Cultivar selection and nitrogen fertilization on wheat protein composition and on the expression of toxic epitopes studied by proteomic analysis

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Introduction

In addition to the fundamental technological role, gluten proteins of soft wheat are also the primary cause of the **celiac disease** (CD) and other **gluten-related diseases**. Despite the numerous researches in the field, a deeper knowledge of genomic variations in different wheat species is required to support food technological application of the wheat flour.
We aimed to evaluate the impact of N fertilization rate on the variation of gluten peptide composition in different wheat cultivars (cv): old variety (landrace), modern cv and a tritordeum cv (hybrid of wild barley and durum wheat).

Materials and methods

Field experiment in North-West Italy (Carmagnola, TO), 2016-17 growing seasons, by comparing according to a factorial design:

- 3 cv: Andriolo (**landrace**), Bologna (**modern** improver wheat), Bulel (**tritordeum**)
- 2 N fertilization treatments: **low** (80 kg N/ha) **high** (160 kg N/ha).

Refined flours were analysed through grain protein content (GPC) and were qualitatively characterised using several **proteomic techniques** such as 1D and 2D electrophoresis, reversed-phase high-performance liquid chromatography (RP-HPLC).
The competitive enzyme immunoassay (ELISA R5, R-Biopharm) was used to quantify peptide fragments of prolamins recognizing the potentially toxic motifs of the gliadin fraction as QQPFP.
A validated static **in vitro digestion** model (INFOGEST 2.0), consisting in protein digestibility at peptide level by RP-HPLC analysis followed by mass spectrometry analysis of immunoreactive peptides allowing the identification of epitopes and allergens through *in silico* analysis (figure 1).

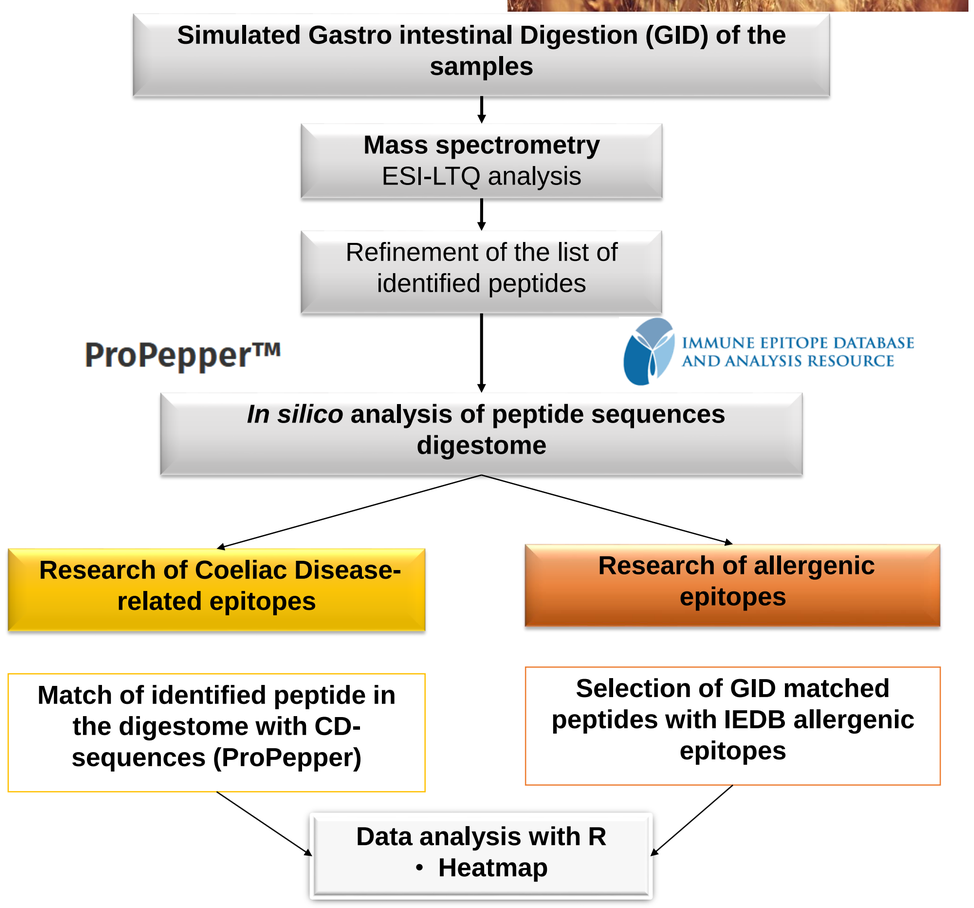

Figure 1 *Flow chart of in silico analysis after vitro digestion and* **Mass spectrometry analysis** (ESI-LTQ). The interrogation of epitope and allergen databases allowed the identification of already indexed ones.

Results

Although the N rate fertilization did not impact significantly on grain yield, the GPC was clearly influenced by the combination of cv and N application. GPC was higher in landrace, followed by tritordeum and modern wheat. In all genotype, N rate significantly increase protein content (figure 2,a). The ratio glutenin/gliadin as well as the ration of high molecular (HMW) and low molecular weight glutenin (LMW-GS) extrapolated from the integration of the chromatographic peaks (figure 2,b and 2,c) seem not to be affected by the increasing amount of N rate fertilization.

Tritordeum presented the lowest amount of toxic epitopes (177 g/kg) compared to landrace (304 g/kg) and modern wheat (364 g/kg) varieties, according to ELISA-R5 enzyme immunoassay.

A static *in vitro* digestion model was applied to the six samples monitoring the kinetic of digestion to identify the gluten resistant peptides to the GID proteolysis. SDS-PAGE analysis of the digestome showed the loss of gluten protein bands (MW>10 kDa, data not shown) while HPLC-ESI-MS analysis allowed the identification of several peptides arising from the digestion.
A deep understanding of the products of digestion is essential as these peptides are responsible of triggering the celiac disease. Further studies, using high resolution mass spectrometry may help identifying a greater number of peptides.

Varietes	25-mer LGQQQPFPPQQPY PQPQPFPSQQPY	33-mer LQLQPFPQPQLPYP QPQLPYPQPQLPYP QPQPF
Landrace_L	LGQQQPFPPQQP	QLQPFPQPQ PFPQPQLPYP
Landrace_H	LGQQQPFPPQQP	QLQPFPQ QLQPFPQPQ
Modern_L	LGQQQPFPPQQP	
Modern_H	LGQQQPFPPQQP	
Tritordeum_L	LGQQQPFPPQQP	
Tritordeum_H	LGQQQPFPPQQP	QLQPFPQ

Table 1 *Summary of aligned GID epitopes with main immunodominant peptide 25 and 33-mer, from iedb DB.*

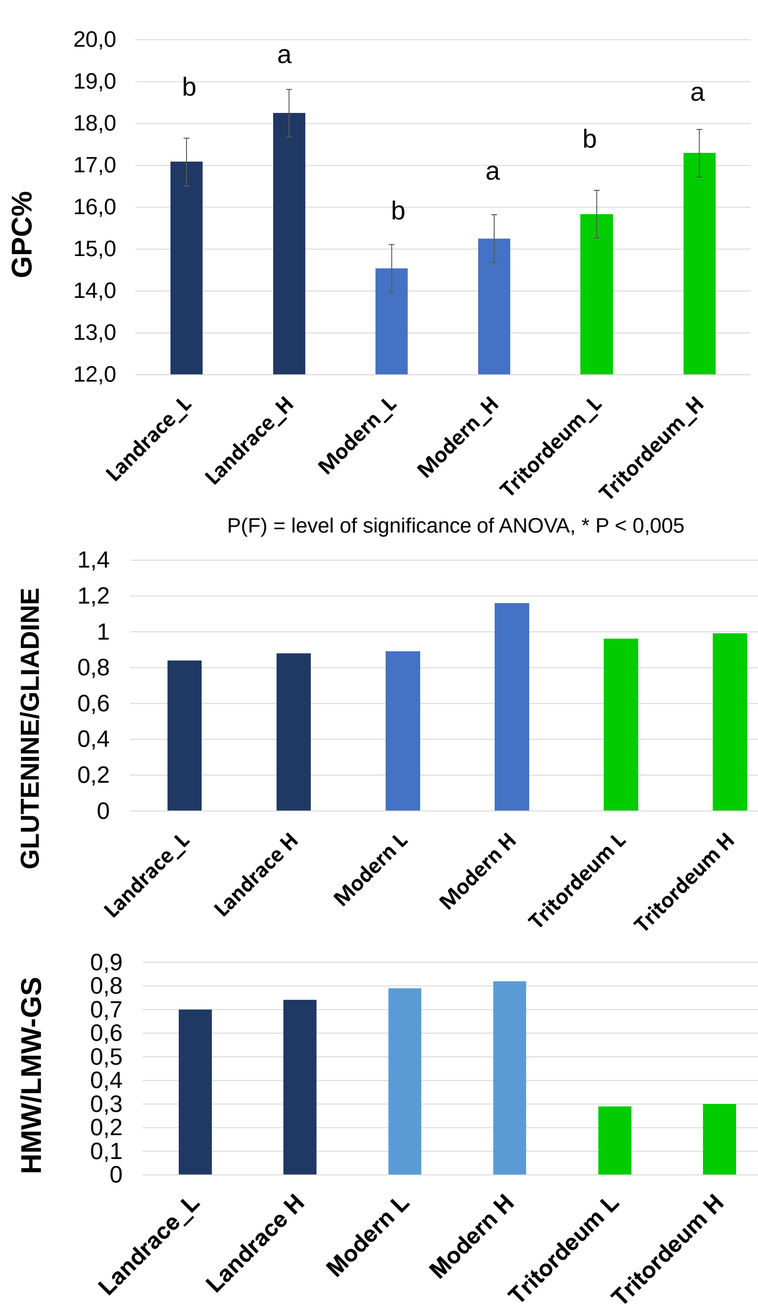

Figure 2: *Effect of cultivar and N rate on GPC % (a), glutenin/gliadin ratio (b) and* **HMW/LMW-GS (c)**

The *in silico* evaluation of celiac and allergenic peptides arising from the digestion was performed using know celiacogenic and allergenic sequences in Propepper and iedb.org public repositories. The number of sequences identified carrying epitopes differed across species and N-rates of fertilization. From a qualitative point of view, no difference in terms of toxic and allergenic sequences can be to the genetic or fertilization effect. In Table 1 are reported the 25 and 33-mer sequences identified across the samples. The two peptides are known to be resistant to digestion and are involved in the celiac disease.

We also analysed the presence of allergenic sequences for the wheat allergenic proteins: TRI A 19 (ω5), 20 (γ), 21 (α), 26 (HMW-GS) and 36 (LMW-GS) (figure 4). Limited number of sequences carrying the known epitopes for TRI A 19 and 26 allergens could be identified.

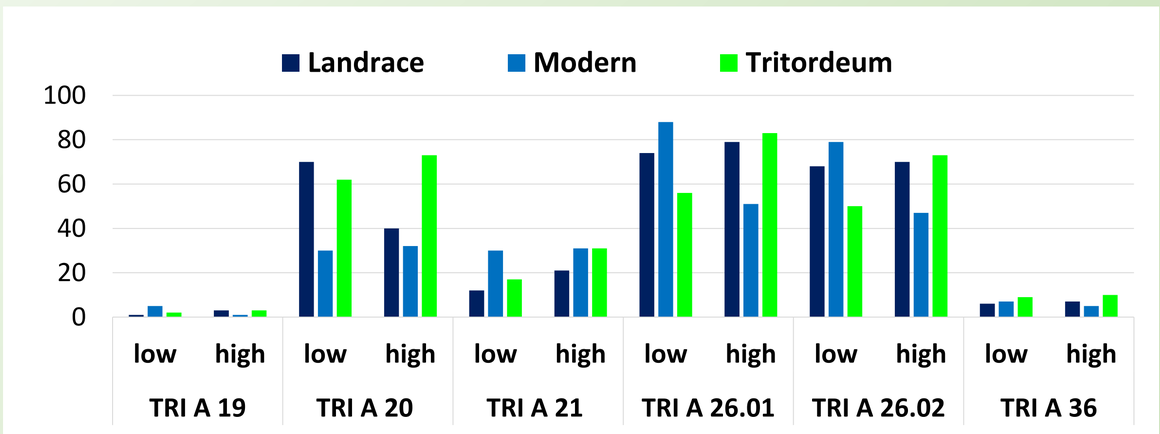

Figure 4: *number of GID epitopes aligned with 5 main allergens*

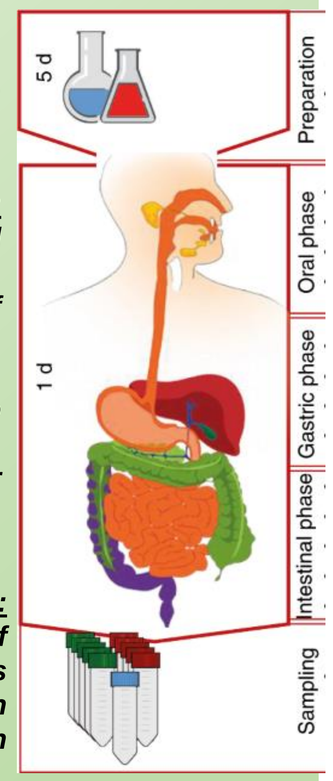

Figure 3: *Timing and flow diagram of the INFOGEST 2.0 in vitro digestion method for food.*

Conclusions

- **N rate fertilization has a strong influence in the protein content in all different varieties, while the role on the gluten composition and occurrence of gluten epitopes was limited;**
- **Genetic effect played a major role in the variation of gluten composition;**
- **Tritordeum show lowest values in terms of immunotoxicity R5 (ELISA Competitive);**
- **Interesting matches of known toxic and allergenic epitopes after in silico analysis.**

Future work

- Characterization of a **major number of genetic variety pool** by **quantitative proteomic analysis**