**Hunting for new wheat allergens: a 2D Immunoblot and mass spectrometry approach**

**Aim of the project:**
Wheat is a complex allergenic food containing a lot of different proteins that are difficult to isolate and to identify. Specific molecular IgE detection in wheat allergic patients is limited since only a few molecular allergens are available on the market. Hence this research aimed to develop a diagnostic method linking a patients specific allergenic 2D western blot profile to a particular clinical symptom in wheat allergy. Afterwards, mass spectrometry (LC-MS/MS) was used to identify the specific molecular allergens.

**Methods**
A total protein extract of wheat seeds was separated on the basis of the isoelectric point and the molecular weight of the proteins. Twenty-five patients presenting positive specific IgE (sIgE) for wheat were classified into 3 different phenotypes: wheat dependent exercise induced anaphylaxis (WDEIA), atopic dermatitis (AD) and pollen rhinitis (PR). Their sera were analyzed by 2D immunoblotting on a standardized wheat seeds extract in order to evaluate their sIgE reactivity against the protein spots. Their sIgE sensitization profiles were compared and protein spots of interest were identified by LC-MS/MS.

**Results:** Comparison of sensitization profiles related on clinical symptoms and identification of interesting allergens by LC-MS/MS.

1. **WDEIA**
   - Serpin (Tri a 33)?
   - ω5-gliadin (Tri a 19)?
   - Tri a 20
   - Tri a 27
   - Tri a 31
   - Tri a 32

   Fig.1: Superposition of 2D immunoblot of four WDEIA patients: case 1 (black), case 2 (blue), case 3 (yellow) and case 4 (red).

2. **Pollen rhinitis (PR)**
   - ω5-gliadin (Tri a 19)?

   Fig.2: Superposition of 2D immunoblot of seven PR patients: case 15 (pink), case 16 (black), case 17 (green), case 18 (red), case 19 (dark blue), case 20 (yellow) and case 21 (light blue).

3. **Atopic dermatitis (AD)**
   - ω5-gliadin (Tri a 19)?
   - Tri a 26?
   - Tri a 12
   - Tri a 14
   - Tri a 44
   - Tri a 45
   - Tri a 7K-LTP

   Fig.3: Superposition of 2D immunoblot of four AD patients: case 6 (red), case 7 (yellow), case 9 (blue) and case 11 (black).

4. **Analyses LC-MS/MS (under investigation)**
   - Currently identified:
     - Spots 1, 2, 4: tri a 26
     - Spot 5: tri a bA
     - Spot 11: Tri a 34
     - Spot 14: tri a tritin

   Fig.4: Identification of a several 2D electrophoresis spots from total wheat proteins extract.

**Conclusion:**
At this stage, specific sensitization profiles were identified for the 3 phenotype groups (WDEIA, AD, PR). The protein spots of interest detected by sIgE concern one or more allergens. Some wheat allergens were identified by LC-MS/MS. Preliminary results show that Tri a 26 seems to be specific to the AD patient group. All four DA patients have Tria 26 specific IgE. At the end of the study, it will be possible to establish a link between all specific symptomatologies and the responsible allergens newly identified.