

## CLONING AND CHARACTERIZATION OF MAIZE CHITINASE A: A TOUGH ALLERGENIC MOLECULE. GENE DIFFUSION AMONG DIFFERENT MAIZE RACES

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Food allergy is recognized as a major health concern, affecting about 4% of total world population, and reaching peaks of 8% in infants. Furthermore, in recent years, the occurrence of food allergy in Westernized countries has shown a steady increasing trend and currently no viable treatments are available for food allergic people.

Maize has only recently been described as a cause of allergy. 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, chitinase A, thoredoxin and trypsin inhibitor [2].

Aim of our research work is the identification of genes coding for allergenic proteins from maize, and the production of recombinant allergens. This 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].

We focused our initial efforts on the chitinase protein (chiA). The portion of the *chiA* gene coding for the mature protein has been cloned and expressed in *Pichia pastoris* [3]. Protein purification and characterization, including activity assays at increasing temperatures and low pHs, allow to confirm the expression of an active molecule, highly resistant to heat and acid environments. 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.

In order to identify possible variants of the *ChiA* genes present in the genomes of maize plants of different origins (teosinte, landraces and inbred lines) we are currently applying a previously established genome walking procedure [4] to different species of maize: four teosinte subspecies (*Zea luxurians*, *Z. nicaraguensis*, *Z. perennis*, *Z. diploperennis*), four maize *Z. mays* landraces (*Arrocillo*, *Cacahuacintle*, *Confite*, *Conico*) and the *Z. mays* inbred B73 line.

### References

1. Valenta *et al.*, 2011, Recombinant allergens for allergen-specific immunotherapy: 10 years anniversary of immunotherapy with recombinant allergens. *Allergy*, 66: 775-783.
2. Fasoli *et al.*, 2009, Searching for allergens in maize kernels via proteomic tools. *J. Proteomics*, 72: 501-10.

3. Naumann, 2011, Modification of recombinant maize ChitA chitinase by fungal chitinase-modifying proteins. *Molec. Plant Pathol.*, 12: 365-372.
4. Leoni *et al.*, 2011, Genome Walking in Eukaryotes. *The FEBS Journal*, 278: 3953-3977.