer protein Tri a 14	Q8GZB0 (AJ852536)	Q8GZB0_WHEAT	[19]
Proteins involved in food allergy			
α/β-gliadin A-III	P04723 (M11076)	GDA3_WHEAT	[20]
γ-gliadin	P04853 (M16064)	GDB2_WHEAT	[20]
HMW glutenin subunit (y-type repetitive domain)	P10387 (X12929)	GLT0_WHEAT	[20]
HMW glutenin subunit DX5 (x- and y-type)	P10388 (X12928)	GLT5_WHEAT	[20]
LMW glutenin subunit	P10385 (X07747)	GLTA_WHEAT	[12,20]
LMW glutenin subunit 1D1	P10386 (X13306)	GLTB_WHEAT	[12,20]

EMBL: European Molecular Biology Laboratory; HMW: High-molecular-weight; LMW: Low-molecular-weight.
Data from [201].

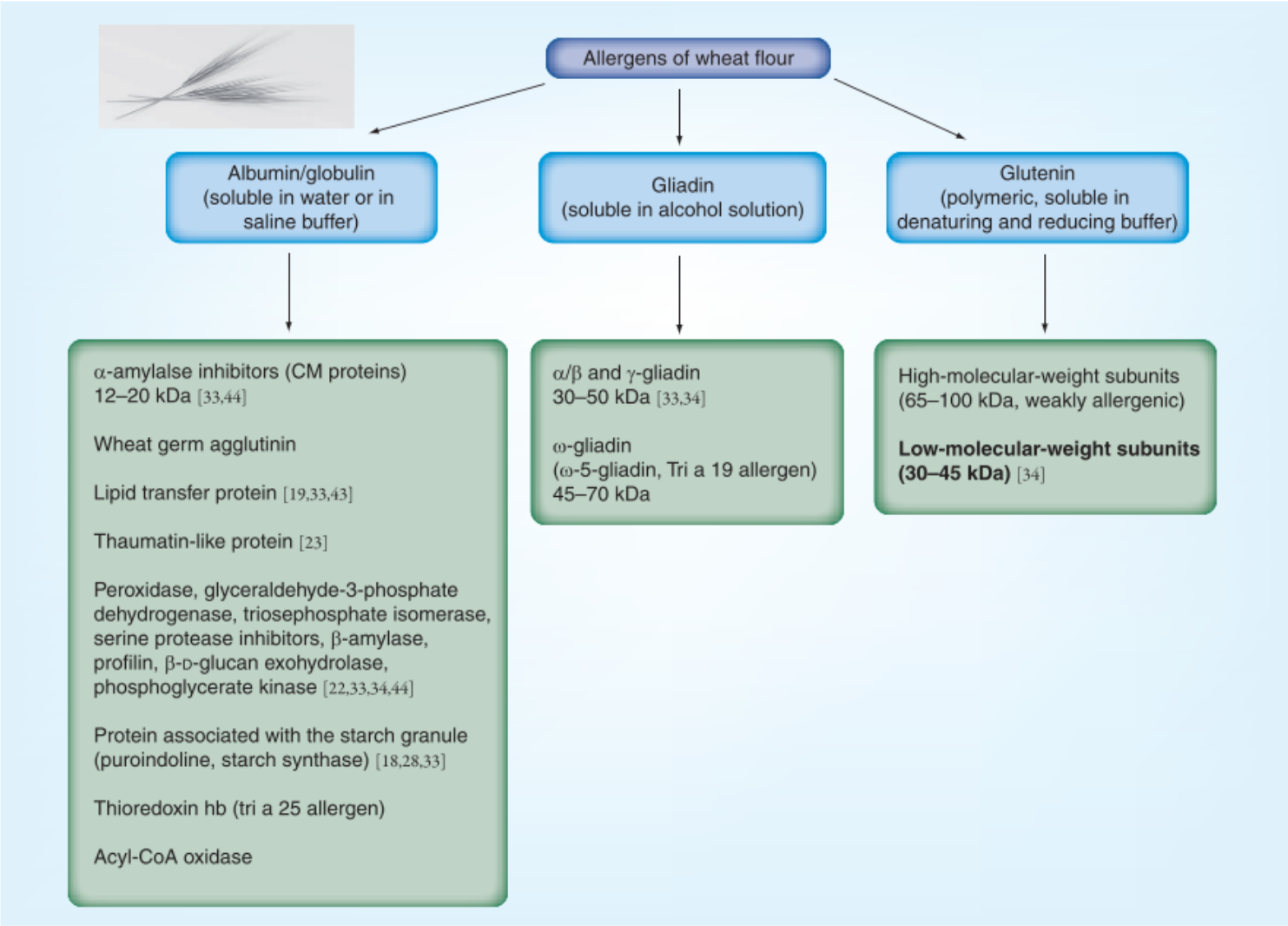


Figure 2. Classification of the different groups of allergenic proteins identified in wheat flour. The most important group is in bold. Many allergens have been identified through the application of proteomic strategies in combination with genetic, clinical and immunological approaches. The reference numbers are reported in those cases where the contribution of proteomic science to allergen definition has been fundamental.

SCIENTIFIC REPORTS

natureresearch

OPEN

Comparative quantitative LC–MS/MS analysis of 13 amylase/trypsin inhibitors in ancient and modern *Triticum* species

Sabrina Geisslitz[1,2], C. Friedrich H. Longin[3], Peter Koehler[4] & Katharina Anne Scherf[1,2]✉

Amylase/trypsin inhibitors (ATIs) are major wheat allergens and they are also implicated in causing non-celiac gluten sensitivity and worsening other inflammatory conditions. With only few studies on ATI contents in different *Triticum* species available so far, we developed a targeted liquid

UniProtKB accession	UniProtKB name	Abbreviation	Amino acids	Number of theoretical peptides[a]	Number of identified peptides[b]
P01083	Alpha-Amylase Inhibitor 0.28	0.28	153	9	7
P01085	Alpha-Amylase Inhibitor 0.19	0.19	124	7	7
P01084	Alpha-Amylase Inhibitor 0.53	0.53	124	7	6
P16850	Alpha-Amylase/Trypsin Inhibitor CM1	CM1	145	5	3
P16851	Alpha-Amylase/Trypsin Inhibitor CM2	CM2	145	4	2
P17314	Alpha-Amylase/Trypsin Inhibitor CM3	CM3	168	9	5
P16159	Alpha-Amylase/Trypsin Inhibitor CM16	CM16	143	5	4
Q41540	CM17 Protein	CM17	143	4	4
P16347	Endogenous Alpha-Amylase/Subtilisin Inhibitor	WASI	180	8	6
Q43723	Trypsin/alpha-Amylase Inhibitor CMX1/CMX3	CMX1/3	121	5	1
Q43691	Trypsin/alpha-Amylase Inhibitor CMX2	CMX2	121	5	1
P83207	Chymotrypsin Inhibitor	WCI	119	3	3
–[c]	Wheat Trypsin Inhibitor	WTI	137	7	3

Table 1. Overview of the 13 ATIs from wheat quantitated by targeted LC–MS/MS. [a]Derived from in silico tryptic digestion with a minimal number of eight and a maximal number of 26 amino acids. [b]Identified by at least five transitions at the same retention time. For amino acid sequences of all identified peptides see Supplementary Table S1. [c]Sequence according to Altenbach et al.[19]; similar to UniProtKB number A0A1D5UB33 (AAI domain-containing wheat protein), 70% identity.

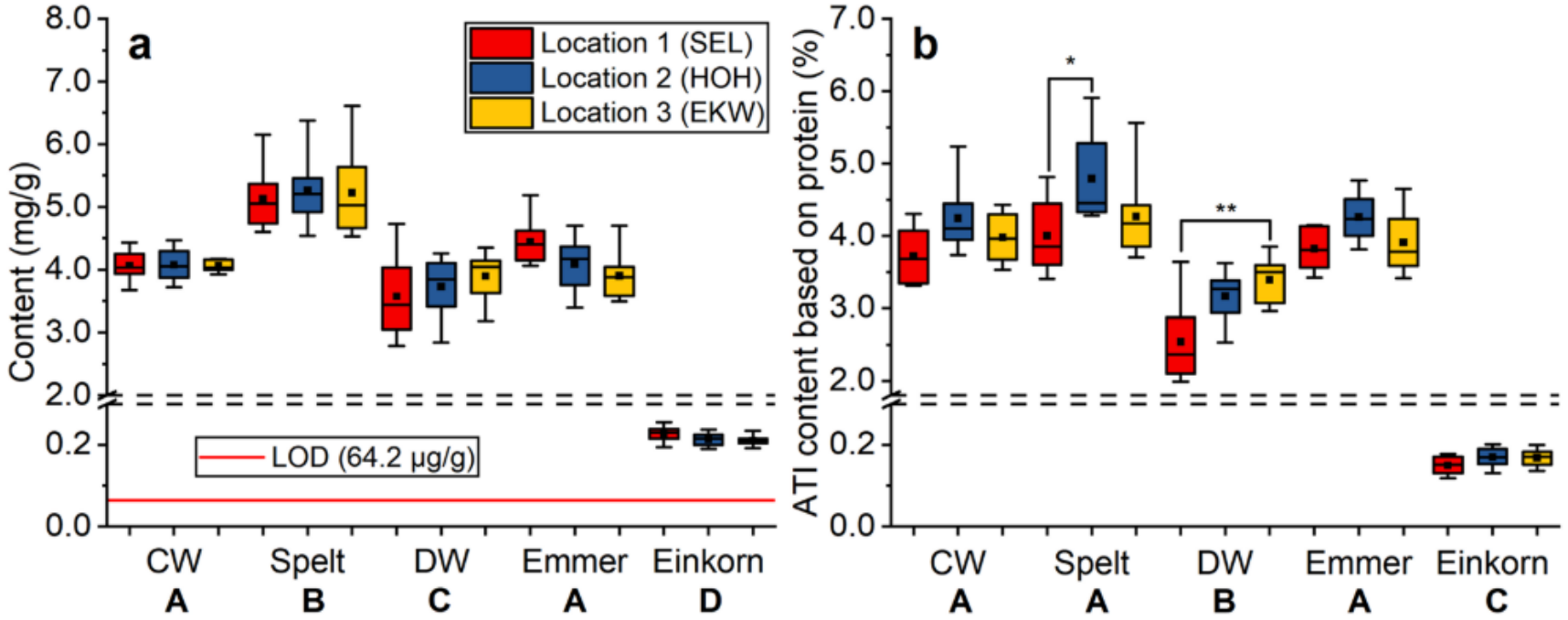


Figure 2. Total ATI contents (mg/g) **(a)** and percentage of the total ATI content relative to total protein content **(b)** of eight cultivars per box grown at the three locations Seligenstadt (SEL), Hohenheim (HOH) and Eckartsweiher (EKW). Boxes, lines and whiskers are presented as in Fig. 1. Boxes marked with asterisks are significantly different (one-way ANOVA with Tukey's test, * $p < 0.05$, ** $p < 0.01$) and different capital letters below the x-axis indicate significant differences between the wheat species.

CW = trigo comum; DW = trigo duro

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Gluten-related disorders: Celiac disease, wheat allergy, and nonceliac gluten sensitivity

Beatriz Cabanillas

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Table 2. Wheat proteins implicated in IgE-mediated wheat allergy.

Wheat allergen	Biochemical name	Molecular weight (kDa)
Tri a 12	Profilin	14
Tri a 14	Nonspecific lipid transfer protein 1	9
Tri a 15	Monomeric alpha-amylase inhibitor 0.28	13.2
Tri a 17	Beta-amylase	56
Tri a 18	Agglutinin isolectin 1	21.2
Tri a 19	Omega-5 gliadin	65
Tri a 20	Gamma gliadin	35–38
Tri a 21	Alpha-beta-gliadin	32.6
Tri a 25	Thioredoxin	13.3
Tri a 26	High molecular weight glutenin	88
Tri a 27	Thiol reductase homolog	27
Tri a 28	Dimeric alpha-amylase inhibitor 0.19	13
Tri a 29	Tetrameric alpha-amylase inhibitor CM1/CM2	13
Tri a 30	Tetrameric alpha-amylase inhibitor CM3	16
Tri a 31	Triosephosphate-isomerase	26.8
Tri a 32	1-cys-peroxiredoxin	23.9
Tri a 33	Serpin	43.3
Tri a 34	Glyceraldehyde-3-phosphate-dehydrogenase	36.5
Tri a 35	Dehydrin	11.5
Tri a 36	Low molecular weight glutenin GluB3-23	40
Tri a 37	Alpha purothionin	12
Tri a 39	Serine protease inhibitor-like protein	9.9
Tri a 40	Chloroform/methanol-soluble 7 protein (alpha amylase inhibitor)	15.96
Tri a 41	Mitochondrial ubiquitin ligase activator of NFKB 1	6.9
Tri a 42	Hypothetical protein from cDNA	8.2
Tri a 43	Hypothetical protein from cDNA	11.6
Tri a 44	Endosperm transfer cell specific PR60 precursor	11.9
Tri a 45	Elongation factor 1 (EIF1)	9.7

Source: Allergen Nomenclature Sub-Committee IUIS.

Celiac Database Search

Peptide Exact Match

Candidate proteins derived from members of the Pooideae subfamily of grasses would be screened as query sequences by comparison against the 1013 peptides using an EXACT MATCH algorithm. This is the primary screening tool and most likely to identify a protein representing a risk of eliciting CD. However, exact matches to a 9 AA peptide may occur to some non-Pooideae, non-gluten proteins, see peptide 68 as an example. It matches a

BROWSE ENTRIES

By Peptides '1013'

By References '72'

By Proteins '72'

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CONSIDERAÇÕES FINAIS

Com o surgimento de metodologias há um estudo crescente para tentar elucidar, dentre as atuais proteínas no trigo e outros cereais, quais estão relacionadas com DRG.

- Quais fatores estão relacionados com o desencadeamento de resposta alérgica ou expressão de uma doença imunomediada?
- Caso de países com mais e menos celíacos
  - Álcool?
  - Tabaco?
  - Agrotóxicos?
  - Estresse?
  - Poluição?

ALERGENICIDADE EM TRIGO

BRUNA MATTIONI
brunamattioni@gmail.com
UFSC