Chapter

MOLECULAR FEATURES OF MAIZE ALLERGENS AND THEIR IMPLICATIONS IN HUMAN HEALTH

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ABSTRACT

Foods from plant origin, particularly nuts and seeds, represent the major source of registered food allergy due to the high variability of plant allergenic molecules, which depends on plant growing conditions, seed/fruit ripening, environmental stresses and/or industrial processing.

Maize, one of the major human diet components, is one of the most world widely consumed cereals. It has become therefore one of the major causes of food allergy due to the widespread corn derived food products, which make difficult itsto avoid it. However, detailled biochemical knowledge of maize allergy is lacking. There are several unanswered questions including the symptoms and mechanisms involved in maize allergenic reactions, its prevalence in adults and children, the implicated allergen molecules and the clinical cross-sensitization. Therefore, diagnostic tests and maize allergy management constitute a field of great interest.

Currently, maize allergen proteins are classified into 20 different families, displaying diverse structures and functions. They are responsible for many IgE cross-reactions between unrelated pollen and plant food allergen sources. The most relevant maize

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allergen molecules belong to the expansin and the Ole e 1 superfamilies, the panallergen profilin, and the Lipid Transfer Proteins (LTPs), i.e. Zea m 14, the major maize allergen.

Agricultural biotechnology promises plant food production through genetically modified (GM) crops with improved agronomic characteristics and enhanced consumer benefits. Numerous efforts in plant breeding programs have resulted in producing low-allergen or allergen-null plants that could moderate the allergic response. For this purpose, identification of new genes, and knowledge of allergen protein structures and function is crucial for the understanding of biochemical processes inducing maize allergy. Computational biology and protein modeling are increasingly used to evaluate whether a novel protein correspons to a known allergen, has a potential to become an allergen or could possibly cross-react with another existing allergen.

In order to answer the above crucial questions and bring insights into the processes of food allergy, we focus our attention in this review on maize allergens protein families, their biochemical, structural and immunological properties, while discussing possible strategies to predict biological consequences of allergen sensitization and cross-reactivity as well as therapies to mitigate maize allergy.

Keywords: *Zea mays*, corn, pollen, seed, allergen proteins, lipid transfer proteins, Ole e 1, profilin, plant breeding, genetically modified food, computational biology, protein modeling, allergy, therapy.

INTRODUCTION

Zea mays, commonly called maize, corn, sweet corn, Indian corn or field corn, belongs to the *Poaceae* (*Gramineae*) family. Cultivars within the genus may be divided into 6 general types: Popcorn (*everta*), Flint corn (*indurata*), Dent corn (*indenta*), Flour corn (*amylacea*), Sweet corn (*saccharata*) and Podcorn (*tunicata*).

There are, however, only 2 basic types: "Sweet corn" is distinguished from "Field corn" by the high sugar content of the kernels at the early "dough" stage and by wrinkled, translucent kernels when dry.

The plant is single-stemmed annual, and grown from one seed, though sucker shoots rise from the base. The single stalk, terminating in the tassel or staminate flowers, can grow to over 3 m at a fast rate. The smooth leaves, usually drooping and green, can be over half a meter long.

The flowers are monoecious (individual flowers are either male or female, but both sexes can be found on the same plant) and are pollinated by wind. The female flowers are born on a receptacle, termed "ear," which arises at a leaf axil near the mid-point along the stem. The flower organs, and later the grain kernels, in quite longitudinal rows, are enclosed in several layers of papery tissue, termed husks. Strands of "silk", actually the stigmas from the flowers, emerge from the terminals of the ears and husks at the same time the pollen from the terminal tassels is shed. In the Northern Hemisphere, corn is in flower between July and October, and the seeds ripen between September and October. The grains are variable as to size, shape, and color.

Maize is now grown almost anywhere summers are reasonably warm, although approximately 50% of the world's Maize is produced in the US. It is a staple cereal of the human diet in Central and South America and in many parts of Africa. It is extremely

important in livestock rearing, food processing and other commercial activities in developed countries. Few plants are grown more extensively or put to more diversified uses than maize.

Maize grain accounts for 15 to 56% of total daily dietary caloric intake in numerous developing countries. It is one of the 3 major grain crops worldwide for human and animal consumption. Over 750 million metric tons are produced each year, the United States, the European Union, China, Brazil, Mexico, and India being the world's leading suppliers. Maize seeds are storage organs that contain essential components for plant growth and reproduction, including starch, protein, and some micronutrients. For this reason, maize has become highly integrated into agriculture on a global scale and the human diet, providing an estimated 15% of the world's protein and 20% of the world's calories (Brown et al., 1988). Normal maize contains about 7-13% protein, which can be fractionated into various solubility classes. The salt-extractable fraction (albumins and globulins) mainly comprises proteins with metabolic functions. Extraction with aqueous alcohol isolates the prolamin fraction that contains the storage proteins of the seed. These constitute around 60-70% of the maize endosperm proteins and are called zeins. These are various polypeptides, classified as alpha, beta, delta and gamma zeins. The reduced soluble proteins are alcohol-extractable and soluble in water. Also present is an alcohol-insoluble glutelin fraction (Pasini et al., 2002).

More than 200,000 plant species are catalogued, but only 50 plant species (including maize) are registered in the official allergen list of the International Union of Immunological Societies (IUIS) Allergen Nomenclature Subcommittee (*http://www.allergen.org/*) as capable of inducing pollen allergy in susceptible individuals (Mothes et al., 2004). Pollinosis is the allergy phenomena associated to pollen, where plants are capable to produce a high amount of anemophilous pollen as a characteristic intrinsic to the pollinosis-associated plants. They can be grouped as (1) trees (Fagales, Pinales, Rosales, Arecales, Scrophulariales, Junglandales, Salicales, and Myrtales), (2) grasses (Bambusioideae, Arundinoideae, Chloridoideae, Panicoideae, and Poideae), and (3) weeds (Asteraceae, Chenopodiaceae, and Urticaceae). Allergies are immunological disorders, characterized by immune responses directed against harmless environmental substances, such as airborne grass pollen. Production of IgE antibodies in the allergic individual leads to allergic symptoms, ranging from rhinoconjunctivitis, eczema, and asthma. Allergy represents a major health problem that affect up to 40% of the population in industrialized countries (almost 500 million people worldwide (ISAAC 1998). Pollen allergens are classified according to their biochemical structure and immunological reactivity, leading to the identification of different allergen groups. Identification, isolation, and characterization of allergens is a necessary task to improve the diagnosis and treatment of this increasing clinical disorder and can help to explain relationships among biologic function, protein structure, and allergenic activity. Unfortunately, a relatively small number of allergens have been biochemically characterized among pollen allergens (Aalberse 2000).

Allergenic pollen contains a complex mixture of several molecules, mainly proteins where are both major and minor allergens included. Major allergens represent components to which the majority of patients (>50%) reacting to a given allergen source are sensitized. Instead, minor allergens are recognized by a limited number of patients. Due to the worldwide distribution and heavy pollen production, grasses represent the major outdoor source of inhalant allergens against which 40% of allergic patients are sensitized. Grass pollens are a major trigger of seasonal allergic rhinitis and asthma worldwide but the contribution of the subtropical grasses to the allergenic burden remains largely undefined. Grass pollen contains

about 1000 different components, but only some are IgE reactive and can cause allergic reactions. The determination of the allergenic potential of cultivated maize and its genetically engineered variants are of public interest (Schubert et al., 2005).

Clinical manifestations seem to be tightly connected with geographical and exposure factors. For diagnosis and specific immunotherapy (hyposensitization therapy) (Twaroch et al., 2011), the extracts of allergy-eliciting pollen are commonly used. However, in order to improve diagnosis and treatment, known allergens should be applied individually instead (component-resolved diagnosis), to determine a patient-specific reaction pattern. In the subsequent therapy, only those individual components that were previously identified as IgE reactive may then be applied to form well defined therapeutics (Jutel et al., 2005). The precise knowledge of the allergenicity of individual pollen proteins is crucial. Maize pollen proteins and their putative function as aeroallergens have not been studied in detail yet.

In this work, we focus our attention in every single family of known allergenic proteins of maize, i.e. Zea m 1 (the mayor maize allergen belonging to the Expansins Superfamily), Zea m 12 (Profilins), Zea m 14 (Lipid Transfer Proteins), Zea m 25 (Thioredoxin), among 21 allergen protein families. We have built for the first time the structure of 17 families of maize allergenic proteins not yet characterized by using computational biology (structural homology modeling), or already crystallized and deposited in the PDB database (*http://www.rcsb.org/*), showing how different structurally these families are, and bringing insights about their biochemical, structural and immunological properties.

MATERIAL AND METHODS

The Maize Allergen Proteins Sequence Database

The protein sequences of all maize allergenic protein families were collected from the Allergome database (*http://www.allergome.org*). Other different allergen protein databases were compared to obtain the maximum information about every single maize allergen protein family, such as Allergen (*http://www.allergen.org*), SDAP (*http://fermi.utmb.edu/SDAP/*), or ADFS (*http://allergen.nihs.go.jp/ADFS/*).

Full-length amino acid sequences for members of all families were compiled to be checked by using Blast P tool (*http://blast.ncbi.nlm.nih.gov/Blast.cgi/*) against world protein databases. Conservational regions and protein sequence consensus were further analyzed for the presence of putative functional protein areas by using the PROSITE database (Sigrist et al., 2010). The biologically meaningful motif descriptors were derived from multiple alignments using ScanProsite program (de Castro et al., 2006) and the Expert Protein Analysis System (ExPASy) proteomics server of the Swiss Institute of Bioinformatics (Gasteiger et al., 2003).

Allergens 3D-Building and Structural Analysis

To understand and compare the structural and molecular conformation of maize allergens, protein sequences of selected family members were modeled using the top ten PDB

closest template structures by SWISS-MODEL, a protein structure homology-modeling server, via the ExPASy web server (Arnold et al., 2006). An initial structural model was generated for the members from the different allergen protein families and subjected to energy minimization with GROMOS96 force field energy (Christen et al., 2005) implemented in DeepView/Swiss-PDBViewer v3.7 (Guex and Peitsch 1997) to improve the van der Waals contacts and correct the stereochemistry of the model. For each sequence analyzed, the quality of the model was assessed by checking the protein stereology with PROCHECK (Laskowski et al., 1993) and the protein energy with ANOLEA (Melo and Feytmans 1998). The Ramachandran plot statistics for the models were calculated to show the number of protein residues in the favored regions. The 2D protein structural analysis, protein superimpositions and surface protein contours analysis were performed and visualized in PyMol 2006 software (DeLano 2002).

The Electrostatic Poisson-Boltzmann (PB) potentials for all the structures were obtained using APBS (Baker et al., 2001) molecular modeling software in PyMol 0.99 (DeLano Scientific LLC) with AMBER99 (Wang et al., 2000) to assign the charges and radii to all of the atoms (including hydrogens), which were added and optimized with the Python software package PDB2PQR (Dolinsky et al., 2007). Fine grid spaces of 0.35Å were used to solve the linearized PB equation in sequential focusing multigrid calculations in a mesh of 130 points per dimension at 310.00 K. The dielectric constants were 2.00 for the protein and 80.00 for water. The output mesh was processed in the scalar OpenDX format to render isocontours and maps onto the surfaces with PyMol 0.99. Potential values are given in units of kT per unit charge (k Boltzmann's constant; T temperature).

MAIZE ALLERGEN PROTEIN FAMILIES

1. Zea m 1, the major maize allergen belongs to the expansin superfamily

EXPB1 (also called Zea m 1) from maize pollen, is a member of the β-expansin subfamily known as group-1 grass pollen allergens, and the major allergen in maize.

Four major isoforms of Zea m 1 can be discriminated using chromatography (Li et al., 2003) or by two-dimensional (2D) gel/blot analysis after deglycosylation (Wang et al., 2004), named as EXPB1, EXPB9, EXPB10, and EXPB1 , included in two rather divergent sequence classes (Li et al. 2003).

EXPB1 is the most abundant of the maize group-1 allergens. The biological function of group-1 allergen in the pollen of grasses remains unknown, and which structure has been built and depicte in the figure 1. It has been suggested that Zea m 1 can facilitate pollen tube penetration by loosening the cell walls of the stigma and style (Cosgrove et al., 1997). Proteomic and functional analysis of Zea m 1 in germinating pollen *in vivo* or *in vitro* will help to elucidate its biological function. The pollen surface consists mainly in two wall layer, an exine coat and an intine. It makes the initial contact with the stigma surface during sexual reproduction. Unfortunately to date, only two proteins have been identified from the maize pollen coat.

Expansins are a large superfamily of proteins that are key regulators of cell wall extensibility in plant growth and development (Lee and Kende 2001). They are primary cell-

wall-loosening agents that directly induce turgor- driven polymer relaxation apparently through the weakening of non-covalent bonds between cellulose microfibrils and cross-linking glycans. Two expansin families with wall-loosening activity have been identified, named α-expansins (EXPA) and β-expansins (EXPB); both are found in all groups of land plants, from mosses to flowering plants (Sampedro and Cosgrove 2005). The biological functions of α-expansins include cell enlargement, fruit softening and abscission (Shin et al., 2005), whereas β-expansin functions are not yet well established because functional studies have been mostly limited to grass pollen (Valdivia et al., 2007b; 2009). They have only 20% amino acid identity, however EXPA and EXPB proteins sizes are similar (27 kDa). They contain a number of conserved residues and characteristic motifs distributed throughout the length of both proteins. EXPA and EXPB appear to act on different cell wall components, but their native targets have not yet been well defined. It is known that EXPB1 induces extension and stress relaxation of grass cell walls (Andersson and Lidholm 2003).

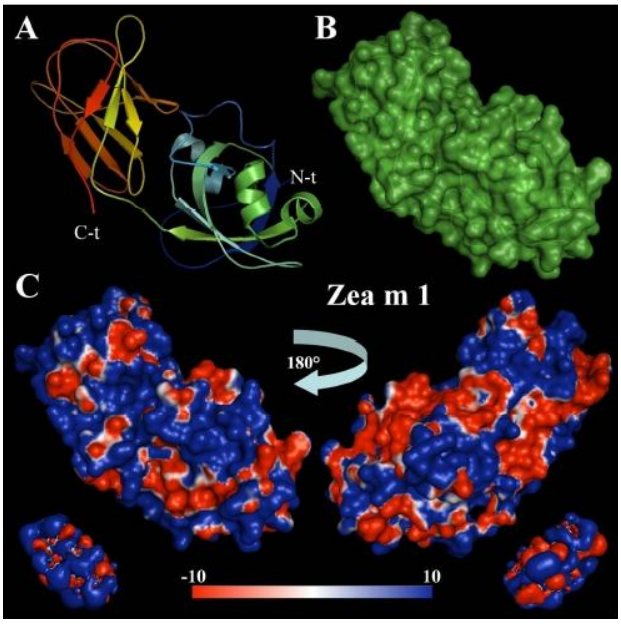

Figure 1. Three-dimensional structures of Zea m 1 allergen. Secondary structure elements (A) are displayed in rainbow colors, with N- and C-terminal in blue and red colors respectively. Surface molecule structure (B) is solid green colored, showing secondary structure elements inside. The distribution of charges (electrostatic potential) over the molecular surface is depicted in (C), where positive (+10) and negative (-10) charges are depicted in blue and red color respectively, in two molecular views rotated 180°, including Both representations as isocontours (value of 10 kT/e). PDB crystallographic model 2HCZ was obtained from the Protein Structure Database *http://www.pdb.org/pdb/home/home.do* and visualized with PyMol (*http://www.pymol.org/*).

The EXPBs are abundantly and specifically expressed in grass pollen, causing hay fever and seasonal asthma in an estimated 200–400 million people (Focke et al., 2001). β-expansins expressed in maize pollen are encoded by multiple genes (Valdivia et al., 2007a). Genetic disruption of ZmEXPB1 – the most abundantly expressed of these genes – impaired the pollen's ability to penetrate maize silks and to compete with wild-type pollen for reproductive success (Valdivia et al., 2007b, 2009). The pollen EXPBs have a marked loosening action on

cell walls from grasses, but not from dicots, whereas the reverse is true for EXPAs; therefore, it seems that the two forms of expansin target different components of the cell wall (Li et al., 2003).

Expansins are readily extracted from grass pollen, purified in milligram quantities, stable in solution, and form crystals suitable for X-ray diffraction analysis of protein structure (Yennawar et al., 2006). They consist of two domains as it can be appreciated in the Figure 1. The putative N-terminal domain [domain 1 of EXPB1 (*D1*)] and the catalytic domain of family-45 glycoside hydrolases (GH45). Despite this resemblance, EXPAs do not hydrolyze wall polysaccharides, and so the sequence similarity is enigmatic. The C-terminal domain [domain 2 of EXPB1 (*D2*)] has sequence similarity (from 35% to 10% identity) to another class of allergens, the group 1 grass pollen allergens, whose biological function is unknown (De Marino et al., 1999).

Common epitopes among corn and grass pollen allergens have been located on the protein portion of these expansins, but these epitopes of group 1 grass allergens are not conserved among all members of the expansin superfamily. Maize EXPB1 and its orthologs in turf grasses share common epitopes, with the predominant epitopes found in the protein portion of the molecule and the glycosyl residues (Petersen et al., 1998). The dominant group-1 allergenic epitopes, can be readily located on the surface of EXPB1, i.e. the 15-residue C98 epitope identified by Ball *et al.* (1994) includes D107 in the conserved catalytic site of EXPB1. ‘‘Site D’’ identified by Hiller *et al.* (1997) overlaps part of the extended conserved groove of *D1*, whereas ‘‘site A’’ identified by Esch and Klapper (1989) includes the small conserved pocket containing W232 and Y238, found on the far side of *D2*. This pocket is also part of the so-called ‘‘peptide 5’’ (Focke et al., 2001), a synthetic peptide derived from B cell epitopes of Phl p 1, the group-1 allergen of timothy grass pollen, which has become into a consideration as an useful component of an epitope-based vaccine for treating patients with severe allergies to grass pollen (Focke et al., 2001). It is surprising that the dominant antigenic epitopes of the group-1 allergens are not shared by vegetative EXPBs or by EXPA members, but indeed fortunate, because otherwise persons with strong allergies to grass pollen would also be allergic to fresh fruits, vegetables, grains, and other plant tissues expressing members of this large gene family that is ubiquitous in plants.

2. Zea m 2

Maize pollen also contains the allergenic protein Zea m 2, which integrates the Group 2 of grasses. In this group is also included other protein members such as Ant o 2 (sweet vernal), Ave s 2 (*Avena sativa*), Cyn d 2 (*Cynodon dactylon*), Dac q 2 (*Dactylis glomerata*), Hol l 2 (*Holcus lanatus*), Hor v 2 (*Hordeum vulgare*), Lol m 2 (*Lolium multiflorum*), Lol p 2 (*Lolium perenne*), Phl p 2 (*Phleum pratense*), Poa p 2 (*Poa pratensis* L.), Sec c 2 (*Secale cereale* L.), and Tri a 2 (*Triticum aestivum*) all members of the *Poaceae* genus (*http://www.allergome.org/*).

Searching gene sequences for this group of proteins in the UNIPROT database (*http://www.uniprot.org/*), it is possible to find molecule sequences like B4FK70, B6T4H8, B6TDY2, B6TUA7, B6TXY8, belonging to the family Expansin cellulose binding-like domain (PS50843), and more precisely to the Expansin EG45 or expansin family-45 endoglucanase-like domain (S50842) that exhibits different structural characteristics. They

can be appreciated in the following structures deposited in the PDB database (*http://www.rcsb.org/*): 1bmv, 1n10, 1who, 1whp, 2hcz and 2vxq.

This group that contains expansin-like proteins is also found in some fungi. In *Trichoderma reesei*, an expansin-like protein (Cel12A) acts as a glycoside hydrolase on xyloglucan and 1-4 β-glucan. These hydrolytic actions differ from the action of expansins, which induce wall extension by a non-hydrolytic mechanism (Yuan et al., 2001).

Structurally, it consists of two domains closely packed and aligned so as to form a long, shallow groove with potential to bind a glycan backbone. The N-terminal cysteine-rich domain has distant sequence similarity to the family-45 of endoglucanases (EG45-like domain). It has noteworthy, but incomplete conservation of the catalytic site identified in the EG45 enzymes. The ~120-residue EG45-like domain has three disulfide bonds and the six participating cysteines are highly conserved. Its fold is dominated by a six-stranded β-barrel flanked by short loops and α-helices. The ~90-residue C-terminal domain may function as a cellulose-binding domain (CBD). It is composed of eight β-strands assembled into two antiparallel β-sheets. The two β-sheets are at slight angles to each other and form a β-sandwich similar to the Ig fold (Yennawar et al., 2006, *http://www.bio.psu.edu/expansins/*).

Three types of molecular architectures have been differentiated between the protein members of this family, containing the following domains:

1) PROKAR_LIPOPROTEIN, EXPANSIN_EG45, EXPANSIN_CBD architecture; 2) EXPANSIN_EG45, EXPANSIN_CBD architecture; 3) EXPANSIN_CBD architecture.

The exact biological function of this group of proteins is unknown, but it has been implicated in sexual reproduction of the plant. An example of structural representation of this group is depicted in the figure 2.

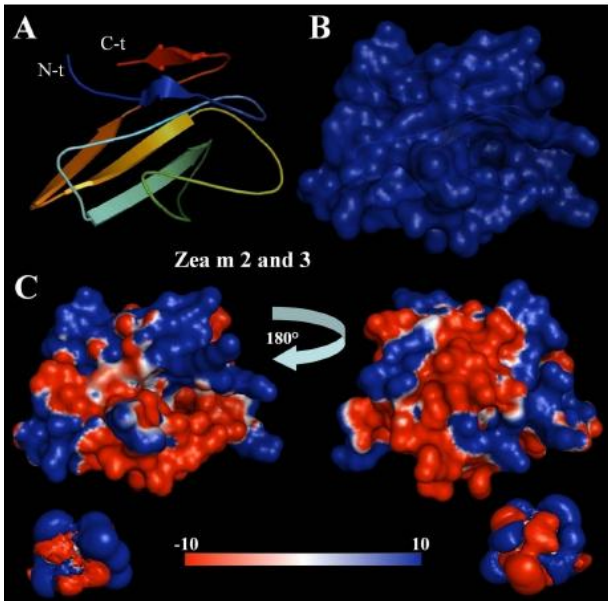

Figure 2. Three-dimensional structures corresponding to the allergen proteins Zea m 2 and Zea m 3. Partial secondary structure elements (A) are displayed in rainbow colors, with N- and C-terminal in

blue and red colors respectively. Surface molecule structure (B) is solid blue colored, showing secondary structure elements inside. The distribution of charges (electrostatic potential) over the molecular surface is depicted in (C), where positive (+10) and negative (-10) charges are depicted in blue and red color respectively, in two molecular views rotated 180°, including Both representations as isocontours (value of 10 kT/e). PDB from the best protein model built for these allergens (see Material and Methods section) was visualized with PyMol *http://www.pymol.org/.*

3. Zea m 3

Maize pollen allergen protein Zea m 3 belongs to the C-terminal domain Expansin superfamily (Prosite accession number PS50843), which also is integrated by other 22 members such as Cyn d 1, Cyn d 2, and Cyn d 15 (*Cynodon dactylon*), Dac g 1, Dac g 2 and Dag g 3 (*Dactylis glomerata*), Hol l 1 (*Holcus lanatus*), Lol p 1, Lol p 2 and Lol p 3 (*Lolium multiflorum*), Ory s 1 (*Oryza sativa*), Pas n 1 (*Paspalum notatum*), Pha a 1 (*Phalaris arundinacea*), Phl p 1, Phl p 2 and Phl p 3 (*Phleum pratense*), Poa p 1 and Poa p 2 (), Tri a 1 and Tri a 2 (*Triticum aestivum*), Zea m 2 (*Zea mays*) (*http://www.allergome.org/*).

Searching in the protein UNIPROT database (*http://www.uniprot.org*/), we only found a unique sequence corresponding to Zea m 3 (Q7XBA3), which has been involved in the sexual reproduction functions of the plant. An example of structural representation of this group is depicted in the figure 2.

4. Zea m 4

Pollen Zea m 4 allergen protein is integrated into the group 4 of grasses, to which also belong Agr q 4 (*Agrostis palustris*), Ant o 4 (*Anthoxanthum odoratum*), Ave s 4 (*Avena sativa*), Cyn d 4 (*Cynodon dactylon*), Fes p 4 (*Festuca pratensis*), Hol l 4 (*Holcus lanatus*), Hor v 4 (*Hordeum vulgare*), Imp c 4 (*Imperata cylindrica*), Lol p 4 (*Lolium multiflorum*), Phl p 4 (*Phleum pratense*), Phr a 4 (*Phragmites australis*), Poa p 4 (*Poa pratensis*), Sec c 4 (*Secale cereale*), and Tri a 4 (*Triticum aestivum*) (*http://www.allergome.org/*).

No gene/protein sequences have been characterized up to now for maize, and the biological function for this maize allergen protein has not been elucidated yet.

5. Zea m 5

The presence of this allergen in maize pollen an other grass species has been inferred by cross-reactivity using antibodies made against recombinant Phl p 5, the mayor timothy grass pollen allergen (Madritsch et al 2011).

No gene/protein sequences have been characterized up to now for maize, and the biological function for this maize allergen protein has not been elucidated yet.

6. Zea m 7

This protein is a calcium-binding allergenic protein in maize pollen, which belongs to the Polcalcin superfamily.

Polcalcins are calcium-binding proteins (CBP) sharing common domains termed EF-hands (helix-loop-helix motifs). They have been classified as EF-Hand 1 (Prosite accession number PS00018) domain signature (*http://prosite.expasy.org/*), with the following characteristic motif:

D-{W}-[DNS]-{ILVFYW}-[DENSTG]-[DNQGHRK]-{GP}-[LIVMC]-[DENQSTAGC]-x(2)-[DE]-[LIVMFYW].

In addition to maize, with Zea m 7, many different species contribute to this group 7 of grass allergens which include the allergens Agr c 7 (*Agropyron cristatum*), Aln g 4 (*Alnus glutinosa*), Amb a 10 (*Ambrosia artemisiifolia*), Amb a 9 (*Ambrosia artemisiifolia*), Ant o 7 (*Anthoxanthum odoratum*), Art v 5 (*Artemisia vulgaris*), Ave s 7 (*Avena sativa*), Bet pu 4 (*Betula alba*), Bet v 3 and Bet v 4 (*Betula pendula*), Bra n 4 and Bra n 7 (*Brassica napus*), Bra r 4 and Bra r 7 (*Brassica rapa*), Bro i 7 (*Bromus inermis*), Car b 4 (*Carpinus betulus*), Che a 3 (*Chenopodium album*), Cry j 4 (*Cryptomeria japonica*), cup a 4 (*Cupressus arizonica*), Cyn d 7 (*Cynodon dactilon*), Dac g 7 (*Dactylis glomerata*) (*http://www.allergome.org/*).

Different protein structures have been crystallized within this group, corresponding to 1A03, 1A29, 1A2X, 1A75, 1AHR, 1AJ4, 1AJ5, as the most representatives. Searching for gene/protein sequences matching Zea m 7, we have been found a unique protein sequence named as B6UD40 in the Uniprot database (Figure 3), also called Calcium-binding allergen Ole e 8.

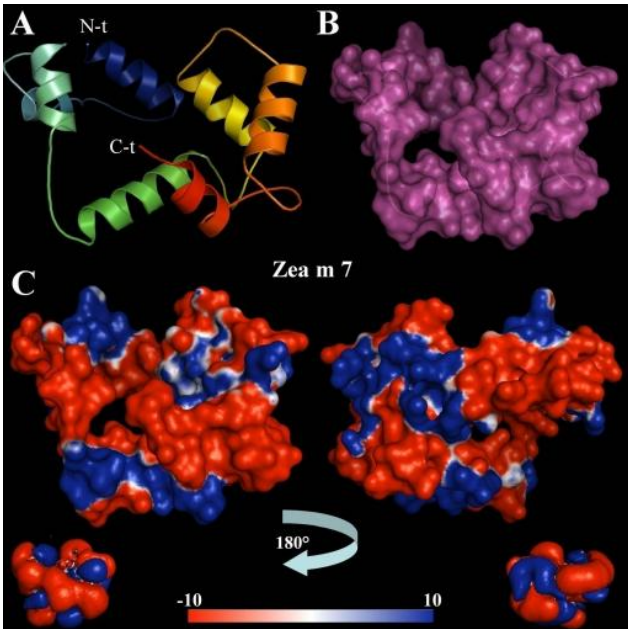

Figure 3. Three-dimensional structures corresponding to the allergen proteins Zea m 7. Partial secondary structure elements (A) are displayed in rainbow colors, with N- and C-terminal in blue and

red colors respectively. Surface molecule structure (B) is solid pink colored, showing secondary structure elements inside. The distribution of charges (electrostatic potential) over the molecular surface is depicted in (C), where positive (+10) and negative (-10) charges are depicted in blue and red color respectively, in two molecular views rotated 180°, including Both representations as isocontours (value of 10 kT/e). PDB from the best protein model built for this allergen (see Material and Methods section) was visualized with PyMol (*http://www.pymol.org/*).

Polcalcins constitute the majority of allergenic CBPs, and their expression seems to be mainly restricted to pollen. Polcalcins are highly cross-reactive calcium-binding allergens that are specifically expressed in pollen tissues. For this reason, sensitization to polcalcins is not associated with allergy to plant-derived foods. Approximately 10% of pollinosis patients react with polcalcins from various trees, grasses, and weeds (Niederberger et al., 1999). Different data indicate that the clinical relevance of polcalcin sensitization is linked to geographical factors and level of exposure to different allergenic sources.

Many calcium-binding proteins belonging to the same evolutionary family share a type of calcium-binding domain, the EF-hand (Kawasaki and Kretsinger 1995). This domain consists of a twelve residue loop, flanked on both sides by a twelve residue α-helical domain. In an EF-hand loop the calcium ion is coordinated in a pentagonal bipyramidal configuration. The six residues involved in the binding are in positions 1, 3, 5, 7, 9 and 12. The invariant Glu or Asp at position 12 provides two oxygens for linking Ca^{2+}. The basic structural/functional unit of EF-hand proteins is usually a pair of EF-hand motifs that together form a stable four-helix bundle domain. The pairing of EF-hand enables cooperativity in the binding of Ca^{2+} ions. The 6th residue in an EF-hand loop is in most cases a Gly, although the number of exceptions to this 'rule' has gradually increased.

According to the number of calcium-binding EF-hand motifs, at least three types of polcalcins have been described in pollen, i.e. those displaying two (Aln g 4, Amb a 9, Art v 5, Bet v 4, Che a 3, Cyn d 7, Fra e 3, Ole e 3, Phl p 7, and Syr v 3), three (Amb a 10 and Bet v 3), and four (Jun o 4, and Ole e 8 or Zea m 7) calcium-binding domains. Monomeric Bet v 4 from birch is composed of two symmetrically arranged EF-hands. Dimeric timothy grass Phl p 7 contains two of these basic structural domains.

The function of polcalcins is still unclear. However, due to their pollen-specific localization and their ability to bind calcium, it has been proposed that polcalcins function in the control of intracellular calcium levels during pollen germination (Wopfner et al., 2007). Interestingly, the calcium-binding ability of polcalcins affects IgE-reactivity. A comparative study between allergens with two, three, and four EF-hand domains revealed that timothy grass Phl p 7 is the most cross-reactive polcalcin. It has therefore been suggested that Phl p 7 could serve as marker molecule for the identification of multiple pollen sensitizations (Tinghino et al., 2002).

7. Zea m 11 belongs to the trypsin inhibitor superfamily

Zea m 11 allergen from pollen and seed belongs to the 2S albumin family, and it is a protein of about 16 kDa that can be recognised by 36% of maize-allergic patients. It was isolated and characterized, showing a role like inhibitor of trypsin in maize (IUIS 2008).

Cereal seeds contain numerous serine proteases and α-amylase inhibitors. These inhibitors can be grouped into families based on structural similarities. The cereal trypsin/α-amylase inhibitor family consists of proteins of about 120 amino acids which contain 10 cysteine residues, all of which are involved in disulfide bonds. Some of these inhibitors are specific to trypsin, others to α-amylase, and a few are bifunctional (Figure 4).

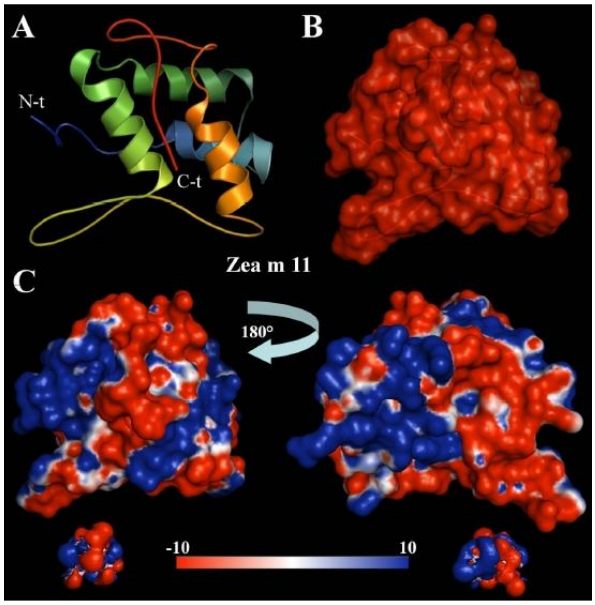

Figure 4. Three-dimensional structures corresponding to the allergen proteins Zea m 11. Secondary structure elements (A) are displayed in rainbow colors, with N- and C-terminal in blue and red colors respectively. Surface molecule structure (B) is solid red colored, showing secondary structure elements inside. The distribution of charges (electrostatic potential) over the molecular surface is depicted in (C), where positive (+10) and negative (-10) charges are depicted in blue and red color respectively, in two molecular views rotated 180°, including Both representations as isocontours (value of 10 kT/e). PDB from the best protein model built for this allergen (see Material and Methods section) was visualized with PyMol (*http://www.pymol.org/*).

The signature pattern characteristic for this cereal trypsin/α-amylase inhibitor family of proteins is the following:

C-x(4)-[SAGDV]-x(4)-[SPAL]-[LF]-x(2)-C-[RH]-x-[LIVMFYA](2)-x(3,4)-C.

where the 3 C's are involved in disulfide bonds (*http://prosite.expasy.org/*).

Different genes have been sequenced belonging to this group of allergens, which display serine-type endopeptidase inhibitor activity. They have been deposited in the database with the following Uniprot accession number: B4FCC1, B6T2Z8, B6TNI5, B6UGR3, B6UHB4, B8QVB7, B8QVC9, P01088, Q09HU3, Q2XWW2, Q946V1 (*http://www.uniprot.org/*).

This group of trypsin inhibitors of grasses is integrated by many other different allergen proteins from diverse species: Ara d 2 (*Arachis spegazzinii*), Ara h 2 (*Arachis hypogaea*), Ara i 2 (*Arachis ipaensis*), Che a 1 (*Chenopodium album*),Fag e TI (*Fagopyrum esculentum*), Gly m TI (*Glycine max*), Hor v 15 (*Hordeum vulgare*), Hor v 28 (*Hordeum vulgare*), Hor v 33

(*Hordeum vulgare*), Hor v BTI (*Hordeum vulgare*), Lol p 11 (*Lolium perenne*), Ole e 1 (*Olea europaea* L.), Ory s 11 (*Oryza sativa*), Ory s 17kD (*Oryza sativa*), Ory s aA_TI (*Oryza sativa*), Phl p 11 (*Phleum pratense*), Sec c aA_TI (*Secale cereale*), Tri a 30 (*Triticum aestivum*), Tri a 33 (*Triticum aestivum*), Tri a CM16 (*Triticum aestivum*), Tri td aATI (*Triticum durum*) (*http://www.allergome.org/*).

8. Zea m 12, a maize panallergen belonging to the profilin superfamily

Maize profilin (Zea m 12) belongs to the group actin binding proteins (ABPs), which regulate the function and activities of the cellular cytoskeleton. In maize, this group exhibits 5 isoforms (Zea m 12.0101 to Zea m 12.0105) (Figure 5).

Numerous profilin from grasses pollen have been identified and named as Cyn d 12, Lol p 12, Ory s 12, Phl p 12, Poa p 12, and Zea m 12 (*http://www.allergome.org/*). Many maize profilin genes have also been sequenced, characterized and deposited in the database Uniprot with the following accession numbers: A4KA55, A4KA56, A4KA57, A4KA58, A4KA59, A4KA60, A4KA61, B4FTX3, B4FV68, B6T699, B6T6Y5, P35081, P35082, P35083, O22655, and Q9FR39 (*http://www.uniprot.org/*). They have in common the characteristic profilin motive or pattern defined by the following amino acids sequence: x(0,1)-[STA]-x(0,1)-W-[DENQH]-x-[YI]-x-[DEQ] (*http://prosite.expasy.org/*).

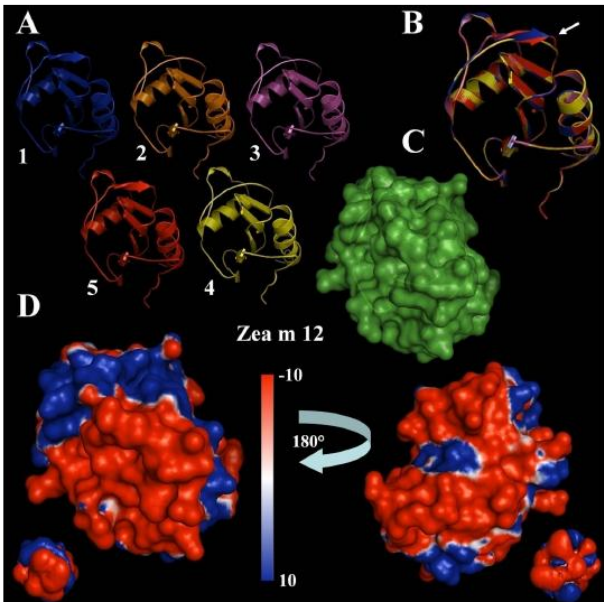

Figure 5. Three-dimensional structures of the allergen protein Zea m 12. Secondary structure elements (A) are displayed in blue, orange, purple, yellow and red color for the isoforms 1 to 5 respectively. Superimposition of the 5 structures (B) had shown a high structural similarity, although two groups (1, 3, 5 and 2, 4) are differentiated by existence of an α-helix. Surface molecule structure (C) is solid green colored, showing secondary structure elements inside. The distribution of charges (electrostatic potential) over the molecular surface is depicted in (D), where positive (+10) and negative (-10) charges are depicted in blue and red color respectively, in two molecular views rotated 180°, including Both representations as isocontours (value of 10 kT/e). PDB from the best protein model built for these five

allergen isoforms (see Material and Methods section) were visualized with PyMol (*http://www.pymol.org/*).

Profilins represent a family of small (12 to 15 kDa), highly conserved molecules sharing sequence identities of more than 75%, even between members of distantly related organisms. This sequence conservation is reflected by highly similar structures (Figure 5) and biologic function (Hauser et al., 2008). Profilins can be found in all eukaryotic cells and are involved in processes related to cell motility by regulating of microfilament polymerization upon binding to actin (Valenta et al., 1992).

In plant cells, profilins play a role in cytokinesis, cytoplasmatic streaming, cell elongation as well as growth of pollen tubes and root hairs (Ramachandran et al., 2000; Valster et al., 1997; Witke et al., 2004).

Besides actin, a plethora of profilin ligands have been described, e.g. phosphoinositides and poly-L-proline stretches, providing evidence for profilin involvement in other cellular processes like membrane trafficking and organization as well as signaling pathways (Gibbon et al., 1998). Cellular localization of profilins in pollen is mayoritary cytoplasmatic, although they can be found close to the site of pollen tube emergence upon pollen hydratation and germination, forming a ring-like structure around the 'effective' apertural region. They can be also detected in the pollen exine of the germinating pollen grains and in the germination medium (Morales et al., 2008). Recently, the putative functional and regulatory consequences of the sequence polymorphism in pollen profilins has been described, and the mechanisms to generate multiple profilin isovariants among plant species have been proposed (Jimenez-Lopez et al., 2012).

Profilins are widely distributed throughout nature and can therefore be viewed as panallergens that are responsible for many cross-reactions between inhalant and nutritive allergen sources (Valenta et al., 1991; 1992). Plant panallergens share highly conserved sequence regions, structure, and function. They are responsible for many IgE cross-reactions even between unrelated pollen and plant food allergen sources. Known panallergens presently comprise only a few protein families, including profilins, polcalcins, and non-specific lipid transfer proteins (nsLTP).

Allergenic profilins have been identified in pollen of trees, grasses, and weeds, in plant derived foods, as well as in latex. IgE cross reactivity resulted from the common three-dimensional profilin fold composed of two α-helices and a five stranded anti-parallel β-sheet, as described for the class of α-β proteins (Hauser et al., 2008).

Due to this conserved structure, profilin-specific IgE may cross-react with homologues from virtually every plant source. Therefore, profilin sensitization is a risk factor for allergic reactions to multiple pollen and food allergen sources (Asero et al., 2003). Multiple allergies to both pollen and food allergen sources seem to be determined by sensitization to such ubiquitously spread allergens (Mari 2001). Initial exposure to panallergens may subsequently drive the allergic immune response towards major allergens through a mechanism called intramolecular epitope spreading (Gould and Sutton 2008).

Maize seed contains the panallergen profilin, but at much lower levels than those found in maize pollen. This profilin may be of low clinical significance, as heat processing destroys the protein. Nonetheless, the presence of profilin in maize seed may play a role in occupational asthma where inhalation of maize flour or dust is possible. As profilins are sensitive to heat denaturation and gastric digestion, they cannot cause sensitization via the

gastrointestinal tract. Consumption of raw foods by profilin-sensitized patients leads to reactions that are usually restricted to the oral cavity (Breiteneder and Radauer 2004; Rodriguez-Perez Ret al., 2003). Such properties are typical for class II food allergens. In contrast to non-pollen-related class I food allergy that mainly affects young children, class II food incompatibility is frequently observed in adults as a consequence of sensitization to aeroallergens (Breiteneder and Ebner 2000).

Profilin sensitization varies between 5 to 40% among allergic individuals. This variability was suggested to be attached to different factors such as levels of exposure, and geographical factors.

The clinical relevance of profilin sensitization is still a matter of debate. The cross-reactivity patterns of IgE antibodies from birch pollen-allergic patients with concomitant food allergy (Wensing et al., 2002) showed that in contrast to Bet v 1-specific IgE, antibodies directed against birch profilin have a broad cross-reactivity spectrum. Bet v 2 sensitization was associated with positive RAST (radio allergosorbent test) to all investigated plant-derived foods except apple, peach, and melon. However, their clinical relevance was low or even absent. Olive profilin Ole e 2 has been reported to cross-react with grass profilins (Ledesma et al., 1998), and Ole e 2-specific IgE antibodies were detected in 95% of olive pollen-allergic patients with concomitant oral allergy syndrome to peach, pear, melon, kiwi, and nuts (Quiralte et al., 2007).

Taken together, these studies emphasize the importance of identifying the responsible allergens as they might have an impact on the clinical features of allergic reactions to fruits and vegetables. Despite the fact that many profilin sensitized patients do not exhibit symptoms, careful patient monitoring and a clear distinction between cross-reactivity and genuine sensitization seem to be a necessary task.

9. Zea m 13, is a maize allergen with polygalacturonase activity

Group 13 are widespread and the major allergens in grasses. They are glycoproteins of approximately 42 kDa with sequence homology to polygalacturonases, a family of pectin-degrading enzymes. They are poorly studied in maize, where they present the structure that can be visualized in the Figure 6.

A number of sequences belonging to the Zea m 13 allergen group from pollen have been identified, which can be numbered as follow: P26216, P35338, P35339, Q1ZYQ5, Q1ZYQ6, and Q1ZYQ7 (*http://www.uniprot.org/*).

Zea m 13 group of proteins are integrated by many other different allergen proteins: Ant o 13 (*Anthoxanthum odoratum* L.), Ave s 13 (*Avena sativa*), Cyn d 13 (*Cynodon dactylon*), Dac g 13 (*Dactylis glomerata*), Fes p 13 (*Festuca pratensis*), Hor v 13 (*Hordeum vulgare*), Lol p 13 (*Lolium multiflorum*), Ory s 13 (*Oryza sativa*), Pas n 13 (*Paspalum notatum*), Phl p 13 (*Phleum pratense*), Phr a 13 (*Phragmites australis*), Poa p 13 (*Poa pratensis* L.), Sec c 13 (*Secale cereal*), and Tri a 13 (*Triticum aestivum*) (*http://www.allergome.org/*).

This group belongs to the glycosyl hydrolases family 28 (Pfam accession number PF00295), which comprises enzymes with polygalacturonase, exo-polygalacturonase, or rhamnogalacturonase activity.

Polygalacturonase (pectinase) catalyzes the random hydrolysis of 1,4-α-D-galactosiduronic linkages in pectate and other galacturonans. It exhibits a specific pattern of

amino acid (Prosite accession number PS00502) (*http://prosite.expasy.org/*) that identifies this group of proteins:

[GSDENKRH]-x(2)-[VMFC]-x(2)-[GS]-H-G-[LIVMAG]-x(1,2)-[LIVM]-G-S

Exo-poly-α-D-galacturonosidase (exoPG) (He and Collmer1990) hydrolyzes peptic acid from the non-reducing end, releasing digalacturonate. Prokaryotic, eukaryotic PG and exoPG share a few regions of sequence similarity.

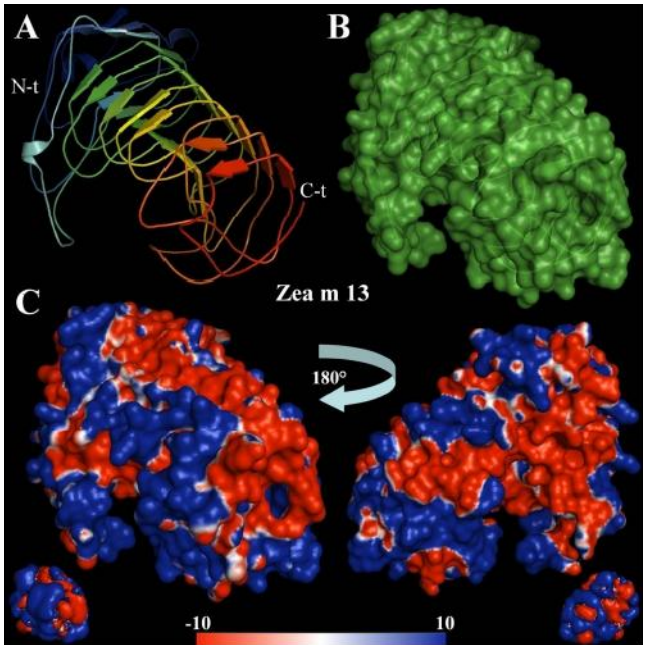

Figure 6. Three-dimensional structures of the allergen protein Zea m 13. Secondary structure elements (A) are displayed in rainbow colors, with N- and C-terminal in blue and red colors respectively. Surface molecule structure (B) is solid green colored, showing secondary structure elements inside. The distribution of charges (electrostatic potential) over the molecular surface is depicted in (C), where positive (+10) and negative (-10) charges are depicted in blue and red color respectively, in two molecular views rotated 180°, including Both representations as isocontours (value of 10 kT/e). PDB from the best protein model built for this allergen (see Material and Methods section) was visualized with PyMol (*http://www.pymol.org/*).

In fruit, polygalacturonase plays an important role in cell wall metabolism during ripening (Willats et al., 2001). They are secreted and display a key role in cell wall biogenesis/degradation. In plant pathogens, polygalacturonase is involved in maceration and soft-rotting of plant tissue. They are also located in reproductive tissues (pollen), where has been detected in the late stages of the pollen development. Group 13 allergens are mainly localized in the cytoplasm of the dry pollen grains (Heslop-Harrison and Heslop-Harrison 1982), thus it is possible that group 13 allergens may be involved in the pollen-stigma interaction during pollination and fertilization by supplying P-particle-derived polysaccharides for the growing pollen tube wall and by dissolving polysaccharides in the

gynoecium (i.e., stigma and pistil) to allow intrusion of the pollen tube (Heslop-Harrison and Heslop-Harrison 1980, 1981).

Due to their heavy pollen production and worldwide distribution, grass pollens belong to the most potent elicitors of seasonal allergic asthma (Freidhoff et al., 1986). They contain several allergens which have been characterized down to the molecular and structural level (Andersson and Lidholm 2003). Among the variety of grass pollen allergens, only two groups of allergens (group 1 and group 13 allergens), occur specifically in all grass pollen subfamilies but not in pollens from other plants.

Polygalacturonases are pollen allergens containing N-linked glycans, which take part in IgE binding (Vrtala et al., 1993). They are major allergens in *Cupressaceae* trees (group 2 allergens) (Grote 1999), as well as they were identified in grass pollen (group 13) (Grote et al., 1993) and in plane tree pollen (Pla a 2). Immunological and biochemical comparison showed that maize pollen contains the grass pollen allergen group 13. Especially the high IgE prevalence of group 1 and 13 (Zea m 1 and Zea m 13) cause cross-reactivity to grasses. The group 13 allergens show a considerable microheterogeneity and degradation, especially after depletion of low-molecular-mass components. Group 13 grass pollen allergens do not cross-react with polygalacturonases from trees and weeds and can therefore be considered as diagnostic markers for genuine grass pollen allergy. Measurement of group 13 allergens in environmental samples may allow us to predict grass pollen allergen exposure more accurately than by pollen counting, which may represent a basis for preventive measures against grass pollen allergy.

10. Zea m Zm13, representative member of the Ole e 1-related proteins superfamily

Zea m Zm13 (Uniprot accession number P33050) (*http://www.uniprot.org/*) is an pollen allergen that belongs to the Ole e 1 protein family (Pfam protein family PF01190), with significant sequence homology with a number of pollen- or anther-specific proteins from monocot and dicot plants, as well as recently described as allergens in olive and rye grass.

Recently has been established a new classification for all the members of the Ole e 1-related protein superfamily (Jimenez-Lopez et al., 2011). In this classification, Zea m Zm13 has been identified as a member of the family Ole e 1_48, concretely classified as Ole e 1_48H6, which is the most extensive family with 63 gene members encoding for different pollen-specific protein C13 (Zm13) homologues.

Included in this pollen proteins Ole e 1 family, there are members as Che a 1 (*Chenopodium album* L.), Cro s 1 (*Crocus sativus*), Fra e 1 (*Fraxinus excelsior*), Lig v 1 (*Ligostrum vulgaris*), Lol p 11 (*Lolium perenne*ryegrass), Ole e 1 (*Olea europaea* L.), Phl p 11 (*Phleum pratense*), Pla l 1 (*Plantago lanceolata*), and Sal k 5 (*Salsola kali*) (*http://www.allergome.org/*).

The polypeptides encoded by the LAT52 gene from tomato and the Zmc13 gene from maize pollens also exhibit a high similarity to Ole e 1 (Twell et al., 1989; Hanson et al., 1989). These plant pollen proteins are structurally related (Figure 7) but their biological function is not yet known; though they have been suggested to be involved in important events of pollen physiology, such as hydration, germination and/or pollen tube growth, and other reproductive functions (Alché et al., 1999; 2004).

Pollen proteins Ole e I family signature or consensus pattern sequences PS00925 (*http://prosite.expasy.org/*), where the Ole e I (Ole) domain defines this family, is characterized by the amino acid sequence: [EQT]-G-x-V-Y-C-D-[TNP]- C-R, where "x" could be any residue. Structurally, the Ole domain contains six conserved cysteines which may be involved in disulfide bonds, since no free sulfhydryl groups have been detected in the native protein (Villalba et al., 1993). Olive Ole e 1 exhibits a high degree of microheterogeneity, mainly concentrated in the third of the molecule closer to the N- terminus (Hamman-Khalifa et al., 2008). There is a high diversity of proteins sharing the Ole domain among plant species. To date, eleven Ole domain-containing genes have been isolated and characterized from olive pollens (Rodríguez et al., 2002). Ole-containing proteins include proline-rich proteins, proteins encoding extensin-like domains, phosphoglycerate mutase, tyrosine-rich hydroxyproline-rich glycoprotein, and hydroxyproline-rich glycoprotein.

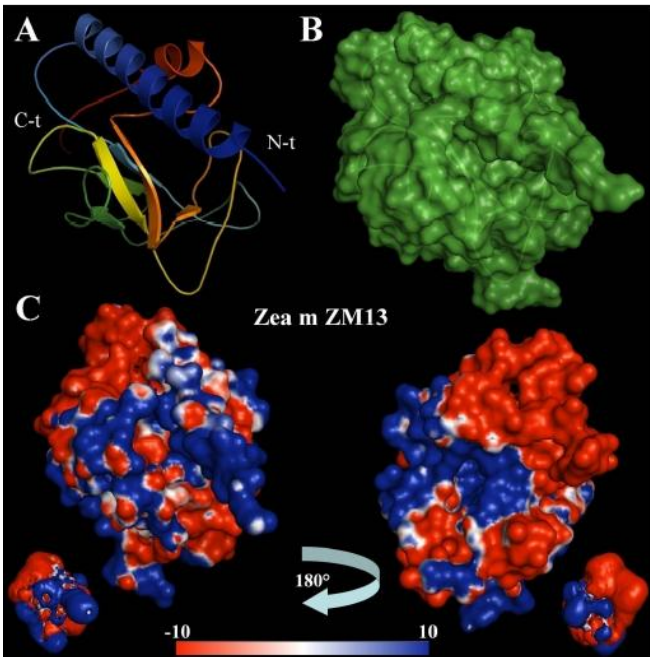

Figure 7. Three-dimensional structures of the allergen protein Zea m Zm13. Secondary structure elements (A) are displayed in rainbow colors, with N- and C-terminal in blue and red colors respectively. Surface molecule structure (B) is solid green colored, showing secondary structure elements inside. The distribution of charges (electrostatic potential) over the molecular surface is depicted in (C), where positive (+10) and negative (-10) charges are depicted in blue and red color respectively, in two molecular views rotated 180°, including Both representations as isocontours (value of 10 kT/e). PDB from the best protein model built for this allergen (see Material and Methods section) was visualized with PyMol (*http://www.pymol.org/*).

According to the established naming system for the Ole e 1 family (Jimenez-Lopez et al., 2011), existing sequences included in this family are integrating 109 Ole e 1 gene families which have been attributed to different functional categories including extensins and extensin-like proteins, proline-rich proteins, hydroxyproline-rich glycoproteins, tyrosine-rich/hydroxyproline-rich glycoproteins, hydrolases, phosphoglycerate mutases, arabinogalactan proteins.

This family of proteins with unknown biological function, contains extracellular plant pollen glycoproteins of about 145 residues in length that contain six conserved cysteine residues that seem to be involved in disulfide bonds.

xxxxxxCxCxxxxxxxxxCxxxxxxxxxxxxxxxxxCxxxxxCxxxxxxxxxxxxxxxxxxxxCxxxxxxx **********

"C": conserved cysteine involved in a disulfide bond.

"*": position of the pollen proteins Ole e I family signature or consensus pattern.

"X" other amino acids.

The prototype member of this family is the major olive pollen allergen, Ole e 1. This allergen binds IgE from 90% of olive pollen allergic patients' sera (Quiralte et al., 2002). Homologous allergens were identified in other trees from the family *Oleaceae* (ash, lilac, privet), from grass pollen (group 11 grass pollen allergens), and from weed pollen (plantain, lamb's quarters). These allergens are glycosylated, and the glycan moieties take part in IgE binding (Batanero et al., 1999). It was shown that IgE cross-reactivity among Ole e 1-related allergens does not extend beyond plant family borders (Lombardero et al., 2002).

11. Zea m 14, a member of the lipid transfer protein superfamily

Zea m 14 is an allergen found in maize seeds, which belongs to the lipid transfer proteins (LTPs) Superfamily, low molecular weight proteins (Figure 8), which are able to facilitate the transfer of phospholipids and other lipids across membranes. These proteins, whose subcellular location is not yet known, could play a major role in membrane biogenesis by conveying phospholipids such as waxes or cutin from their site of biosynthesis to membranes unable to form these lipids. The proteins have been characterized in many plant species and are found in a variety of tissues and developmental stages. Two main families with different molecular masses have been isolated. One is composed by proteins with molecular mass of about 9 kDa (LTP1) and the other, by proteins with molecular mass of 7 kDa (LTP2). The LTP1 proteins are basic, with isoelectric points (pI) of between 9 and 10 (Kuppannan et al., 2011). Plant lipid transfer proteins have a characteristic consensus pattern (Prosite accession number PS00597) (*http://prosite.expasy.org/*), with the following sequence:

[LIVM]-[PA]-x(2)-C-x(1,2)-[LIVM]-x(1,2)-[LIVMST]-x-[LIVMFY]-x(1,2)-[LIVMF]-[STRD]-x(3)-[DN]-C-x(2)-[LIVM],

where the 2 C's are involved in disulfide bonds.

Concretelly, sequences of LTP1 are characterized by having 90-95 amino acid residues, of which 8 are positional conserved cysteines, involved in intramolecular disulfide bridges, following schematic representation:

**********……..xCxxxxCxxxxxxCCxxxxxxxxxCxCxxxxxxxxxxxxCxxxxxxCx

where "C" are conserved cysteines involved in a disulfide bond, and the asterisk "*" represents the position of the consensus pattern.

LTPs do not contain aromatic tryptophan or phenylalanine residues. Two well conserved tyrosine residues are located toward the N- and C-termini of the polypeptide backbone.

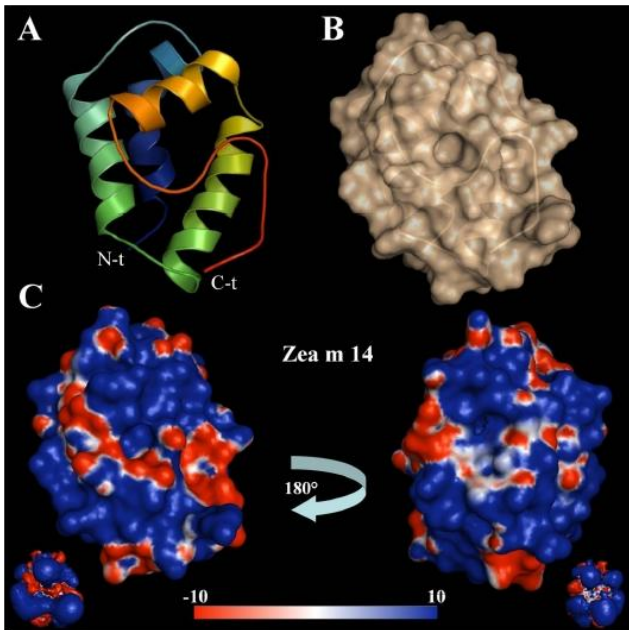

Figure 8. Three-dimensional structures of the allergen protein Zea m 14. Secondary structure elements (A) are displayed in rainbow colors, with N- and C-terminal in blue and red colors respectively. Surface molecule structure (B) is solid orange colored, showing secondary structure elements inside. The distribution of charges (electrostatic potential) over the molecular surface is depicted in (C), where positive (+10) and negative (-10) charges are depicted in blue and red color respectively, in two molecular views rotated 180°, including Both representations as isocontours (value of 10 kT/e). PDB from the best protein model built for this allergen (see Material and Methods section) was visualized with PyMol (*http://www.pymol.org/*).

The functional role of LTPs in plants has been extensively debated. LTP1s have been found in the cell wall in *Arabidopsis thaliana*, *Zea mays*, *Ricinus communis*, and *Vigna unguiculata* seeds. In *R. communis* kernels, a LTP isoform has been found inside an organelle, which was characterized as the glyoxosome, possibly involved in the regulation of the catabolism of lipid storage. In *Brassica oleracea* var. italica, LTP was found associated with the waxy surface of the leaves, and the expression pattern indicates a role of the LTP in the transport of monomers of cutin. Abiotic stress factors such as drought, cold, and salt have been described to upregulate members of the LTP family in some plant species. LTPs have a potential role in plant growth and development, including embryogenesis, germination, and pollen-pistil interaction. The role of LTPs still remains not known, but the role in plant defense mechanisms against phytopathogens such as bacteria, fungi, and viruses seems to be well-established.

LTPs have recently been identified as plant food allergens. They are considered a complete food allergen in that they are capable of inducing specific IgE as well as eliciting severe symptoms. Zea m 14 has also been isolated from maize flour. Skin reactivity and IgE antibodies to this allergen were detected in 86% patients with systemic symptoms following the ingestion of maize, confirming this as the maize major allergen. LTP is an extremely

stable protein that is resistant to both proteolytic attack and food processing, which permits the allergen to reach the gastrointestinal immune system in an immunogenic and allergenic conformation, allowing sensitization and induction of systemic symptoms. Maize lipid transfer protein is highly heat-stable. Treatment at even 100°C for 160 minutes, though it almost totally eliminated the IgE-binding activity of the higher-molecular-weight bands seen in maize, did not affect that of the lipid transfer protein (Pastorello et al., 2003).

12. Zea m 20S, a maize allergen with pectate lyase activity

Zea m 20S, named also as proteasome subunit beta type, belongs to the group of pectate Lyases enzymes, which has been identified in maize seeds. Right until now, only a protein sequence named as Q5XML0 has been found in Uniprot database (*http://www.uniprot.org/*).

There have been included in this family, members from different species such as *Ambrosia artemisiifolia* (Amb a 1, including 11 isoforms), *Ambrosia psilostachya* (Amb p 1), *Artemisia vulgaris* (Art v 6, including 2 isoforms), *Aspergillus fumigatus* (Asp f PL), *Chamaecyparis obtusa* (Cha o 1, with 2 isoforms), *Cryptomeria japonica* (Cry j 1, with 4 isoforms), *Cuppressus arizonica* (Cup a 1.0101, 2 isoforms), *Cupressus sempervirens* (Cup s 1), *Juniperus sabinoides* (Jun a 1, 2 isoforms), *Juniperus communis* (Jun c 10), *Juniperus oxycedrus* (Jun o 1), *Juniperus virginiana* (Jun v 1, 2 isoforms), *Senecio jacobaea* (Sen j PL), and *Thuja plicata* (Thu p 1) (*http://www.allergome.org/*).

Zea m 20S is also classified as a member of the proteasome subunit family of proteins. The proteasome (or macropain) is a multicatalytic proteinase complex (EC 3.4.25.1), which is characterized by its ability to cleave peptides with Arg, Phe, Tyr, Leu, and Glu at neutral or slightly basic pH. The proteasome has an ATP-dependent proteolytic activity. It is an eukaryotic and archaebacterial multicatalytic enzyme involved in an ATP/ubiquitin-dependent non-lysosomal proteolytic pathway. In eukaryotes the proteasome is composed of about 28 distinct subunits which form a highly ordered ring-shaped structure (20S ring) of about 700 Kd.

As a signature pattern (Prosite accession number PS00854) (*http://prosite.expasy.org/*) for proteasome B-type subunits, it has been selected the best conserved region, which is located in the N-terminal part of these proteins:

[LIVMACFT]-[GSA]-[LIVMF]-x-[FYLVGAC]-x(2)-[GSACFYI]-[LIVMSTACF]-[LIVMSTAC]-[LIVMFSTAC]-[GACI]-[GSTACV]-[DES]-x(15,16)-[RK]-x(12,13)-G-x(2)-[GSTA]-D

Structural characteristics of the 26S proteasome have shown a 20S proteasome core and two 19S regulatory subunits. The 20S proteasome core is composed of 28 subunits that are arranged in four stacked rings, resulting in a barrel-shaped structure. The two end rings are each one formed by seven alpha subunits, and the two central rings are each one formed by seven beta subunits (Figure 9).

This proteasome has been located in the cytoplasm and the nucleus, having different molecular functions: 1) Hydrolase, 2) Protease, and 3) Threonine protease.

Subunits that are known to belong to this family are listed as following:

- ✓ Vertebrate subunits C5, β, delta, epsilon, theta (C10-II), LMP2/RING12, C13 (LMP7/RING10), C7-I and MECL-1.
- ✓ Yeast PRE1, PRE2 (PRG1), PRE3, PRE4, PRS3, PUP1 and PUP3.
- ✓ Drosophila L(3)73AI.
- ✓ Fission yeast pts1.
- ✓ Thermoplasma acidophilum β-subunit. In this archaebacteria the proteasome is composed of only two different subunits.

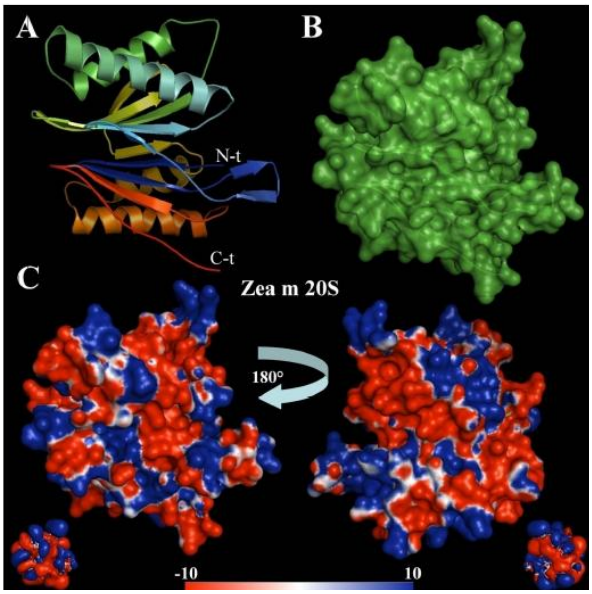

Figure 9. Three-dimensional structures of the allergen protein Zea m 20S. Secondary structure elements (A) are displayed in rainbow colors, with N- and C-terminal in blue and red colors respectively. Surface molecule structure (B) is solid green colored, showing secondary structure elements inside. The distribution of charges (electrostatic potential) over the molecular surface is depicted in (C), where positive (+10) and negative (-10) charges are depicted in blue and red color respectively, in two molecular views rotated 180°, including Both representations as isocontours (value of 10 kT/e). PDB from the best protein model built for this allergen (see Material and Methods section) was visualized with PyMol (*http://www.pymol.org/*).

13. Zea m 22, an enolase with glycolytic function

Zea m 22, a maize pollen allergen belongs to the Enolase (EC 4.2.1.11) family of proteins, which is a glycolytic enzyme that catalyzes the dehydration of 2-phospho-D-glycerate to phosphoenolpyruvate (2-phospho-D-glycerate = phosphoenolpyruvate + H2O) (Zhang et al., 1997). It is therefore alyase structurally, it is a dimeric enzyme that requires magnesium both for catalysis and to stabilize the dimer (Figure 10).

A unique sequence has been deposited and characterized as an allergen (P42895) in Uniprot database (http://www.uniprot.org/). A characteristic signature sequence pattern (Prosite accession number PS00164) (http://prosite.expasy.org/), has been found for this

group of proteins, located in the C-terminal end of the sequence. This consensus pattern has the following sequence:

[LIVTMS]-[LIVP]-[LIV]-[KQ]-x-[ND]-Q-[INV]-[GA]-[ST]-[LIVM]-[STL]-[DERKAQG]-[STA].

A cDNA clone (pZM245 or pENO1) encoding maize enolase has been previously identified (Lal et al., 1991), and named eno1. The transcript levels detected by the ENO1 did not increase after 24 h of anaerobic stress (Lal et al.,, 1994). Enolase was also purified from maize seeds, and characterizated as three isoforms, although eno1 is a single-copy gene in maize (Lal et al., 1991). Later another cDNA encoding maize enolase (pENO2) was cloned.Its nucleotide sequence, and its expression during the anaerobic-stress response are compared with those of the previously reported pENO1 enolase clone. These two enolase cDNAs, ENO1 and ENO2 are the products of two different genes.

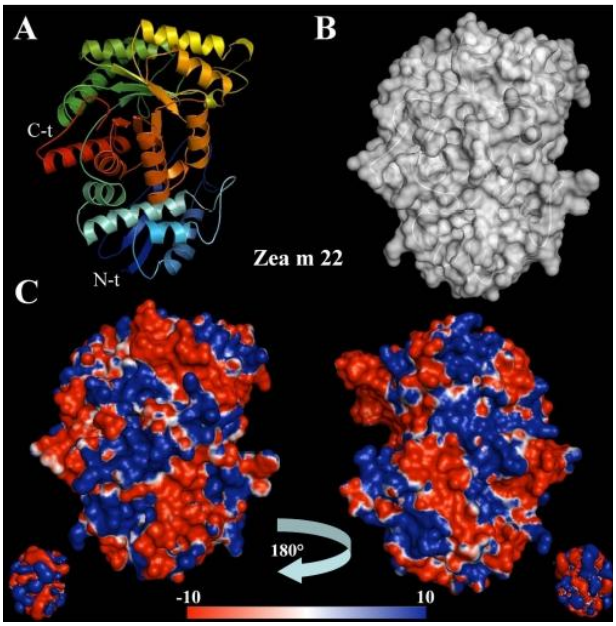

Figure 10. Three-dimensional structures of the allergen protein Zea m 22. Secondary structure elements (A) are displayed in rainbow colors, with N- and C-terminal in blue and red colors respectively. Surface molecule structure (B) is solid white colored, showing secondary structure elements inside. The distribution of charges (electrostatic potential) over the molecular surface is depicted in (C), where positive (+10) and negative (-10) charges are depicted in blue and red color respectively, in two molecular views rotated 180°, including Both representations as isocontours (value of 10 kT/e). PDB from the best protein model built for this allergen (see Material and Methods section) was visualized with PyMol (*http://www.pymol.org/*).

Many members of this family of proteins have been found in different species, such as Alt a 6 (*Alternaria alternata*, 1 isoform) , Asp c 22 (*Aspergillus communis*), Asp f 22 (*Aspergillus fumigates*, 1 isoform), Asp v Enolase (*Aspergillus versicolor*), Bea b Enol (*Beauveria bassiana*), Bla g Enolase (order *Blattaria*), Cand a Enolase (*Candida albicans*), Cla h 6 (*Cladosporium herbarum*, 1 isoform), Cur l 2 (*Curvularia lunata*, 1 isoform), Cyn d

22 (*Cynodon dactylon*, 1 isoform), Dan re 2 (*Danio rerio*), Hev b 9 (*Hevea brasiliensis*, 1 isoform), Meg am 2 (*Megalobrama amblycephala*), Neo fi 22 (*Neosartorya fischeri*), Ore mo 2 (*Oreochromis mossambicus*), Pen c 22 (*Penicillium citrinum*, 1 isoform), Rho m 1 (*Rhodotorula rubra*), Rho m 1.0101 (*Rhodotorula mucilaginosa*), Sac c Enolase (*Saccharomyces cerevisiae*), Sal s 2 (*Salmo salar*, 1 isoform), and Tal st 22 (*Talaromyces stipitatus*) (*http://www.allergome.org/*).

Enolase is probably found in all organisms that metabolize sugars. In vertebrates, there are three different tissue-specific isozymes: α present in most tissues, β in muscles and γ found only in nervous tissues. The cytoplasm is the subcellular localization of this enzyme, where glycolysis processes take place.

The regulation of enolase in plants is complex and, may involve posttranslational modifications (Van Der Straeten et al., 1991; Lal et al., 1994) such as phosphorylations in a highly conserved region around Tyr-46 for different species, including maize enolase (Lal et al., 1991; Van Der Straeten et al., 1991).

14. Zea m 25, a member of the thioredoxin superfamily

Zea m 25 belongs to the thioredoxin (Trx) superfamily, which has been identified in maize seeds. They are ubiquitous proteins of about 12-14–kDa, they are regulatory proteins that reduce intrachain disulphide bridges of target proteins (thioredoxin [–SH HS–] + target protein [–S-S–] gthioredoxin [–S-S–] + target protein [–SH HS–]), such as the wheat storage prolamins (gliadins and glutenins), thus enhancing mobilization of these proteins in germinating wheat seeds (Kobrehel et al., 1992).

There are 2 isoforms of Zea m 25 which have been deposited in Uniprot database (*http://www.uniprot.org/*) with the accession numbers Q4W1F6 and Q4W1F7. This protein has been crystallized and the coordinates are deposited in the PDB database (*http://www.rcsb.org/*).

They serve as general protein disulfide oxidoreductases and interact with a broad range of proteins. Multiple in vitro substrates for thioredoxin have also been identified, including ribonuclease, choriogonadotropins, coagulation factors, glucocorticoid receptor, and insulin. Reduction of insulin is classically used as an activity test.

There are many allergenic representative forms in different species: Alt a 4(Alternaria alternate), Asp f 28 (*Aspergillus fumigatus*), Asp f 28.0101 (*Aspergillus fumigatus*), Asp f 29 (*Neosartorya fumigate*, 1 isoform), Cop c 2 (*Coprinus comatus*), Cur l Trx (*Cochliobolus lunatus*), Fus c 2 (*Fusarium culmorum*, 1 isoform), Hev b Trx (*Hevea brasiliensis*), Hom s Trx (*Homo sapiens*), Mala s 13 (*Malassezia sympodialis*, 1 isoform), Plo i 2 (*Plodia interpunctella*, 1 isoform), and Tri a 25 (*Triticum aestivum*, 1 isoform) (*http://www.allergome.org/*).

This family of proteins is characterizated by a molecular signature sequence pattern, with the Prosite accession number PS00194 (*http://prosite.expasy.org/*), and a defined sequence like [LIVMF]-[LIVMSTA]-x-[LIVMFYC]-[FYWSTHE]-x(2)-[FYWGTN]-C-[GATPLVE]-[PHYWSTA]-C-{I}-x-{A}-x(3)-[LIVMFYWT], where the 2 C's form the redox-active bond.

These redox proteins are known to be present in all organisms. Thioredoxin is present in prokaryotes and eukaryotes and the sequence around the redox-active disulfide bond is well conserved. Bacteriophage T4 also encodes for a thioredoxin but its primary structure is not

homologous to bacterial, plant or vertebrate thioredoxins. It plays a role in many important biological processes. In humans, loss-of-function mutation of either of the two human thioredoxin genes is lethal for the developing embryo. Thioredoxin plays a central role in humans through their response to reactive oxygen species (ROS). In plants, thioredoxins perform a plethora of critical functions, ranging from photosynthesis to growth, flowering and the development and germination of seeds. It has also recently been found to play a role in cell-to-cell communication (Meng et al., 2010). Plants have an unusually complex complement of Trxs composed of six well-defined types (Trxs f, m, x, y, h, and o) that reside in different cell compartments and function in an array of processes.

Thioredoxins are characterized at the level of their amino acid sequence by the presence of two vicinal cysteines in a CXXC motif. These two cysteines are the key to the ability of thioredoxin to reduce other proteins. Thioredoxin proteins also have a characteristic tertiary structure termed the thioredoxin fold (Figure 11).

Allergenic thioredoxins are found in fungi (Asp f 28, Asp f 29, Mala s 13 and Alt a 4) as well as in plants, including cereal allergens associated with baker's asthma, and thioredoxin from natural rubber latex.

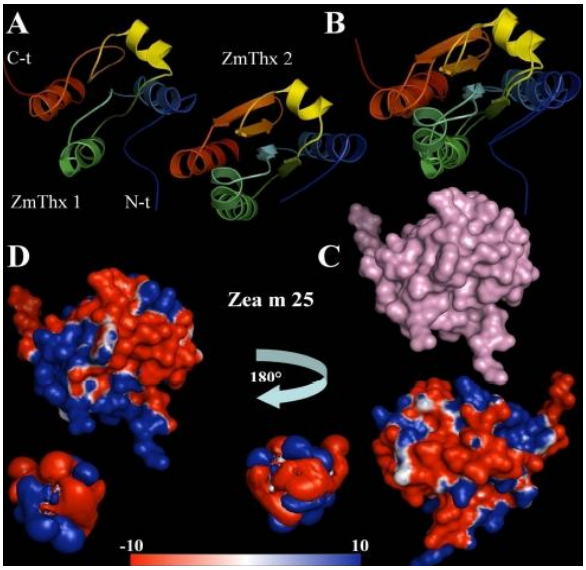

Figure 11. Three-dimensional structures of the allergen protein Zea m 25. Secondary structure elements of ZmThx1 and ZmThx2 (A) are displayed in rainbow colors, with N- and C-terminal in blue and red colors respectively. Superimposition of the 2 structures (B) had shown a high structural similarity, beside the C- and N-terminal regions that are shorter in ZmThx2. Surface molecule structure of ZmThx1 (C) is solid light pink colored, showing secondary structure elements inside. The distribution of charges (electrostatic potential) over the molecular surface is depicted in (D), where positive (+10) and negative (-10) charges are depicted in blue and red color respectively, in two molecular views rotated 180°, including Both representations as isocontours (value of 10 kT/e). PDB from the best protein model built for this allergen (see Material and Methods section) was visualized with PyMol (*http://www.pymol.org/).*

Wheat thioredoxin (Tri a 25) has been identified as a novel allergen related to baker's asthma (Weichel et al., 2006). Both Tri a 25 and its homologous maize thioredoxin Zea m 25

(74% of amino acid sequence identity) have been produced as recombinant proteins in *Escherichia coli* and tested against 17 sera from patients with baker's asthma (Weichel et al., 2006). Sensitization (specific IgE) rates of 47% were found for both recombinant allergens. Specific IgE to the wheat and maize thioredoxin allergens was also detected in 35% and 20% of 20 sera from subjects with grass pollen allergy, but no clinical history of wheat or maize allergy, thus suggesting cross-reactivity between Tri a 25, Zea m 25, and grass pollen thioredoxins. Tri a 25 could also be involved in wheat-dependent food allergy (Weichel et al., 2006), as are other wheat allergens associated with baker's asthma.

15. Zea m 27kD Zein belongs to the prolamin superfamily

The common name for this maize allergen is Zea m 27kD Zein, which belongs to the prolamin superfamily. Two protein sequences of this type have been identified in maize seeds with allergenic properties: P04706 and Q548E9 (*http://www.uniprot.org/*).

The biological function and structural characteristics of this protein correspond to these of the gliadin/glutenin Superfamily (Figure 12), which are alcohol soluble seed storage proteins, mainly located in the endosperm of the seed, concretely a vacuolar (aleurone grain membrane) subcellular location, in the border of the inner part of the membrane of endosperm protein bodies. They serve as nutrient reservoir, filling about 15% of the total endosperm protein content.

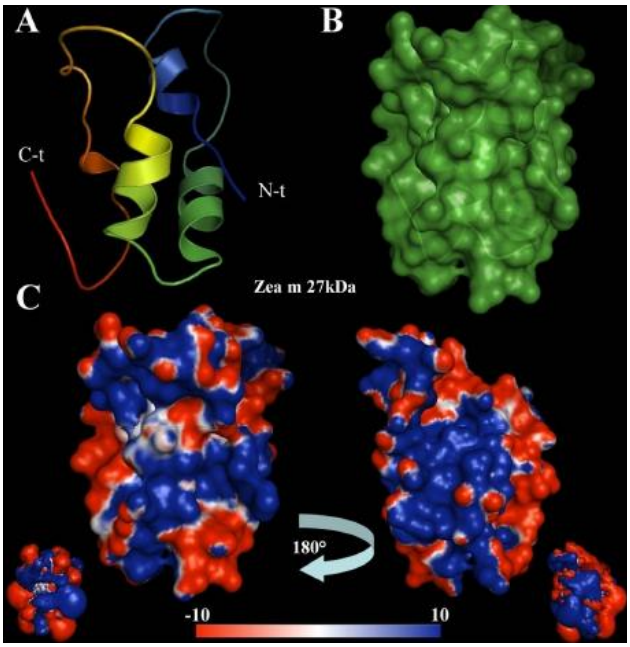

Figure 12. Three-dimensional structures of the allergen protein Zea m 27kD Zein. Secondary structure elements (A) are displayed in rainbow colors, with N- and C-terminal in blue and red colors respectively. Surface molecule structure (B) is solid green colored, showing secondary structure elements inside. The distribution of charges (electrostatic potential) over the molecular surface is depicted in (C), where positive (+10) and negative (-10) charges are depicted in blue and red color respectively, in two molecular views rotated 180°, including Both representations as isocontours (value

of 10 kT/e). PDB from the best protein model built for this allergen (see Material and Methods section) was visualized with PyMol (*http://www.pymol.org/*).

It has been demonstrated that the 27 kDa γ-zein induces a strong immunological response in young pigsUsing fulllength FASTA search against the AllergenOnline database, it was possible to detect sequence homology between maize 27 kDa γ-zein and several known allergens. This bioinformatic analysis enabled to ascertain the potential risk of allergenic cross-reactivity of the 27 kDa γ-zein. Interestingly, the 27 kDa γ-zein contains a peptide (RQQCCQQLRQ) that has been identified as an IgE binding epitope present in the high molecular weight glutenin, a known allergen (Matsuo et al., 2004). Additionally, using sera from individuals sensitive to maize, it was possible to demonstrate specific IgE binding to the 27 kDa γ-zein. These results unveil the allergenic potential of the 27 kDa γ-zein (Krishnan et al 2011).

16. Zea m 50kD Zein belongs to the prolamin superfamily

The common name of this allergen is Zea m 50kD Zein, which belongs to the prolamin superfamily. Only a single protein sequence of this type has been identified in maize seeds with allergenic properties: Q946W1 (*http://www.uniprot.org/*).

The biological function and structural characteristics of this protein corresponds to that of the gliadin/glutenin superfamily (Figure 13). It belongs to the reduced soluble protein (RSP) fraction. It has been isolated and has shown to be stable to heat and digestion.

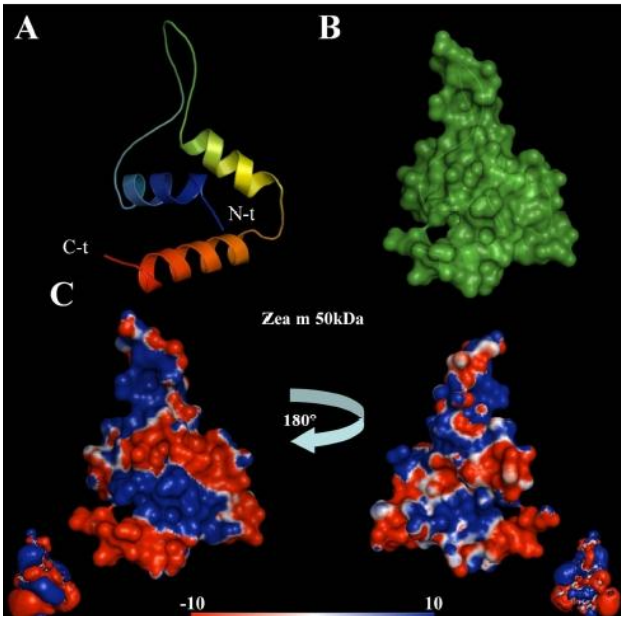

Figure 13. Three-dimensional structures of the allergen protein Zea m 50kD Zein. Secondary structure elements (A) are displayed in rainbow colors, with N- and C-terminal in blue and red colors respectively. Surface molecule structure (B) is solid green colored, showing secondary structure elements inside. The distribution of charges (electrostatic potential) over the molecular surface is depicted in (C), where positive (+10) and negative (-10) charges are depicted in blue and red color

respectively, in two molecular views rotated 180°, including Both representations as isocontours (value of 10 kT/e). PDB from the best protein model built for this allergen (see Material and Methods section) was visualized with PyMol *http://www.pymol.org/.*

Major proteins in maize endosperm are composed of 2S albumins, 7S and 11S globulins in the cytoplasm, and aqueous alcohol-soluble prolamins (zeins) found in storage protein bodies (Shewry et al., 1999).

Zea m 50 kD allergen is resistant to pepsin or pepsin plus trypsin and is also stable to heating (cooking) (Watanabe 1995). This protein also contains a sequence motif (Gln- Gln-Gln-Pro-Gln) similar to the IgE-binding epitope (Gln-Gln- Gln-Pro-Pro) of wheat glutenin (Sung-Ho Lee 2005)**.**

17. Zea m Chitinase

The common name for this allergen is chitinase, which is representatives of its biological function. Class I is also known as Hevein-like domain proteins. Only a single gene of this type has been found in maize seeds (Q6JBK3 as Uniprot accession number) (*http://www.uniprot.org/*) with allergenic properties. These proteins are implicated in catabolic processes of macromolecules of the cell wall.

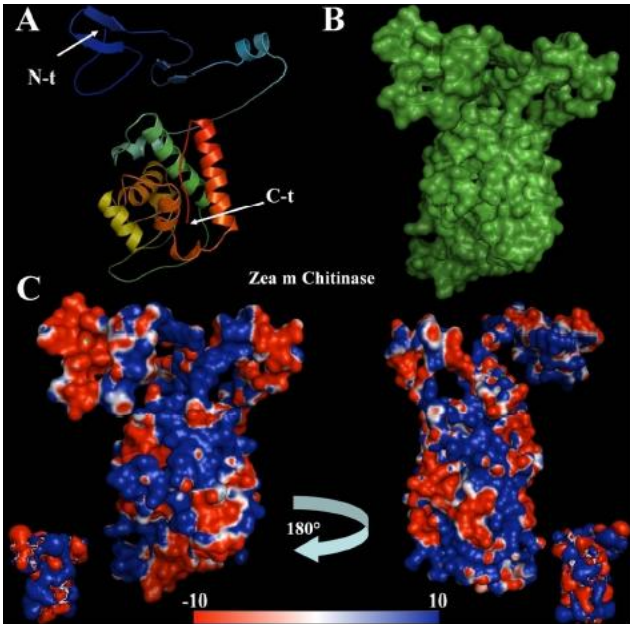

Figure 14. Three-dimensional structures of the allergen protein Zea m Chitinase. Secondary structure elements (A) are displayed in rainbow colors, with N- and C-terminal in blue and red colors respectively. Surface molecule structure (B) is solid green colored, showing secondary structure elements inside. The distribution of charges (electrostatic potential) over the molecular surface is depicted in (C), where positive (+10) and negative (-10) charges are depicted in blue and red color respectively, in two molecular views rotated 180°, including Both representations as isocontours (value of 10 kT/e). PDB from the best protein model built for this allergen (see Material and Methods section) was visualized with PyMol (*http://www.pymol.org/*).

Chitinases are enzymes that catalyze the hydrolysis of the beta-1, 4-N-acetyl-D-glucosamine linkages in chitin polymers. Chitinases belong to glycoside hydrolase families 18 or 19 (Henrisaat 1991). Chitinases of family 19 (also known as classes IA or I and IB or II) are enzymes from plants that function in the defence against fungal and insect pathogens by destroying their chitin-containing cell wall. The catalytic domain of these enzymes consists of about 220 to 230 amino acid residues (Figure 14). This chitin-bind-1 family (Pfam accession number PF00187) has a carbohydrate-binding module (CBM) found in carbohydrate-active enzymes (for example glycoside hydrolases). Zea m Chitinase has been identified as new allergen in maize by proteomic tools (Fasoli et al., 2009).

18. Zea m G1

The common name of this allergen is vicilin-like embryo storage protein. Itbelongs to the cupin superfamily, which is a diverse superfamily of proteins containing a conserved barrel domain (Figure 15). The cupin superfamily includes a wide variety of enzymes, but also contains the non-enzymatic seed storage proteins. The route of exposure to this allergen is by ingestion of seeds. Only a single protein sequences of this allergen is know, with Uniprot accession number Q03865 (*http://www.uniprot.org/*). Zea m G1 has been identified in seeds as new allergen of maize by proteomic tools (Fasoli et al., 2009).

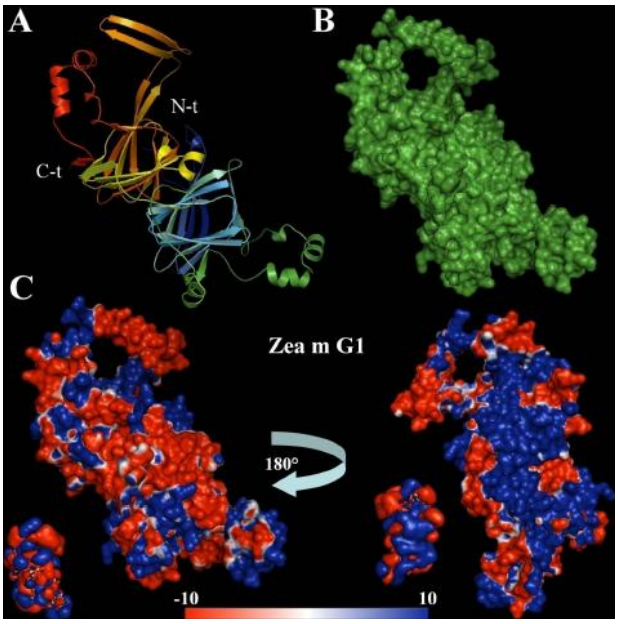

Figure 15. Three-dimensional structures of the allergen protein Zea m G1. Secondary structure elements (A) are displayed in rainbow colors, with N- and C-terminal in blue and red colors respectively. Surface molecule structure (B) is solid green colored, showing secondary structure elements inside. The distribution of charges (electrostatic potential) over the molecular surface is depicted in (C), where positive (+10) and negative (-10) charges are depicted in blue and red color respectively, in two molecular views rotated 180°, including Both representations as isocontours (value of 10 kT/e). PDB

from the best protein model built for this allergen (see Material and Methods section) was visualized with PyMol (*http://www.pymol.org/*).

19. Zea m G2

The common name of this allergen is globulin-2 precursor. It belongs to the cupin superfamily (Figure 16). The route of exposure to this allergen is by ingestion of seeds. Only a protein sequences is know of this allergen, with Uniprot accession number Q7M1Z8 (*http://www.uniprot.org/*).

The majority of these domains have carbohydrate-binding activity. Some of these domains are found on cellulosomal scaffolding proteins. CBMs were previously known as cellulose-binding domains (Gilkes et al., 1991). CBMs are classified into numerous families, based on amino acid sequence similarity. There are currently 64 families of CBM (Cantarel et al., 2009).

They play a central role in the recycling of photosynthetically fixed carbon through their binding to specific plant structural polysaccharides (Szabo et al., 2009). CBMs can recognise both crystalline and amorphous cellulose forms (Jamal et al., 2004).

Zea m G2 has been identified in seeds as new allergen of maize by proteomic tools (Fasoli et al., 2009).

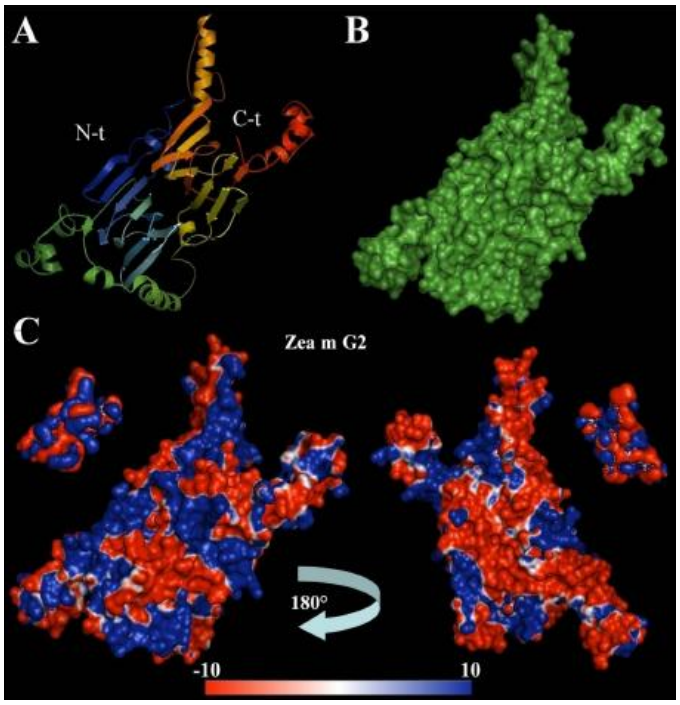

Figure 16. Three-dimensional structures of the allergen protein Zea m G2. Secondary structure elements (A) are displayed in rainbow colors, with N- and C-terminal in blue and red colors respectively. Surface molecule structure (B) is solid green colored, showing secondary structure elements inside. The distribution of charges (electrostatic potential) over the molecular surface is depicted in (C), where positive (+10) and negative (-10) charges are depicted in blue and red color respectively, in two

molecular views rotated 180°, including Both representations as isocontours (value of 10 kT/e). PDB from the best protein model built for this allergen (see Material and Methods section) was visualized with PyMol (*http://www.pymol.org/*).

20. Zea m PAO, a member of the amine oxidases superfamily

The amone oxidase superfamily is a group of flavin-containing amine oxidoreductases that catalyze the oxidation of the secondary amino group of polyamines (spermine, spermidine and their acetyl derivatives).

This group includes leaf maize polyamine oxidase (PAO), L-amino acid oxidases (LAO) and various flavin containing monoamine oxidases (MAO). This protein needs cofactors for its functionality like one FAD per subunit and its structure is monomeric (Figure 17). It plays an important role in the regulation of the intracellular concentration of polyamine.

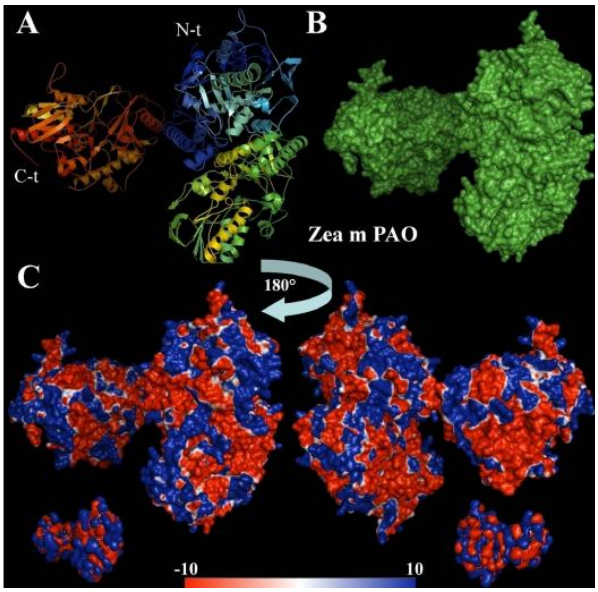

Figure 17. Three-dimensional structures of the allergen protein Zea m PAO. Secondary structure elements (A) are displayed in rainbow colors, with N- and C-terminal in blue and red colors respectively. Surface molecule structure (B) is solid green colored, showing secondary structure elements inside. The distribution of charges (electrostatic potential) over the molecular surface is depicted in (C), where positive (+10) and negative (-10) charges are depicted in blue and red color respectively, in two molecular views rotated 180°, including Both representations as isocontours (value of 10 kT/e). PDB from the best protein model built for this allergen (see Material and Methods section) was visualized with PyMol (*http://www.pymol.org/*).

The catalytic activity can be summarized as follow (Tavladoraki et al., 1998):

Spermidine + O2 + H2O = propane-1, 3-diamine + 4-aminobutanal +H2O2.

N(8)-acetylspermidine + O2 + H2O = propane-1,3-diamine + 4-acetamidobutanal + H2O2.

Spermine + O2 + H2O = N-(3-aminopropyl)-4-aminobutanal + trimethylenediamine + H2O2.

N(1)-acetylspermine + O2 + H2O = N-(3-acetamidopropyl)-4-aminobutanal + trimethylenediamine + H2O2.

Up to now, two genes of this type have been sequenced in maize named O64411 and Q546R6 (*http://www.uniprot.org/*). There is also a crystallographic structure deposited in the PDB database (1B37) (*http://www.rcsb.org/*) (Figure 17). The leaf is the main location for these enzymes.

Other members of this family include tryptophan 2-monooxygenase, putrescine oxidase, corticosteroid-binding proteins, and antibacterial glycoproteins.

Zea m PAO has been identified as allergen in maize (Iacovacci et al., 2001)

POTENTIAL CROSS-REACTIVITY OF MAIZE ALLERGENS

The allergenic potency of maize pollen compared with the native grass *P. pratense* could serve as a standard for assessing the influence of maize pollen on occupational asthma, e.g., for farmers or other strongly exposed persons, and also to assess the importance of altered protein composition in genetically engineered maize varieties if compared to the cultivated wild-type maize. Two structural characteristics of maize pollen may explain the low incidence of maize pollen allergenicity when compared to the pollen of other grasses:

- ✓ Increased pollen size of the grains causes a low aerial spread of maize pollen. In comparison, maize pollen (although having a shape similar to European native grass pollen) is about three times larger in diameter and thus possesses some 27-fold higher volume and probably weight than grass pollen.
- ✓ Pollen grains of maize are more sticky due to pollenkitt present on the exine surface, which causes agglomeration of the pollen and may prevent aerial spread even further (Suen et al., 2003).

However, pollen fragments, or aerobiological particles of low molecular weight such as starch granules, may be released (Suphioglu et al., 1992). These may be of importance considering the tremendous amounts of maize pollen produced in agriculture. This may enhance pre-existing grass pollen allergies in susceptible patients.

Based in these about results, it may to be concluded that maize pollen plays a less important role as a sensitizer when compared to pollen of our common grass species. This may be explained by morphological differences, as well as the apparently lower allergen content and the lower number of allergen groups found in maize pollen.

Immunological and biochemical comparison showed that maize pollen contains the four grass pollen allergen groups (1, 3, 12, and 13). As mentioned before, groups 1 and 13 are responsible for high prevalence and cross-reactivity to grasses growing in temperate climate zones. Potential IgE cross-reactivity of the group 12 allergens (profilins) in maize, which was also identified by detecting a high sequence identity between both plant species of 80.6%.

Because of their fundamental role in the formation of the cytoskeleton, profilins are highly conserved proteins; however, profilin IgE reactivity is weak (IgE prevalence for timothy pollen amounts to about 10% (Valenta et al., 1992).

Zea m 1 represents an expansin. In spite of the important function, the structural variability of Zea m 1 between timothy and maize is high (Petersen et al., 1995; Li et al., 2003), which is also the case among Zea m 1 isoforms. In grasses, the IgE prevalence of 95% as determined in the case of Phl p 1 indicates that this protein represents the single most important grass pollen allergen (Laffer et al., 1996).

The group 3 allergens differ considerably in their protein sequences and reveal only 34.5% sequence identity (Petersen et al., 2006). N-terminal sequencing of the Zea m 3 isoform at pI 4.5 shows additional amino acid differences. Both, group 2 and 3 of allergens were reported to belong to the Expansin group (Cosgrove 1996) and both show sequence similarity to the C-terminal part of group 1 allergens.

A sensitisation rate of 47% among bakers with occupational asthma and of 35% among patients with grass pollen allergy, but without a clinical history of cereal allergy, was demonstrated to Tri a 25, a thioredoxin. Maize thioredoxin (Zea m 25) shares a 74% identity with Tri a 25, and exhibits distinct IgE cross-reactivity with its Wheat homologue. Thioredoxins are cross-reactive allergens that might contribute to the symptoms of baker's asthma and might in addition be related to grass pollen allergy. Therefore similar effects may be postulated for Zea m 25 (Weichel et al., 2006).

LTP, endochitinase, zein, and vicilin precursors have been identified as maize allergens with variable importance as regards maize food allergy. One of the most abundant maize allergen is a lipid transfer protein (Asero et al., 2002; 2003). A 9 kDa kernel LTP or Zea m14 is the major food allergen (Pastorello et al., 2000), which ishighly cross-reactive with peach LTP. Maize LTP is a relevant allergen, probably due to the characteristic resistance to thermal treatment, i.e., it is the only maize allergen that maintains IgE-binding property at 100°C for 161 min. A high degree of cross-reactivity has been demonstrated among the LTPs of peach, apple, walnut, hazel nut, peanut, maize, rice, sunflower seed, french bean and apricot (Pastorello et al., 1999; Asero et al., 2001; Conti et al., 2001; IUIS 2008). Furthermore, this wide cross-reactivity has been demonstrated to be mediated by specific conformational epitopes shared by pathogenesis-related proteins (LTP) between pollen and food allergens (Jimenez-Lopez et al., 2012). Maize LTP was shown to cross-react completely with Rice and Peach LTP but not with Wheat or Barley LTP (IUIS 2008). Lipid transfer protein from Cowpea has a high homology of similarity to lipid transfer proteins of Maize (72%) (Carvalho et al., 2006). A 16 kDa allergen, isolated and shown to be a Maize inhibitor of trypsin, was shown to cross-react completely with Grass, Wheat, Barley, and Rice trypsin inhibitors (IUIS 2008). The primary structure of the Japanese cypress (Chamaecyparis obtUS) allergen, Cha o 2, shows significant identity with the polygalacturonases of Avocado, Tomato, and Maize (Mori et al., 1999). The clinical implications of this finding have not been clarified yet.

Three-dimensional structures are known for three allergens from this family (LTP), namely Pru p 3 (2ALG), Hor v 1 (1JTB), Zea m 14 (1MZM). The molecular determinants of allergenicity for this family may be extracted from the known IgE epitopes, for Ara h 2 (Stanley et al., 1997), Jug r 1 (Robotham et al., 2002), Par j 1 (Asturias et al., 2003), and Par j 2 (Asturias et al., 2003). The T-cell epitopes are known only for Ara h 2 (Glaspole et al., 2005).

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