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MOLECULAR CLONING AND CHARACTERIZATION OF ALLERGENIC PROTEINS FROM MAIZE

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Food allergies are a problem of considerable social impact and increasing diffusion. As part of a larger project (PRIN 2008) aimed at the identification and characterization of allergenic proteins present in low concentrations in foods of plant and animal origin, the research presented in this communication has the specific objective to identify genes coding for allergenic proteins from maize, and the production of recombinant allergens. This type of study fits into the research lines of modern allergology. Indeed, the possibility of obtaining recombinant allergenic proteins opens the possibility of future applications both in diagnosis and therapy [1]. The recombinant allergens can be used in diagnostic tests, instead of natural allergen extracts, eliminating the problem of variability and allowing the accurate determination of the molecular profiles of patients allergic sensitization (component resolved diagnosis). In addition, the availability of recombinant allergens makes it possible strategies of immunotherapy based on the development of hypoallergenic allergens, obtained by applying molecular biology tools (expression of molecular sub-regions, mutagenesis, synthesis of variants of protein folding, gene-shuffling, etc.).

Despite the wide consumption, maize has only recently been described as a cause of allergy (see [2] and references cited within). The first characterized allergen was a "lipid transfer protein" (LTP) of 9 kDa. More recently other proteins have been described as maize allergens: vicilin, globulin-2, gamma-zein, endochitinase, thoredoxin and trypsin inhibitor [2]. The case of maize LTP allergen is particularly interesting because the protein is known to bind IgE even after heating to 100 °C.

We have focused our initial efforts on the proteins LTP (EBI, Q2XX13). The coding sequence has been cloned in different vectors for expression in both *Escherichia coli* and *Pichia pastoris*.

Purified protein will be tested to confirm its allergenic activity, a necessary prerequisite for inclusion in the list of allergens of the International Union of Immunological Societies. Mutagenesis studies will then be initiated and assessment of allergenicity of new forms of allergen established. The analysis of LTP multigene families in different species of maize (*Zea diploperennis*, *Z. luxurians*, *Z. mays*, *Z. nicaraguensis*, *Z. perennis*) by a Genome Walking technique developed in our laboratories [3] is also in progress, so to assess the distribution and prevalence of the different isoforms of the protein.

The same experimental approaches will be used for other maize allergenes.

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