Journal of Cereal Science 59 (2014) 62–69

Contents lists available at ScienceDirect

Journal of Cereal Science

journal homepage: www.elsevier.com/locate/jcs

# Genetic and environmental factors affecting pathogenicity of wheat as related to celiac disease

Barbara Prandi [a], Paola Mantovani [b], Gianni Galaverna [a], Stefano Sforza [a,*]

[a] Department of Food Science, University of Parma, Parco Area delle Scienze 95A, I-43124 Parma, Italy
[b] Società Produttori Sementi S.p.A., Via Macero 1, Argelato, Bologna, Italy

ARTICLE INFO

*Article history:*
Received 8 April 2013
Received in revised form
27 August 2013
Accepted 10 October 2013

*Keywords:*
Gluten peptides
Durum wheat
Celiac disease
Varietal screening

ABSTRACT

Gluten proteins are the basis of the rheological properties of wheat derived products, such as bread and pasta. Their particular amino acidic composition (high proline and glutamine content) is responsible for the poor gluten digestibility. Some of the high molecular weight peptides that are generated in the gastrointestinal tract are involved in an autoimmune entheropathy called celiac disease. In this work we compared the amount of peptides containing sequences involved in adaptive and immune responses, which were produced after simulated gastrointestinal digestion of prolamins extracted from different durum wheat varieties and in-bred lines. Peptides containing sequences involved in celiac disease were quantified using an isotopically labeled peptide as internal standard. The results demonstrated a very high variability in the amount of pathogenic peptides produced by different lines, showing a strong contribution of the genetic component. At the same time, the variability in total protein and gluten content was lower; the weak correlation between pathogenic peptides and the amount of gluten proteins gives rise to the possibility of a varietal selection aimed to maintain good rheological properties, but simultaneously reducing the exposure to peptides eliciting an immunological response in celiac predisposed subjects. These varieties might be useful for celiac disease prevention.



## 1. Introduction

Wheat is one of the most harvested cereals in the world and represents the most important source of complex carbohydrates in the human diet (http://www.fao.org/docrep/W8079E/w8079e0g.htm). It is principally cultivated in North Africa, Western Europe, North America and middle East of Asia (Leff et al., 2004). The main species used for human consumption are *Triticum aestivum* (bread wheat), which is mainly used for baked products, and *Triticum durum* Desf. usually utilized in pasta production and for some traditional breads of the Mediterranean basin (Peña, 2002). In addition to direct consumption, changes in wheat starch and gluten content and composition have expanded its use also for food and non-food additives (Atchison et al., 2010). Gluten represents 80% of the protein fraction in the wheat kernel and plays a key role in bread production and in the pasta industry, conferring elasticity to dough and firmness to pasta during the cooking process. Gluten is composed of an alcohol soluble fraction (gliadins) and an alkaline/acid-soluble fraction (glutenins) that needs denaturing and reducing conditions for its extraction (Van den Broeck et al., 2009); a correct balance of these two fractions is of outmost importance for correct dough development (Uthayakumaran et al., 1999). Gliadins are small monomeric proteins that contribute to dough viscosity and extensibility, while glutenins are present in polymers of high molecular mass formed by the establishment of disulfide bonds between single monomeric subunits. Glutenins confer strength and elasticity to dough (Wieser, 2007). To improve the rheological properties of wheat flour, breeders selected varieties with genetic features promoting high protein and gluten content (Šramková et al., 2009).

As well as for its rheological properties, there is an increasing interest in studying gluten proteins because they are the main factor causing celiac disease, an autoimmune enteropathy. Besides the consumption of gluten, celiac disease needs a genetic predisposition due to an antigluten T-cell response associated with specific histocompatibility antigens, namely HLA-DQ2.5 and HLA-DQ8, and the loss of the oral tolerance to gluten (Schuppan, 2000). The immune response in celiac subjects is due both to toxic motifs triggering the innate immune system and to epitopes influencing

*Abbreviations:* Fmoc, fluorenylmethoxycarbonyl; HLA, human leukocyte antigen; HMW, high molecular weight; LC/MS, liquid chromatography/mass spectrometry; LMW, low molecular weight; tBu, *tert*-butyl; TIS, triisopropylsilane; TPC, total protein content; Trt, trityl; UPLC, ultra performance liquid chromatography.

* Corresponding author. Tel.: +39 0521 905406; fax: +39 0521 905472.
*E-mail address:* stefano.sforza@unipr.it (S. Sforza).

0733-5210/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.jcs.2013.10.006

the adaptive system (Gianfrani et al., 2005). These peptides are generated in the gastrointestinal tract when gluten is partially proteolyzed by digestive enzymes. Gluten breakdown is not complete in human digestion because of the high content of proline whose cyclic structure hinders access to the peptide bond; this leads to the formation of long peptides involved in this autoimmune disorder (Hausch et al., 2002). Celiac disease is, nowadays, one of the most common chronic health disorders. In the recent past, the prevalence of this pathology ranged from 0.5 to 1.26% of children and adolescents (Rostom, 2004), but in the last decades, there was an increase of celiac cases even in adults (Catassi et al., 2010). There are many hypotheses that could explain this trend, such as the higher amount of gluten ingested, its quality, the reduction of leavening time during the baking process or changes of the intestinal microbiota (Gobbetti et al., 2007; Ivarsson et al., 2000). Recent studies have demonstrated the presence of a different content of celiac disease epitopes in modern and old wheat varieties (Van den Broeck et al., 2010). Therefore, it is questionable whether there is a correlation between breeding practices and the increasing incidence of celiac disease. Although the current trend of breeding is to develop wheat varieties with higher protein and gluten content, it is also known that the modest influence of evolutionary pressure on gliadins and glutenins determinates their great variability among wheat cultivars in comparison with functional proteins. The different contributions of the AA, BB, and DD genomes in the production of epitope-containing gliadins have already been demonstrated (Molberg et al., 2005), and in genetic studies, large differences emerged in the content of T-cell stimulatory epitopes in proteins from different hexaploid and tetraploid accessions (Van Herpen et al., 2006). These differences were also confirmed by T-cell proliferation tests and monoclonal antibody competition assays (Spaenij–Dekking et al., 2005).

In a recent study from our group (Prandi et al., 2012), the quantification of celiac-related peptides in samples from various *Triticum* species demonstrated strong differences among the varieties tested. Some samples, belonging to the same varieties and/or cultivation areas, showed a lower $\alpha$-gliadin content, and a smaller amount of peptides involved in adaptive and innate immune responses. This observation can be useful in identifying varieties with a lower content of pathogenic peptides, thus reducing the exposure of non-celiac people (especially of the young population, e.g., baby foods) and the probability of developing the disease.

In order to further confirm and expand those results, in this work, a set of durum wheat inbred lines and varieties were utilized to extract the prolamin fraction, in order to investigate the effect of genotype and environment on the content of peptides containing sequences involved in adaptive and innate immune responses generated after simulated gastrointestinal digestion. A set of inbred lines and cultivars with low genetic diversity, together with a set of germplasm accessions representative of the Mediterranean area with higher genetic diversity than the previous ones, cultivated in different places, were analyzed for their ability to produce pathogenic peptides upon gastrointestinal digestion. The same samples were analyzed for total nitrogen content, and fractionated with the Osborne procedure in order to verify a possible correlation between the total protein content (TPC) and the gliadin/glutenin content.

## 2. Material and methods

### 2.1. Plant material

All the durum wheat samples were provided by Società Produttori Sementi S.p.A. (Argelato, Bologna, Italy). Two sets of samples were collected aiming to evaluate the role of the environment and of the genotype on the pathogenic peptide content.

The set of durum samples was composed of:

a) a total number of 45 samples from five inbred line and cultivars (Aureo, F255, F312, F371, Levante; Table 1) cultivated in three locations well-representing the different Italian wheat cultivation area (Argelato-North Italy, Falconara-AN Central Italy, Lucera-South Italy); for each sample, three replicates/location were collected.
b) Because of the modest genetic diversity among these five samples in terms of pedigree, a second set of 25 samples from a collection of durum wheat accessions were selected and analyzed for the content of peptides involved in celiac disease generated after in vitro digestion of the prolamine extract.

25samples (Table 2) were selected from a collection of durum wheat accessions representative of the germplasm cultivated in the Mediterranean basin, with a suitable level of genetic variation and deeply characterized from a molecular point-of-view (Durum Panel; Maccaferri et al., 2005).

### 2.2. Reagent and solvents

Deionised water was obtained from a Millipore Alpha Q-Waters purification system (Billerica, MA, USA). Pepsin from porcine gastric mucosa, trypsin from porcine pancreas, $\alpha$-chymotrypsin from bovine pancreas, sodium dihydrogenphosphate, acetonitrile, ethanol, Fmoc-glutamine(Trt)-OH, piperidine, diisopropylethylamine, dichloromethane, triisopropylsilane, tyrosine, phenylalanine, tris(hydroxymethyl)-aminomethane, urea, dithiothreitol and diethyl ether were purchased from Sigma–Aldrich (St. Louis, MO, USA). Hydrochloric acid (37% V/V), sulfuric acid (96%), phosphosulfuric acid, copper (I) oxide, sodium hydroxide, bromocresol green, methyl red, 1-propanol and dimethylformamide were purchased from Carlo Erba (Milan, Italy). Fmoc-leucine-OH, Fmoc-proline-OH, HBTU and Fmoc-tyrosine(tBu)-Wang resin were purchased from Novabiochem (Darmstadt, Germany). Formic acid and trifluoroacetic acid were purchased from Acros Organics (Geel, Belgium). Selenium, silicone antifoam and sodium sulfate were purchased from Thompson and Capper (Hardwick, UK). Boric acid was purchased from Fluka (Buchs, Switzerland). Hydrochloric acid 0.1 N and sodium chloride were purchased from AnalaRNormapur (Milan, Italy).

### 2.3. Synthesis of the internal standard

The peptide LQLQPF($d_5$)PQPQLPY (isotopically labeled on the phenylalanine residues) was synthesized on solid phase according to Fmoc/t-butyl strategy using a Syro I Fully Automated Peptide Synthesizer (Biotage, Uppsala, Sweden). The peptide was cleaved from the Wang-resin using a TFA:TIS:$H_2O$ (95:2.5:2.5) solution and purified using a semipreparative RP-HPLC-UV ($\lambda$ = 280 nm). The purified product was quantified by a spectrophotometric method at 280 nm using an external calibration curve made with an equimolar solution of tyrosine and phenylalanine.

### 2.4. Osborne fractionation

Albumin, globulin and gliadin extraction was carried out as described by Lookhart and Bean (1995). Glutenins were extracted as described by Wieser et al. (1998) In order to assess the protein content of the extracted fractions, a Q-bit fluorometer (Invitrogen, Grand Island, NY, USA) was used. The working solution was prepared by mixing 1 μl of the Quant-it reagent in 199 μl of Quant-it buffer; 2 μl of each extract were mixed with 198 μl of the