Classical Innovative approaches for studying proteins involved in adverse reaction to wheat and grain quality

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XXXI Cycle of the PhD course in Plant and Animal Science

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Wheat is a worldwide staple food because of its adaptability, high yields in different environments and its good nutritional value. Wheat kernels contains an elevated amount of carbohydrates, dietary fibers, micronutrients and represent one of the main source of proteins in human diet. The viscoelastic properties of dough, necessary to realize different types of end products (breads, pasta, biscuits etc.…), are mainly related to gluten proteins. Wheat may also cause adverse reactions in sensitive people. Such adverse reactions include true allergies, autoimmune disorders (e.g. celiac disease) and neither immune nor allergic disorders such as Non Celiac Wheat Sensitivity (NCWS). In this view, the candidate aimed at pursuing three main goals:

1.   The genomic and proteomic characterization of bread wheat lines with a lower amount of α/β gliadins, involved in celiac disease

2.   The production and characterization of wheat lines with a lower amount of α-amylase/trypsin inhibitors (ATI) genes using RNA interference and genome-editing technologies.

3.   The investigation of the complete set of genes coding for g- and w-gliadins and low molecular weight glutenin subunits in a specific accession of Aegilops tauschi ssp. strangulata, the donor of the D genome to bread wheat, in comparison with the D genome present in a bread wheat cultivar.

The first goal produced a paper with the candidate as first author: "Production and molecular characterization of bread wheat lines with reduced amount of a-type gliadins". BMC Plant Biology (2017) 17:248. DOI 10.1186/s12870-017-1211-3

The second goal was achieved through two different ways: by silencing with RNA interference and by CRISPR/Cas9 (this latter goal was performed in Rothamsted Research UK by the candidate during his stage). The first approach allowed to obtain several bread wheat lines with a reduced amount of CM3, CM16 and 0.28 ATI subunits. These lines were analysed to evaluate the ATI content, the relative expression of genes encoding each subunit and the potential pleiotropic effects. As regards CRISPR/Cas9 silencing, a durum wheat cultivar was used and the genes coding for subunits CM3 and CM16 were silenced by using a multiple genome editing approach based on the strategy adopted by Xie et al in the 2015 to produce several gRNAs exploiting the endogenous tRNA processing system of plants. High Resolution Melting analysis (HRM) and Sanger sequencing allow to identify 12 mutants plants with an editing efficiency about 6%.

The third goal consisted in the characterization of genes encoding some gluten proteins (LMW-GS, ω- and γ-gliadins) on 1D chromosomes of bread wheats and *Ae. tauschii* ssp. *strangulata,* the D genome donor of cultivated wheat. The high percentage of identity among gene sequences from *Ae. tauschii* ssp. *strangulata* highlighted that allohexaploidization exerted a weak influence on LMW-GS, ω- and γ-gliadins genes' coding regions.

The identification and utilization of agronomically important genes in *Ae. tauschii* is therefore an important genetic improvement of hexaploid bread wheat.

ACTIVITIES

- ✓ V Annual Meeting of the Plant Genetic and Biotechnology Network:
New Breeding Techniques: Genome Editing, Cisgenesis and Genomic Selection

Università Cattolica del Sacro Cuore, Piacenza, June 6-8, 2016

- ✓ 1stImpARAS Training School:
Improving Allergy Risk Assessments Strategy for new food proteins

Polish Academy of Sciences, Warsaw, Poland September 19-20, 2016

- ✓ Training course on:
Advancements in plant breeding, trial design & analysis

Institute of field and vegetable crops, Novi Sad, Serbia, September 27-29, 2016

- ✓ Training course on:
L'ambiente statistico R: una introduzione all'analisi dei dati ecologici

Università degli Studi della Tuscia, Viterbo, June 5-9, 2016

- ✓ Training course on:
Principi di scrittura scientifica

Università degli Studi della Tuscia, Viterbo, June 5-9, 2016

- ✓ SIBV-SIGA Joint Congress:
Sustainability of agricultural environment: contributions of plant genetics and physiology

Poster award winner.

- ✓ **Visiting Ph.D student**:
Rothamsted Research, Plant Sciences Department

West Common, Harpenden, Hertfordshire, UK. March 1 – June 1, 2017.

- ✓ Training course on:
High-throughput wheat phenotyping

Bologna - Italy, 17-18 September 2018.

- ✓ International conference:
From Seed to Pasta III: A Sustainable Durum Wheat Chain for Food Security and Healthy Lives.

Bologna - Italy, 19-21 September 2018.

- ✓ International conference:
From Seed to Pasta III: A Sustainable Durum Wheat Chain for Food Security and Healthy Lives.

Bologna - Italy, 19-21 September 2018.

PUBBLICATIONS

Francesco Camerlengo, Francesco Sestili, Marco Silvestri, Giuseppe Colaprico, Benedetta Margiotta, Roberto Ruggeri, Roberta Lupi, Stefania Masci, Domenico Lafiandra**. Production and molecular characterization of bread wheat lines with reduced amount of a-type gliadins.** BMC Plant Biology (2017) 17:248. DOI 10.1186/s12870-017-1211-3

Francesco Camerlengo, Damiano Martignago, Caroline Sparks, Angela Doherty, Alison Huttly, Domenico Lafiandra, Stefania Masci, Francesco Sestili. **CRISPR-Cas9 MULTIPLEX EDITING OF DURUM WHEAT CULTIVAR SVEVO FOR REDUCING THE LEVEL OF ALLERGENS**. Proceedings of the Joint Congress SIBV-SIGA Pisa, Italy – 19/22 September, 2017 ISBN 978-88-904570-7-4.