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## Introduction



Wheat is one of the most cultivated cereal grains in the world and is used for the manufacture of a wide range of bakery products, although contains some proteins linked to several gluten-related diseases.

At the moment the complex protein structure of gluten, deeply investigated for the quality and technological aspects, needs a greater understanding as a trigger for extremely widespread diseases such as **celiac disease** (CD) and Non-Celiac Gluten Sensitivity (NCGS).

It is very important to know the key field factors that are able to modulate qualitatively and quantitatively the flour protein profile.

### Aim:

Investigation of the role of environmental conditions, different wheat genotypes and two nitrogen (N) fertilization on the variation of gluten peptide composition, potentially toxic/immunodominant.

Our attention has focused on a new commercial hybrid variety which according to some early scientific evidence suggests a low content of gluten immunogenic peptides, Tritordeum.



The N rate fertilization did not impact significantly grain yield, while the **GPC was clearly influenced by the combination of cv and N application**: higher in landrace (17,65%), followed by tritordeum (16,55%) and modern wheat (14,85%), all three cvs showed an increase of values with N160 rate (datas not shown).

After *in vitro* digestion of flours, *in silico* analysis permits to observe the 50% fewer **CD** epitopes belonging to the  $\omega$  gliadins of tritordeum, and the 23% and 44% lower epitopes numbers of LMW-GS class compared to landrace and modern cv. An interesting result for **allergenic epitopes** identified was for tritordeum values which were less than 50% in comparison to modern and landrace wheats.

The greatest number of cd epitopes was found in the class of alpha gliadins in all three varieties. Unlike, the allergenic epitopes they were mainly belonging to the gamma gliadins class (Figure 1).

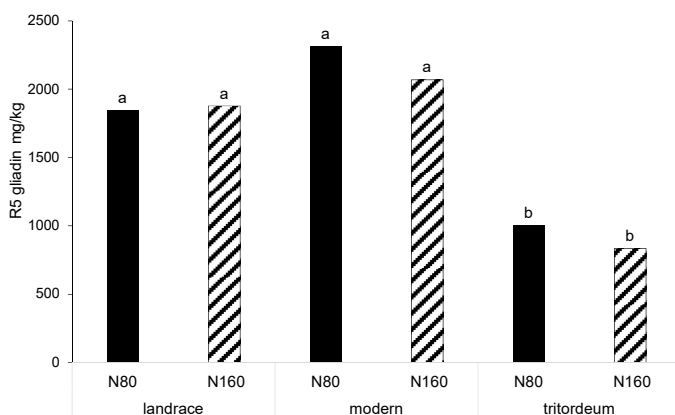


Figure 2: Quantification of celiac toxic motif "QQFPF" recognized by the R5 monoclonal antibody in soft wheat (landrace and modern) and tritordeum cultivars under N80 (80 kg n ha<sup>-1</sup>) and N160 (160 kg N ha<sup>-1</sup>) fertilization rate.

Bars with different letters are significantly different (p-value <0.05), according to the REGW-F test.

## Materials and methods



landrace



modern



tritordeum

Factorial experimental design in North-West Italy, 2016-17 growing seasons, to evaluate:

- 3 cvs: Andriolo (**landrace**), Bologna (**modern** improver wheat), Bulel (**tritordeum**)

- 2 N fertilization treatments: **low** (80 kg N/ha, **N80**) **high** (160 kg N/ha, **N160**).

Refined flours of all samples (cultivar X N rate) were analysed for grain yield and kernel quality traits and through advanced proteomic approaches: chromatography (RP-HPLC), electrophoresis.

After were subjected to **in vitro** simulated human **digestion** using the static model system (INFOGEST 2.0), followed by LC-MS/MS analysis.

The identified digested peptide were evaluated through *in silico* study of celiac and allergenic peptides using know epitopes sequences in Propepper and iedb.org public repositories.

A quantification of CD-toxic epitopes using a **competitive R5 ELISA** test was made on *in vitro* digested flours.

## Results

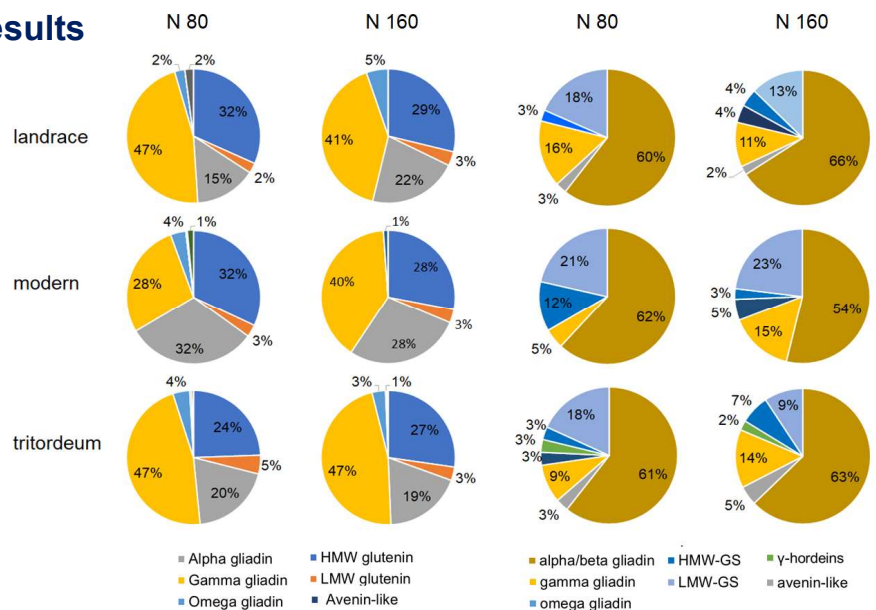


Figure 1: Percentage of gluten sources of allergenic (on the left) and celiac (on the right) and epitopes in soft wheat (landrace and modern) and tritordeum cultivars under N80 (80 kg n ha<sup>-1</sup>) and N160 (160 kg N ha<sup>-1</sup>) fertilization rate.

Digested **tritordeum flours** showed the lowest amount of **CD toxic epitopes** (919,6 mg/kg) compared to landrace (1861,5 mg/kg) and modern wheat (2192,2 mg/kg) varieties, according to competitive R5 ELISA immunoassay (Figure 2).

## Conclusions

- N rate fertilization influenced gluten 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) and epitopes of some gluten fraction
- Probable lower presence of highly celiac epitopes compared to an ancient and modern wheat, caused by the absence of **D genome**