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

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**Aims:** Wheat is a complex allergenic food containing a lot of proteins that are difficult to isolate and to identify. Hence we aimed to develop a diagnostic method linking specific allergenic 2D western blot profiles to a particular clinical symptom in wheat allergy. Afterwards, we used mass spectrometry (LC-MS/MS) to identify 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:** Specific sensitization profiles were identified for each phenotype group. For WDEIA, protein spots around 37 kDa (pH 6-9) and 37-50 kDa (pH 5-6) were identified. For AD, spots were observed around 50 kDa (pH 9), 10 kDa (pH 9) and 20 to 75 kDa (pH3). For PR, specific spots were situated around 90 kDa (pH 9). The LC-MS/MS analysis of these identified spots pointed out several potential interesting allergens: tri a 26, tri a bA, tri a 34, tri a tritin.

**Discussion:** Our study answers to the request of many allergists wishing to get an accurate diagnosis of wheat allergy in order to determinate the risk of cross-reaction, to adapt the diet and to limit the risk of anaphylactic choc. Nevertheless, it is necessary to consider the 2D immunoblot results with the medical history of each patient. Moreover, there are different clinical manifestations of wheat allergy depending on the involved allergen and the way of exposure: WDEIA, AD, PR or baker's asthma. The present project pointed out new wheat allergens that could be associated to a specific phenotype. The identification of further protein spots is still under investigation.

**Conclusion:** At this stage, specific sensitization profiles were identified for the 3 phenotype groups (WDEIA, DA, PR). The protein spots of interest detected by sIgE concern one or more allergens. Some wheat allergens were identified by LC-MS/MS. At the end of the study, it will be possible to establish a link between a specific symptomatology and the responsible allergens newly identified.