WFL Publisher
Science and Technology

Food, Agriculture & Environment Vol.2 (2) : 14-24. 2004

www.world-food.net

Food and Health

Pathogenesis-related proteins – an important group of plant-derived allergens

Raina Niskanen* and Ramdane Dris

*World-Food RD Ltd, Meri-Rastilantie 3C, FI-00980 Helsinki, Finland. *e-mail:raina.niskanen@world-food.net*

Received 22 January 2004, accepted 17 April 2004.

Abstract

Several protein families that contribute to the defence mechanisms of food plants have members which are allergens or putative allergens. Cross-reactivity between pollens, fruit and vegetables relies upon pan-allergens: pathogenesis-related (PR) proteins, profilins and enzymes with the same functional activity. A number of plant-derived allergens and PR-like proteins found in various species all over the plant kingdom are homologous proteins responsible for cross reactivities between different allergen sources. A considerable percentage of identified plant allergens can be grouped to the families 2, 3, 4, 5, 8, 10 and 14 of pathogenesis-related proteins. Characteristics of these PR groups are reviewed in this paper.

Key words: β-1,3-glucanases, chitinases, intracellular proteins, lipid transfer proteins, thaumatin-like proteins.

Introduction

Almost 20% of people in western populations suffer from IgE-mediated allergies, and individuals with familial atopic background are susceptible to development of allergic reactions to food and/or environmental allergens[115]. The prevalescence of food allergy has been considered to be between 2 and 8% for infants and children, decreasing to 1% for the adult population[107]. However, of the individuals suffering from atopic dermatitis, approximately 20% of the children and 10% of the adults experience clinically significant food allergies[73].

The main sources containing allergens are plants, mites, fungal spores and insects. Plant-derived allergens may either be taken in from the upper respiratory tract or they are present in a vast range of plant food causing food allergic reactions[41]. Inhalative plant allergens present predominantly in pollen, spores, and plant-derived products like cosmetics and rubber articles may cause rhinoconjunctivitis, asthma, edema, urticaria and anaphylaxis. Plant food allergens can cause symptoms ranging from pruritus, swelling of lips, tongue and soft palate, often accompanied by mild laryngeal symptoms as a sensation of tightness, itching, cough and pruritus of the ear canals to gastrointestinal symptoms, rhinitis, asthma, cutaneous reactions and most severe systemic anaphylaxis[41].

Antigens able to elicit the production of IgE antibodies are called allergens. During the last ten years, identification and purification of allergens have been important for structural and immunological studies in order to discover how these molecules stimulate IgE antibody production[107]. The primary structure of some 200 allergens is known; however, only a relatively small number of plant food allergens have been characterized. Allergens are designated according to the accepted taxonomic name. The first three letters describe the genus, followed by the first letter of the species and an Arabic number assigned in the order of the identification. Most of the known plant food allergens are storage proteins (soybean, peanuts) and proteinase inhibitors (wheat, rice, soybean) that are present in large amounts in these foods[63]. In addition, a number of pathogenesis-related (PR) proteins occurring in fresh fruits and vegetables (apple Mal d 1, celery Api g 1) function as food allergens[6].

Allergens tend to be ovoid in shape and structural motifs are important for allergenicity [47]. The protein structure of an allergen can be described at different levels known as primary (amino acid sequence), secondary, tertiary and quaternary structures[13]. From the immunological point of view, most important are the structures defined as epitopes (antigenic determinants), through which allergens are recognized by the T- and B-cells. B-cell epitopes are considered to be discontinuous, conformational epitopes, and T-cell epitopes "linear" continuous epitopes [63]. The term "cross-reactive B-cell epitope" is used when the antibodies are able to recognize similar structures in at least two different antigens. This may occur, for instance, between botanically related species or between conserved proteins having sequence similarity. IgE-binding to cross-reactive carbohydrate determinants (CCDs) between structurally unrelated and glycosylated allergens has become an important issue[1].

Oligosaccharides are added to many proteins during or shortly after their synthesis in the endoplasmic reticulum. Glycosylation involves the covalent attachment of oligosaccharides either to asparagine (N-linked) or to serine/threonine (O-linked) amino acid residues within the primary amino acid sequence [47]. The α(1,3)-fucosylation and β(1,2)-xylosylation of N-glycan core structures are typical for plant-glycoproteins but are not found im mammals. Both the α(1,3)-fucose and β(1,2)-xylose units have been found partly responsible for the immunogenicity of plant-glycoproteins in mammals [32, 128].

Because the ability of food allergens to reach the intestinal mucosa is prerequisite to allergenicity, the stability of an allergen has become an important parameter in distinguishing food allergens from "nonallergens" [3]. Most known plant food allergens (soybeans, peanuts) are stable molecules that resist the effects of food processing and digestive processes, and remain immunologically active through the intestinal tract [55]. Most food allergens appear to be 10 to 70 kDa water-soluble glycoproteins relatively resistant to acids and proteases [63, 73, 94].

Plant proteinase inhibitors are widely distributed in nature and are present in many common food *e.g.* legumes, cereal grains, potatoes, and other tubers [65]. Proteinase inhibitors, such as Kunitz-type soybean trypsin inhibitor (KSTI) [83] and α-amylase trypsin inhibitors in rice and wheat are food allergens [74]. Proteinase inhibitors are extremely stable molecules being involved in interfering with the digestion of food proteins in the gut [3, 65]. Potato tuber is by nature a rich source of proteinase inhibitors whose primary role is in defending the host pathogen attacks [61].

Hypersensitivity reactions against raw fruits and vegetables have been associated with individuals suffering from pollinosis, and the immunological basis for these reactions has been considered to be IgE-mediated cross-reactivity [6]. Particularly, birch pollen-allergic individuals are known to experience hypersensitivity reactions when handling raw potatoes [21, 38, 94], and heat-labile proteins in the sap of potato were responsible for these reactions [38, 59, 85, 90]. Also cooked potato could cause atopic eczema in infants suffering from atopic dermatitis [37]. Wahl et al.[124] detected IgE-binding to potato proteins ranging from 16 to 65 kDa in size. Protein homologues of the birch (*Betula verrucosa*) pollen allergens Bet v 1 [8] and Bet v 2 [114] in fruits and vegetables have been suggested to be responsible for hypersensitivity to potato in patients suffering from birch pollen allergy [6, 24].

The most frequent clinical syndrome caused by cross-reactive IgE antibodies is the oral allergy syndrome (OAS), an association of food allergies to fruits, nuts and vegetables in patients with pollen allergy. In the majority of the pollen allergic individuals suffering from OAS, it is caused by IgE cross-reactivity of Bet v 1 and its homologous proteins. About 90% of birch pollen allergic patients react with this major pollen allergen and they frequently display crossreactivity to pollen from related trees like alder, hazel, hornbeam, beach and European chestnut [92].

Pollen allergens interact with the human immunal system and the resulting IgE antibodies provide specific probes for their identification and characterisation. Grass allergenic proteins are expressed late in pollen development coincident with the laying down reserves [56]. Sequence similarity of allergens has indicated possible functions for some allergens. The major birch (*Betula verrucosa, B. pendula*) pollen allergen shows similarity with pathogen-related proteins, which form a secondary response in plant host-pathogen interactions and show anti-microbial activity. Some allergens of unknown function are cysteine-rich proteins, while some others have cysteine-rich regions; for example, the major allergen from rye-grass (*Lolium perenne*) pollen, Lol p 1, has a cysteine-rich N-terminal region, while at the C-terminal region four tryptophan residues together with tyrosine and phenylalanine residues resemble those of cellulose- or sugar-binding domains of other proteins. Several pollen allergens show sequence similarity to cell wall-associated enzymes, while others show hydrolytic enzyme activity often associated with cell walls.

Profilin is a small (12-15 kDa) actin- and phospholipid-binding protein that interacts with at least three different ligands. Previously profilin was known only from studies on animals and lower eukaryotes but recently identified as a birch pollen allergen[105]. The role of profilin in the cell is perhaps due to interactions with polyphosphoinositides and proline-rich proteins, or due to the ability to lower the critical concentration for actin assembly at the fast-growing barbed end of actin filaments [33]. Three members of the profilin multigene family from maize were identified and cDNAs were utilized in a tissue-specific manner and were anther or pollen specific [105]. Profilin is an actin-binding protein in higher plants. Tobacco profilin was detected in different somatic tissues, but the expression was 50-100-fold higher in mature pollen [77]. Profilin is a G-actin monomer-binding protein which has been shown to participate in actin-based tip-growth of animal cells. The abundance of profilin in pollen and its occurrence in several vegetable foods raises the question of the role of profilin in plants. Profilin was present in most if not all parts of the tomato plant [17]. Many food plants contain proteins with high homology to the birch pollen allergens Bet v 1 and Bet v 2 (birch profilin) [24]. Two classes of functionally distinct profilin isoforms in maize were found which were dependent on the concentration of free calcium[57]. Possibly profilin alters cellular concentrations of actin polymers in response to fluctuations in cytosolic calcium concentration.

A homologue of the major birch pollen allergen Bet v 1 was detected in four of eight bell pepper cultivars [51,52]. A 23-kD allergen of bell peppers was shown to correspond to the 23-kD major paprika allergen. Its N-terminal sequence showed 100% identity to P23 from tomatoes. The appearance of profilin in all and Bet v 1 in 50% of the tested cultivars indicates that bell peppers have to considered potentially dangerous for Bet v 1- and profilin-sensitized patients. Moreover, in four of eight bell pepper cultivars a homologue of the osmotin-like protein P23 from tomatoes is responsible for substantial IgE binding. Contact with Bet v 1 and P23 homologues in bell peppers can therefore be minimized by avoidance of the respective cultivars.

The amino acid sequences of two ribonucleases from a callus cell culture of Panax ginseng (*Panax pseudoginseng*) differed at 26% of the amino acid positions [82]. Homology was found with a large family of intracellular pathogenesis-related proteins, food allergens and tree pollen allergens from both dicotyledonous and monocotyledonous plant species. There is about 30% sequence difference with proteins from species belonging to the same plant order (Apiales: parsley and celery), 60% with those from four other dicotyledonous plant orders and about 70% from that of the monocotyledonous asparagus. High antigenic and allergenic activity in native celery was reduced by thermal processing [50]. The heat-stability of the known celery allergens decreased in the following order: carbohydrate epitopes> profilin> Api g 1. The allergenicity was only mildly reduced by non-thermal processing.

A number of plant-derived allergens and PR-like proteins can be found in various species all over the plant kingdom. These homologous proteins are responsible for cross reactivities between different allergen sources and represent at least in part the molecular basis for syndromes such as the 'pollen-related food syndrome', 'latex-fruit syndrome', or the 'birch-mugwort-celery-spice syndrome'. Therefore, all new insights in the plant cell metabolism and the defence-related strategies plants have developed may contribute to a better understanding for the existence of allergy syndromes.

Pathogenesis-Related Proteins

A considerable percentage of identified plant allergens can be grouped to the families of pathogenesis-related proteins (PR) based on sequence characteristics [120, 133]. Plant-derived allergens have been identified with sequence similarity to PR families 2, 3, 4, 5, 8, 10 and 14. For individual allergens like Pers a 1 and Mal d 1, a PR-inducible expression and function has already been shown [41].

The common biochemical features of PR proteins are low molecular weight (5-70 kD), stability at low pH and resistance to proteases[120, 133]. These features are of use for plant proteins during pathogen attack and stress conditions, while the plant metabolism is trying to cope with unwanted situations. On the other hand, those proteins, which remain stable and are easily soluble, represent good candidates evoking an immune response in humans, once coming into contact with mucosal surfaces. Since they are usually protease-resistant they can produce immune responses in the intestinal tract.

Since plants lack an immune system, they have to protect themselves against pathogen attack as well as against phytophagous insects by different strategies. Besides, establishing a physical barrier by strengthtening their cell walls, plants produce antibiotic compounds called phytoalexins and accelerate cell death to suppress the spread of infectious pathogens. Furthermore, a number of proteins is encoded by the host and induced by various types of pathogens (viruses, bacteria and fungi) or by chemicals such as ethylene and salicylic acid, mimicking the effect of pathogen infection and thus inducing stress.

Especially, fruits and vegetables with high water content and possibly sweet tasting (such as cherries, avocados and bananas) are very likely to be attach by either phytophagous insects or fungi. In these fruits and plant organs, proteases and antifungal proteins are present in reasonable amounts and represent an allergenic potential. Roots and floral tissues and leaves are also known to contain a certain amount of PR proteins. Homologous PR proteins are grouped into small multigene families in various plant species. The broad range of stress factors inducing PR protein expression and the constitutive expression in certain stages points to a more general function of various PR proteins within the plant than strictly pathogen-related. PR proteins could be part of general recognition processes and contribute and/or are part of a signal transduction cascade [72].

The term PR proteins now comprises very different plant proteins such as chitinases, glucanases, endoproteinases, peroxidases, as well as small proteins like defensins, thionins and lipid transfer proteins. Some of the PR proteins are not only induced *de novo* upon pathogen attack or wounding or other physical or chemical stress, but they are constitutively expressed in some organs or during developmental stages. In strict terms these forms are not PR proteins themselves but are designated PR-like. Usually, the acidic forms of PR protein families are secreted to the extracellular space, the basic forms are transported to the vacuole by a signal located at the C-terminus. Induction of gene expression during pathogen infection takes place by various signalling molecules like salicylic acid and reactive oxygen species, which mediate the expression of acidic PR genes [41]. Expression of basic PR genes is mediated by gaseous phytohormone ethylene and methyl jasmonate [129]. A number of protein factors like kinase and DNA-binding protein, regulating the expression of these genes, have been identified in recent years. Basic PR genes have been found to be expressed constitutively in some organs, including roots, parts of seedlings and cultured cells.

After virus infection, and most probably also when ethylene production is stimulated by chemicals, ethylene is the physiological inducer of PR proteins. The induction of PR proteins by benzoic acid, and particularly by its more effective derivative salicylic acid must then be explained by assuming that stimulated ethylene production triggers the synthesis of a presumably aromatic compound that mimics the action of salicylic acid [118]. Ethylene is known to stimulate aromatic biosynthesis in several plant species, including tobacco, and during the hypersensitive reaction of tobacco to tobacco mosaic virus TMV extensive changes in phenol metabolism have been observed [62]. Induction of PR proteins by ethephon or salicylic acid in tobacco is inhibited by cycloheximide or D-2-(4-methyl-2,6-dinitroanilino)-N-methylpropionamide, both inhibitors of protein synthesis in chloroplasts and mitochondria [117].

Cytoplasmic protein synthesis is necessary for appearance of PR protein. PR proteins clearly differ from heat-shock proteins, which are rapidly synthesized upon a raise in temperature but subsequently disappear when adaptation has occurred [97]. The expression of PR proteins is controlled at the level of translation. A substantial increase in ethylene production apparently initiates a series of events which make the mRNAs available for translation [117]. The largest part of the PR proteins is present in the intercellular spaces and it is difficult to envisage how those could be effective in reducing multiplication and spread of viruses present within the cells. Perhaps only the tiny fraction inside the protoplast is active.

PR Protein Families which Include Allergens

PR-2 β-1,3-glucanases: The PR-2 family comprises monomeric β-1,3-glucanases (glucan endo-1,3-β-glucosidases) with a molecular mass of 25-35 kD. These enzymes catalyse endo-type hydrolytic cleavage of the 1,3-β-D-glucosidic linkages in β-1,3-glucans, the abundant component of plant cell walls. The β-1,3-glucanases are highly regulated enzymes, especially in seed-plant species [44]. These enzymes are thought to play a role in response of plants to microbial pathogens, and are implicated in a number of physiological and developmental processes in uninfected plants, like pollen germination and tube growth, fertilisation, fruit ripening, seed germination, mobilisation of storage products in the endosperm of cereal grains and represent a response to wounding, cold, ozone and UV-B [64].

PR-2 proteins are grouped into three classes by differences in size, isoelectric point, primary structure, cellular localisation and pattern of expression. Class I enzymes (size approximately 33 kD) are known from tobacco, tomato, potato and other plant species [64]. The intracellular enzymes are basic proteins localised in the vacuoles, translated as a preprotein consisting of a N-terminal signal peptide and a C-terminal extension N-glycosylated at a single site. The acidic members of class II and III (34-36 kD) are secreted into the extracellular space. During seed germination the embryo has to overcome the physical barrier by disrupting the covering layers. β-1,3-glucanases have been shown to be induced during tobacco seed germination and there is evidence that ethylene induces the glucanase expression [64]. The ethylene responsive element present in class I glucanase and class I chitinase seems to play a major role in the regulation and function of these two class I enzymes [87].

In grapevine leaves, the less agressive *Botrytis cinerea* strain, T4, enhanced the accumulation of many defence products including secondary metabolites and the PR-proteins, chitinase and β-1,3-glucanase [18]. Secondary metabolites were formed in cells around a small group of dead cells. The more aggressive strain, T8, had larger necrotic spots, no secondary metabolite biosynthesis and accumulations of chitinases and β-1,3-glucanases that were more delayed, yet only slightly weaker.

Allergens with sequence similarity to β-1,3-glucanases have been identified. Complementary DNA coding for basic β-1,3-glucanase isolated from the latex of the tropical rubber tree *Hevea brasiliensis* has been sequenced and characterised as Hev b 2, a relevant latex allergen [109]. The association between *Hevea* latex allergy and hypersensitivity to foods, especially avocado, banana, chestnut, fig and kiwi has been termed latex-fruit syndrome[5]. Phylogenetically conserved, homologous proteins present in these fruits and vegetables are responsible for the cross-reactivity. The basic β-1,3-glucanases from *Hevea* latex are recognised by specific IgE from food allergic patients suffering from hypersensitivity to banana, potato and tomato, rather than to latex products. The amount of β-1,3-glucanase expression increases significantly in banana fruit during ripening [12] thus possibly increasing the risk of developing allergic symptoms by reactive atopic patients.

PR-3 Chitinases (Class I, II, IV): Plant chitinases are mostly endochitinases which hydrolyse chitin within the biopolymer. They are usually monomers of 25-35 kD. Chitinases produce 2-6 N-acetyl-glucosamine units and they frequently also possess lysozyme activity hydrolysing the β-1,4-linkages between the N-acetyl, uramic acid and the N-acetyl glucosamine. Chitin is the major component of the exoskeleton of insects as well as of the cell wall of most fungi and nematodes. Related substrates are the bacterial peptidoglycan and chitosan [15]. The PR-3 family comprises chitinases class I, II and IV. Chitinases from class I are found in PR-4 and PR-11 and chitinases from class III are related to PR-4. In general, the basic isoforms are vacuolar and antifungal, whereas the acidic ones are extracellular with little antifungal activity. The intracellular localisation depends on the presence of a C-terminal vacuolar targeting propeptide [86].

Class I chitinases contain a cysteine-rich 40 amino acid residue domain at the N-terminus, the chitin-binding hevein domain, a hypervariable domain, which is a proline-rich region, and a catalytic domain. A signal peptide is removed from the mature protein and a target sequence directing the protein to the vacuole is located at the C-terminus. The hevein domain is not a targeting signal and does not play a role in the catalytic activity of chitinases, yet its presence seems to be essential for chitin binding and for substrate affinity. Possibly the chitin-binding domain is helping to attach the catalytic domain onto the substrate and thus strongly improve the enzymatic cleavage of the polymer [41].

Class II chitinases lack the hevein domain and the vacuolar target sequence. They are acidic, extracellular enzymes. Class II enzymes display 60-65% sequence similarities to class I chitinases. Class II chitinases may release elicitors and thus induce antifungal activity in living plant cells rather than killing the invading fungus, a function that has already been postulated for β-1,3-glucanases class II.

Class IV chitinases are related to class I and class II enzymes by sequence similarities, yet there are a number of deletions, especially in the hevein region. Class V and VI representatives display either two chitin binding domains arranged tandemly or present one truncated chitin binding domain. Chitinases have been shown to exert an antifungal activity *in vitro* [108].

Chitinases are developmentally regulated in several tissues like flowers, roots and lower leaves from tobacco and *Arabidopsis* [68, 93]. Furthermore, chitinase activity is induced by various factors like pathogen and pest attack, treatment with elicitors, abiotic factors like heavy metals and plant hormones. Higher enzyme activity, protein and mRNA levels of chitinases have been reported in resistant cultivars, compared to susceptible cultivars. A number of transgenic plants have already been produced in order to improve fungus resistance in crops [35].

Relevant allergens of chestnut and avocado have been identified by N-terminal amino acid sequences and cDNA cloning as class I chitinases containing such hevein (chitin binding) domains [19]. Class I chitinases with hevein-like domains, but not class II enzymes, are relevant chestnut and avocado allergens. Two major IgE-binding proteins of 32 and 34 kD from banana were identified as class I chitinases with hevein-like domain further explaining the cross-sensitization between *Hevea* latex and fruits [95].

PR-4 Chitinases: Prohevein is a chitin-binding protein from the rubber tree. Hevein has sequence similarity for chitin-binding proteins, whereas the C-terminus is homologous to wound-inducible proteins. From potato, wound-inducible proteins isolated have designated win1 and win2 with high sequence similarity to prohevein [106]. Among the PR-4 proteins, class II chitinases have been identified from tobacco and tomato with sequence similarity to win proteins, yet lacking the chitin-binding domain [31, 66].

Wounding and chemical treatment of turnip (*Brassica rapa*) plants induced the expression of an 18.7 kD protein with allergenic characteristics, that was recognised by IgE of natural rubber latex allergic individuals [48]. Peptide sequences of the turnip allergen revealed 70% identity to prohevein, and high similarities to wound-induced proteins from tomato (74%) and potato (71%).

A ‘chimeric’ hevein-like chitin-binding protein, SN-HLPf, homologous to PR-4 and class V chitinase (PR family 11) genes, displaying a hevein-like N-terminus and a C-terminus related to chitinases was isolated from mature elderberry (*Sambucus nigra*) fruits [116]. The protein is accumulated in elderberry fruits and displays a rather weak antifungal activity, but a distinct chitin-binding activity.

Prohevein is a major allergen from *Hevea* latex designed Hev b 6.01. Furthermore, its N-terminal domain represents allergen Hev b 6.02 (hevein) and the carboxyterminal domain Hev b 6.03. Possibly due to the sequence similarities to proteins with allergenic properties from the PR-3 and PR-4 family present in plant derived food, the prevalence of latex-allergic patients to develop food allergies to a number of fruits and vegetables can be explained.

PR-5 Thaumatin-like proteins (TLPs): Members of this PR group originally described from tobacco were induced upon infection of tobacco mosaic virus [119]. These proteins were isolated from acidic extracts from leaves. Their sequence is similar to the intensely sweet tasting protein thaumatin, originating from the African shrub *Thaumatococcus daniellii*. PR-5 proteins are designated thaumatin-like proteins, although none of the other proteins has been described to have a sweet taste. Osmotin from tomato and another tobacco protein, NP24, both accumulated after osmotic stress, are also homologous to thaumatin. TLPs have been detected in leaves of young plants, but they rapidly accumulate to high levels upon biotic or abiotic stress. Especially in roots, epidermal peel, and corolla from tobacco plants high mRNA levels coding for osmotin have been identified [60].

TLPs are developmentally expressed in high levels in flower buds of *Brassica* and overripe fruits of cherries [11, 29]. According to their molecular mass, TLPs are grouped into two sections: one group of proteins has a size ranging from 22 to 26 kD whereas the other group comprises proteins of 16 kD due to an internal deletion of 58 amino acids. There are acidic, basic and neutral TLPs.

Zeamatin does not have the sweet-taste but exerts an antifungal acitivity, whereas thaumatin, the sweet tasting protein, displays weak or no antifungal activity [70]. Possibly PR-5 proteins are inserted directly into the fungal membrane forming a transmembrane pore and causing influx of water followed by osmotic rupture [91]. Tobacco pathogenesis-related (PR) proteins of group 5, (PR-S and osmotin), serologically related to zeamatin, an antifungal protein of maize seeds [132], have a direct antifungal activity by a membrane permeabilization mechanism similar to that demonstrated for zeamatin with specificity for different fungal species.

Recently six TLPs from barley, tomato, cherry, and tobacco have been shown to exert a β-1,3-glucanase activity on polymeric glucan, thus proposing a new type of enzymes, thaumatin-like β-1,3-glucanases [34].Transgenic tobacco and potato plants overexpressing osmotin led enhanced resistance to *Phytophtora infestans*, the potato late blight pathogen [131].

In bell pepper, a 23-kD protein was identified as an important allergen [51, 52]. The N-terminal sequence of this protein was found to be identical to a corresponding portion of the osmotin-like protein P23 from tomatoes.

An apparent 31 kD major apple allergen whose amino-terminal sequence shared 46% identity with PR-5 proteins was the first thaumatin-like protein described as an allergen Mal d 2 [46]. In sweet cherry (*Prunus avium*), a 23.3-kD thaumatin-like protein was identified as an important allergen, designated as Pru av 2 [49]. A 29-kDa polypeptide is the most abundant soluble protein in ripe cherry (*Prunus avium*) fruit; accumulation begins at the onset of ripening as the fruit turns from yellow to red [29]. The cherry thaumatin-like protein did not have a sweet taste and any antifungal acitivity. Expression of the protein was probably regulated at the gene level, with mRNA levels at their highest in the ripe fruit.

PR-8 Chitinases (Class III): PR-8 proteins are chitinases of class III type originally described as lysozymes [4] and do not display any similarities to either class I or class II chitinase. One of the major latex proteins, hevamine, displays lysozyme and chitinase activity. Hevamine, a 30-kD basic protein from the lutoids of *Hevea* latex has been identified as an allergen present in latex products. The cucumber chitinase is acidic, from tobacco an acidic and basic form is known [75].

PR-10 Intracellular proteins with unknown enzymatic function: A large number of intracellular, possibly cytosolic proteins has been isolated from various species such as asparagus, parsley, bean, pea, potato and apple. Due to their sequence similarities and uniform size they have been grouped together into family PR-10. PR-10 proteins are not only directly involved in pathogen perception of defence but exert a more common function as a biochemical component in pathways switched on during generalised stress and/or during physiological changes in distinct developmental stages.

Pathogenesis-related proteins of the PR10 class found in many plant species are induced under various stress conditions and act as common allergens [102]. PR-10 proteins have been identified from roots of Leguminosae after mite attack as well as in the early stages of interaction with microsymbiontic bacteria which are often considered as refined parasites [9]. Proteins from ginseng have been shown to display ribonuclease activity [82]. All these proteins are encoded by multiple genes, forming a monophyletic group and representing potential phylogenetic markers within or among closely related families in flowering plants [127]. Most of the genes have been induced upon microbial attack, by fungal elicitors, wounding or stress stimuli. PR-10 type proteins are also expressed tissue-specifically during developmental stages.

Two PR10 proteins were present in yellow lupin (*Lupinus luteus* cv. Ventus), both 17 kDa proteins are composed of 156 amino acids and have 91% similarity [102]. Identity to homologues from other plants ranged 46-82% similarity. The proteins were localised in the parenchymatous tissues of the root and senescent nodule, primary in the cortex, but were not found in nodule bacteroid tissue. Expression in aerial parts of the plant is generally lower and only one of the proteins is expressed constitutively in the stem, leaf and petiole, while the other is induced in senescent leaves.

The proteins of PR-10 family may appear in plants not only as the effect of pathogen attack or abiotic stress but also at some stages of development. Heavy metals are strong oxidants, leading to the formation of reactive forms of oxygen, such as free radicals, which may damage the cell metabolism. The level of free radicals influences in turn the activity of an antioxidant enzyme, superoxide dismutase (SOD) [96]. PR-10 proteins might be involved in the programmed cells death, which occurs during the processes of senescence [14]. Also antifreeze proteins in winter rye are similar to PR proteins [45]. The hypothesis is that slight modifications of the amino acid composition happened during evolution enabled the originally pathogen-related proteins to gain an additional function in defence against abiotic stresses like freezing and excess heavy metals proteins [88].

The Bet v1 gene family of birch (*Betula pendula*) encodes the major pollen allergens as well as pathogenesis-related proteins that are induced by microbes in somatic tissues [111]. Bet v 1 is a member of the PR-10 family, and its homologues form an ubiquitous group of proteins which have been identified in a wide range of flowering plants [127]. A number of isoforms and homologous proteins from closely related species (alder, hazel and hornbeam) have been isolated previously and their cDNAs cloned and characterized [41]. The exon-intron formation is highly conserved throughout this family of PR proteins in dicot plants and is also found in Aopr 1 (*Asparagus officinalis*), a monocot species.

A number of Bet v 1 homologous allergen sequences have been isolated and cloned from fruits, including Pru av 1 from sweet cherry, Pru ar 1 from apricot and Pyr c 1 from pear. From vegetables, allergen encoding cDNA-sequences are known from celery, Api g 1 and from carrot, Dau c 1 [7, 42]. Additional Bet v 1- homologous proteins capable of binding anti-Bet v 1 IgE were described as pcPR1 and pcPR2 from parsley and pSTH-2 and pSTH-21 from potato [43]. Phylogenetic analysis suggested a possible common origin of the intron position in these homologous proteins at codon 62 in various families of flowering plants, including Fagaceae, Rosaceae and Apiaceae. This conserved 'proto-splice site' may point to a structure/function relationship.

Isoforms from Bet v 1 are inducible upon stress, pathogens and heavy metals.These PR proteins belong to a group of conserved intracellular defence-related proteins that have been termed 'ribonuclease-like' PR proteins, on the basis of the partial sequence homology observed between PR1, a Bet v 1-homologue from parsley and a ginseng ribonuclease [111]. It was shown that Bet v 1 proteins possess an intrinsic ribonucleolytic activity as they can digest different RNA substrates *in vitro*, but show no activity on single or double-stranded DNA.

The root is of a fairly simple architechture consisting of epidermis, cortex, endodermis and stele and basic structure of the root tissue is fairly common in all species of plants. Extensins and P33, which are hydroxyproline-rich glycoproteins have been identified as cell wall proteins in roots and the transcripts increased markedly after wounding [113]. A major root specific protein (CR16) of carrots has a high homology to intracellular pathogenesis-related proteins, stress-induced proteins and also the major allergen protein of celery (Api g 1) [130]. The CR16 protein gene formed a super gene family and transcripts of this protein gene are predominant in root tissue, not in leaves or flowers. Three major proteins with a molecular mass of 41, 40 and 16 kD, each about 10% of the total protein, were found in extracts of mature roots cv. Kuroda-gosun. The protein concentration of root tissue was about ten to twenty-fold lower than in leaves, and stems. The 16 kD one was the only protein with root-specific expression pattern. The relative content increased as the carrot plants were developing to mature forms. Two 41 and 40 kD proteins, were found in leaves and stems as well as in roots. These two proteins in aerial parts are identical ones in roots but their contents were fairly constant through all stages of development. The content of the CR16 protein of root increased during development. Expression levels and patterns in three other cultivars Early Chantenay, Imperator and Nantes Scarlet were much the same as found in Kuroda-gosun. Various intracellular PR protein genes which are highly homologous to the CR16 root-specific protein gene have been isolated from parsley, bean, potato and asparagus [16, 98, 103, 123, 126]. The amino-terminal sequence of CR16 has high homology to parsley PR protein, strongly induced by viruses and pathogens[103]. No common features were found between the CR16 and other proteins found in carrot roots. A fructose-1,6 bis phosphate aldolase[81], carotenoprotein[10], DcDrP1 protein[22], and 6-hydroxymellein synthetase[58] have been isolated and contents of these proteins in carrot roots were reported to be relatively high.

Pathogen infection induces many intracellular PR proteins with a high homology to CR16 proteins. In potato tuber, the defense-related STH-2 protein is rapidly induced by infection of *Phytophtora infestans* zoospores[16]. The *Asparagus officinalis* intracellular PR1 (AoPR1) gene is also expressed in response to pathogen attack [126]. Elicitor treatments also induce intracellular PR proteins with high homology to CR16 proteins in peas, beans and soybeans [123]. Cellular signal compounds such as arachidonic acid and salicylic acid induced PR10 (formerly STH2)[71] and AoPR1 gene expression [125], respectively. Furthermore, the chemically synthesized compounds, probenazole (3-allyloxy-1,2-benzisothiazole-1,1-dioxide)[76] 2,6-dichloroisonicotinic acid and benzo (1,2,3)thiadiazole-7-carbothioic acid S-methyl ester (BHT)[36] also induced acquired disease resistance in plants and one of the probenazole induced proteins was identified as intracellular PR protein. These agrochemicals induce resistance to disease in a variety of plants including monocot and dicot, suggesting that a common signal pathway for establishment of the disease resistance is evolutionaly conserved. These signal compounds might also function as cellular signals for enhanced gene expression of CR16 protein gene. The major pollen allergen Bet v 1 is highly homologous to CR16 protein.

Mal d 1, a PR-10 protein from apple is constitutively expressed in ripe apples as well as in old leaves. Pathogen attack, abiotic stress factors as well as fungal elicitors increased Mal d 1 mRNA as well as protein in young leaves [89]. Mal d 1 is the first major allergen characterized in fruits of apple (*Malus pumila*). The cDNA sequence of the major allergen of apple Mal d 1 showed 72% identy with the coding region of one of the known isoforms of Bet v 1 [99]. The deduced amino acid sequence resulted in a 158-residue protein with MW of 17.5 kDa and 63% amino acid sequence indentity to Bet v 1. Further protein alignments showed a high degree of identity with allergens from other tree pollens and some PR proteins from food plants. Allergy to fresh apples, as well as allergies to nuts, stone fruits and other fruits and vegetables, is very common in patients with hay fever caused by birch pollen. The observed clustering of hypersensitivities is due to cross-reactions of allergen-specific IgE antibodies [20, 26, 84, 112]. Bet v 1, the major allergen of birch pollen (MW 17 kD), and an 18 kD allergen from apples are important cross-reactive compounds [23].

Apple-allergic patients frequently report that some apple cultivars usually are highly allergenic (i.e. 'Granny Smith', 'Golden Delicious') whereas others (i.e. 'Jamba', 'Gloster', 'Boskoop') are tolerated without or with moderate symptoms and that the symptoms are often more severe after ingestion of mature fruits[121]. In a study on 16 apple cultivars (Alkmene, Altländer Pfannkuchenapfel, Apollo, Boskoop, Braeburn, Cox Orange, Ellison's Orange, Gloster, Golden Delicious, Granny Smith, Hammerstein, Jamba, Jonagold, Macoun, Prime Rouge, Sternrenette) the allergenic potency of mature apples depended on the occurrence of the 18 kD allergen and the amount of this allergen varied greatly between cultivars [122]. Not all of the apple cultivars are capable of expressing the 18 kD allergen. Five of seven "highly allergenic" apple cultivars are very common on the German market (Braeburn, Cox Orange, Golden Delicious, Granny Smith, Jonagold). Most of the "low allergenic" apple strains are of minor economic importance (Altländer, Ellison's Orange, Hammerstein, Macoun). Up to seven allergen bands of molecular mass of 12-67 kD were found in various apple cultivars (Golden Delicious, Granny Smith, Boskoop). The allergenic potency of the apples quantified as "medium" was mediated by the 30-67 kD allergens. Divergent allergenicity of apple cultivars mainly depends on different expression levels of the major allergen. Mutational analysis of Mal d 1 allergens identified serine in position 111 as essential for IgE binding [104]. Introduction of a proline residue in position 111 of Mal d 1 and in position 112 of Bet v 1 led to a drastic reduction of allergenicity of both the pollen and the food allergen obviously also removing the cross-reactive epitopes.

In the case of apple allergy, the symptoms are more severe after ingestion of mature fruits [121]. The allergic potency increased strongly during maturation of 'Golden Delicious' apples and to lesser degree during ripening of 'Boskoop' apples [121]. The extract with the highest allergenic activity was obtained from mature 'Golden Delicious'. The differences in the potency of IgE binding were due to the occurrence of an 18 kD allergen in 'Golden Delicious' apples, whereas only very low amounts of this protein were detected even in extracts of mature 'Boskoop'. The allergenic potency of apples depends on the occurrence of the 18 kD allergen, and the property of expressing high amounts of this protein is not inherent in all apple cultivars. However, several additional allergens of higher molecular weights were detected in extracts of 'Boskoop' and 'Golden Delicious' apples. The common modern use of controlled atmosphere storage of fruits may represent one stress factor leading to increased apple allergen concentrations [121].

A mean amount of 10-50 mg per kg fresh weight of the 18 kD allergen was assumed in mature 'Golden Delicious' apples [121]. Therefore, one bite (approximately 10 g) which is able to elicit an allergic reaction represents 0.1-0.5 mg of ingested apple allergen. A net increase in protein synthesis which occurs during development and during cold storage of apples [39, 69] may extend the measured differences in allergenicity between immature and mature fruits.

Peels of Rosaceae fruits such as apple, peach, and pear, have a higher allergenicity than pulps. More than 40% of patients allergic to apple and pear tolerated the ingestion of the pulp of these fruits, and reactions were only elicited by the intake of the whole fruit [27]. An important cross-allergenicity was found between the peel and pulp of apple and peach, although the amount of shared allergenic epitopes seemed to be higher in peels.

PR-14 Lipid transfer proteins: Plant lipid transfer proteins (LTPs) are small proteins (9 kD) which facilitate the transfer of phospholipids and other lipids across membranes. These proteins ase widely distributed across the plant kingdom. LTPs can take part in plant defence, as some LTPs have potent antifungal and antibacterial activities [78]. These proteins could play a major role in membrane biogenesis by conveying phospholipids like waxes and cutin from their place of synthesis to membranes unable to form these lipids. LTPs contain eight conserved cysteine residues forming four disulfide bridges which makes them highly resistant to harsh temperature and pH changes [54]. Coldness inducible genes encoding LTP in barley show differential response to bacterial pathogens [78]. Plant lipid transfer proteins are generally located in the outer cell layer of plant organs.

LTPs are the most important allergens of the Prunoideae, such as peach, apricot, plum and cherry, when no pollinosis is involved [2, 27, 28]. An 8- to 10-kD protein doublet, exclusive to peach skin extracts largely accounted for the allergenicity of that fruit [67]. IgE antibodies to Rosaceae LTPs reacted to a broad range of vegetable foods, including cereals, peanut, walnut, pistachio, broccoli, carrot, celery, tomato, melon and kiwi [2]. Due to its extreme resistance to pepsin digestion, LTP is a potentially severe food allergen.

Conclusions

Several protein families that contribute to the defence mechanisms of food plants have members which are allergens or putative allergens and some of these proteins are used in molecular approaches to increase resistance. These include α-amylase and trypsin inhibitors, lectins and pathogenesis-related proteins. The source of the transgene is of great importance for the application of immunological assays. Vegetable allergens are increasingly involved in food allergens. Cross-reactivity between pollens, fruit and vegetables relies upon pan-allergens: pathogenesis-related proteins, profilins, enzymes with the same functional activity, etc. The evolution of the allergenicity of transgenic foods is carried out by *in vitro* analysis of binding of the transgenic protein to specific IgEs from patients allergic to the source of the gene. The search for homology with known allergens, the degradability of antigenicity by proteolytic enzymes are needed for proteins originating from non-allergenic sources [80]. *De novo* sensitizations are unpredictable and zero risk cannot be asserted.

New complications may arise from genetically modified plant foods. Since one major goal is to improve the disease resistance in economically useful plants, insertion of PR-encoding genes or enhancing their expression levels is the method of choice. Since a number of allergens are PR-like proteins, the potential allergenic features of newly introduced proteins have to be carefully evaluated. The first food products derived from transgenic plants that are resistant to diseases, insects or viruses are now reaching the market and there is growing public concern about problems of allergenicity and toxicological changes in such transgenic food plants [30].

References

[1]Aalberse, R.C., Koshte, V. and Clemens, J. G. J. 1981. Immunoglobulin E antibodies that crossreact with vegetable foods, pollen and Hymenoptera venom. Journal of Allergy and Clinical Immunology **68**: 356-364.

[2]Asero, R., Mistrello, G., Roncarolo, D., Vries, S. C., de, Gautier, M. F., Ciurana, C. L. F., Verbeek, E., Mohammadi, T., Knul-Brettlova, V., Akkerdaas, J. H., Bulder, I., Aalberse, R. C., Ree, R. van, de Vries, S. C. and van Ree, R. 2000. Lipid transfer protein: a pan-allergen in plant-derived foods that is highly resistant to pepsin digestion. International Archives of Allergy and Immunology **122**:20-32.

[3]Astwood, J. D., Leach, J. N. and Fuchs, R. L. 1996. Stability of food allergens to digestion in vitro. Nature Biotech. **14**:1269-1273.

[4]Bernasconi, P., Locher, R., Pilet, P. E. and Jolles, P. 1987. Purification and N-terminal amino acid sequence of a basic lysozyme from *Parthenocissus quinquifolia* cultured in vitro. Biochim. Biophys. Acta **915**:254-260.

[5]Blanco, C., Carrillo, T., Castillo, R., Quiralte, J. and Cuevas, M. 1994. Latex allergy: clinical features and cross-reactivity with fruits. Annals of Allergy **73**:309-313.

[6]Breiteneder, H. and Ebner, C. 2000. Molecular and biochemical classification of plant-derived food allergens. Journal of Allergy and Clinical Immunology **106**: 27-36.

[7]Breiteneder, H., Hoffmann-Sommergruber, K., O'Riordain, G., Susani, M., Ahorn, H., Ebner, C., Kraft, D. and Scheiner, O. 1995. Molecular characterization of Api g 1, the major allergen of celery (*Apium graveolens*), and its immunological and structural relationships to a group of 17 kDa tree pollen allergens. European Journal of Biochemistry **233**:484-489.

[8]Breiteneder, H., Pettenburger, K., Bito, A., Valenta, R., Kraft, D., Rumbold, H., Scheiner, O. and Breitenbach, M. 1989. The gene coding for the major birch pollen Bet v 1, is highly homologous to a pea resistance response gene. The EMBO Journal **8**:1935-1938.

[9]Broderick, K., Pittock, C., Arioli, T., Creaser, E., Weinman, J. J. and Rolfe, B. G. 1997. Pathogenesis-related proteins in *Trifolium subterraneum*: A survey and subsequent characterisation of a protein inducible by ethephon and redlegged earth mite attack. Australian

Journal of Plant Physiology **24**:819-829.
[10]Bryant, J. D., McCord, J. D., Uniu, L. K. and Erdman, J. W., Jr. 1992. Isolation and partial characterization of alfa- and beta-carotene-containing carotenoprotein from carrot (*Daucus carota* L.) root chromoplasts. Journal of Agriculture and Food Chemistry **40**: 545-549.
[11]Cheong, N. E., Choi, Y. O., Kim, W. Y., Chang, S., Cho, M. J. and Lee, S. Y. 1997. Purification of an antifungal PR-5 protein from flower buds of *Brassica campestris* and cloning of its gene. Physiologia Plantarum **101**:583-587.
[12]Clendennen, S. K. and May, G. D. 1997. Differential gene expression in ripening banana fruit. Plant Physiology **115**:463-469.
[13]Creighton, T. E. 1997. The general properties of protein structures. In: Proteins, structures and molecular properties. Second Edition. W.H. Freeman and Company. New York.
[14]Cromwell, D. N., Maliyakal, E. J., Russel, D. and Amasino, R. M. 1992. Characterization of a stress-induced, developmentally regulated gene family from soybean. Plant Molecular Biology **18**:459-466.
[15]Collinge, D. B., Kragh, K. M., Mikkelsen, J. D., Nielsen, K. K., Rasmussen, U. and Vad, K. 1993. Plant chitinases. Plant Journal **3**:31-40.
[16]Constabel, C. P. and Brisson, N. 1992. The defence-related STH-2 gene product of potato show race-specific accumulation after inoculation with low concentrations of *Phytophtora infestans* zoospores. Planta **188**:289-295.
[17]Darnowski, D. W., Valenta, R. and Parthasarathy, M. V. 1996. Identification and distribution of profilin in tomato (*Lycopersicon esculnetum* Mill.). Plant **198**:158-161.
[18]Derckel, J. P., Baillieul, F., Manteau, S., Audran, J. C., Haye, B., Lambert, B. and Legendre, L. 1999. Differential induction of grapevine defences by two strains of *Botrytis cinerea*. Phytopathology **89**:197-203.
[19]Diaz-Perales, A. Collada, C., Blanco, C., Sanchez-Monge, R., Carillo, T. and Aragoncillo, C. 1998. Class I chitinases with hevein-like domains, but not class II enzymes, are relevant chestnut and avocado allergens. Journal of Allergy and Clinical Immunology **102**:127-133.
[20]Dreborg, S. 1988. Food allergy in pollen-sensitive patients. Annals of Allergy **61**:41-46.
[21]Dreborg, S. and Foucard, T. 1983. Allergy to apple, carrot and potato in children with birch pollen allergy. Allergy **38**:167-172.
[22]Ebener, W., Fowler, T. J., Suzuki, H., Shaver, J. and Tieney, M. L. 1993. Expression of DcPRP1 is linked to carrot storage root formation and is induced by wounding and auxin treatment. Plant Physiology **101**:259-265.
[23]Ebner, C., Birkner, T., Valenta, R., Rumpold, H., Breiteneder, H., Scheiner, O. and Kraft, D. 1991. Common epitopes of birch pollen and apples - studies by western and northern blot. Journal of Allergy and Clinical Immunology **88**: 588-594.
[24]Ebner, C., Hirschwehr, R., Bauer, L., Breiteneder, H., Valenta, R., Ebner, H., Kraft, D. and Scheiner, O. 1995. Identification of allergens in fruits and vegetables: IgE cross-reactivities with the important birch pollen allergens Bet v 1 and Bet v 2 (birch profilin). Journal of Allergy and Clinical Immunology **95**: 962-969.
[25]Eriksson, N. E. 1978. Food sensitivity reported by patients with asthma and hay fever. Relationship between food sensitivity and birch pollen-allergy and between food sensitivity and acetylsalicylic acid intolerance. Allergy **33**:189-196.
[26]Eriksson, N. E., Formgren, H. and Svenonius, E. 1982. Food hypersensitivity in patients with pollen allergy. Allergy **37**: 437-443.
[27]Fernandez Rivas, M. and Cuevas, M. 1999. Peels of Rosaceae fruits have higher allergenicity than pulps. Clin. Exp. Allergy **29**:1239-1247.
[28]Fernandez Rivas, M., Van Ree, R. and Cuevas, M. 1997. Allergy to Rosaceae fruits without related pollinosis. Journal of Allergy and Clinical Immunology **100**: 728-733.
[29]Fils-Lycaon, B. R., Wiersma, P. A., Eastwell, K. C. and Sautiere, P. 1996. A cherry protein and its gene, abundantly expressed in ripening fruit, have been identified as thaumatin-like. Plant Physiology **111**: 269-273.
[30]Franck-Oberaspach, S. L. and Keller, B. 1997. Consequences of classical and biotechnological resistance breeding for food toxicology and allergenicity. Plant Breeding **116**: 1-17.
[31]Friedrich, L., Moyer, M., Ward, E. and Ryals, J. 1991. Pathogenesis-related protein-4 is structurally homologous to the carboxy-terminal domains of hevein, Win-1 and Win-2. Molecular & General Genetics **230**: 113-119.
[32]Garcia-Casado, G., Sanchez-Monge, R., Chrispeels, M. J., Armentia, A., Salcedo, G. and Gomez, L. 1996. Role of complex asparagine-linked glycans in allerginity of plant glycoproteins. Glycobiology **6**: 471-477.
[33]Gibbon, B. C., Ren Hai, Yun, Staiger, C. J. and Ren, H. Y. 1997. Characterization of maize (*Zea mays*) pollen profilin function in vitro and in live cells. Biochemical Journal London **327**:909-915.
[34]Grenier, J., Potvin, C., Trudel, J. and Asselin, A. 1999. Some thaumatin-like proteins hydrolyze polymeric β-1,3-glucans. Plant Journal **19**: 473-480.
[35]Grison, R., Greezes-Besset, B., Schneider, M., Lucante, N., Olsen, L., Leguay, J. J. and Toppan, A. 1996. Field tolerance to fungal pathogens of *Brassica napus* constitutively expressing a chineric chitinase gene. Nature Biotechnology **14**:643-646.
[36]Gorlach, J., Volrath, S., Knauf-Beiter, G., Hengy, G., Beckhove, U., Kogel, K.-H., Oostendorp, M., Staub, T., Ward, E., Kessmann, H. and Ryals, J. 1996. Benzothiadiazole, a novel class of inducers of systemic acquired resistance, activates gene expression and disease resistance in wheat. Plant Cell **8**:629-643.
[37]Hannuksela, M. 1987. Diagnosis of dermatologic food allergy. Annals of Allergy **59**:153-156.
[38]Hannuksela, M. and Lahti, A. 1977. Immediate reactions to fruits and vegetables. Contact Dermatitis **3**:79-84.
[39]Hansen, E. 1970. Proteins. In: Hulme, A. C. (ed.). The biochemistry of fruits and their products. Academic Press, New York. pp. 147-157.
[40]Helbling, A., Lopez, M., Schwartz, H. J. and Lehrer, S. B. 1993. Reactivity of carrot-specific IgE antibodies with celery, apiaceous spices, and birch pollen. Annals of Allergy **70**:495-499.
[41]Hoffmann-Sommergruber, K. 2000. Plant allergens and pathogenesis-related proteins. What do they have in common? International Archives of Allergy and Immunology **122**(3):155-166.
[42]Hoffmann-Sommergruber, K., O'Riordain, G., Ahorn, H., Ebner, C., Laimer Da Camara Machado, M., Pühringer, H., Scheiner, O. and Breiteneder, H. 1999. Molecular characterization of Dau c 1, the Bet v 1 homologous protein from carrot and its cross-reactivity with Bet v 1 and Api g 1. Clin. Exp. Allergy **29**:840-847.
[43]Hoffmann-Sommergruber, K., Vanek-Krebitz, M., Radauer, C., Wen, J., Ferreira, F., Scheiner, O. and Breiteneder, H. 1997. Genomic chraracterization of members of the Bet v 1 family; genes coding for allergens and pathogenesis-related proteins share intron positions. Gene **197**:91-100.
[44]Hoj, P. B. and Fincher, G. B. 1995. Molecular evolution of â-glucan endohydrolases. Plant Journal **7**:367-379.
[45]Hon, W.-Ch., Grittith, M., Mlynarz, A., Kwok, Y. C. and Yang, D. S. 1995. Antifreeze proteins in winter rye are similar to pathogenesis-related proteins. Plant Physiology **109**:879-889.
[46]Hsieh, L. S., Moos, M. and Lin, Y. 1995. Characterization of apple 18 kD and 31-kD allergens by microsequencing and evaluation of their content during storage and ripening. Journal of Allergy and Clinical Immunology **96**:960-970.
[47]Huby, R. D. J., Dearman, R. J. and Kimber I. 2000. Why are some proteins allergens? Toxicol. Sci. **55**:235-246.
[48]Hänninen, A. R., Mikkola, J. H., Kalkkinen, N., Turjanmaa, K., Ylitalo, L., Reunala, T. and Palosuo, T. J. 1999. Increased allergen production in turnip (*Brassica rapa*) by treatments activating defence mechanism. Journal of Allergy and Clinical Immunology **104**:194-201.
[49]Inschlag, C., Hoffmann-Sommergruber, K., O'Riordain, G., Ahorn, H.,

Ebner, C., Scheiner, O. and Breiteneder, H. 1998. Biochemical characterization of Pru a 2, a 23 kDa thaumatin-like protein representing a potential major allergen in cherry (*Prunus avium*). International Archives of Allergy and Immunology **116**:22-28.

[50]Jankiewicz, A., Baltes, W., Bogl, K. W., Dehne, L. I., Jamin, A., Hoffmann, A., Haustein, D. and Vieths, S. 1997. Influence of food processing on the immunochemical stability of celery allergens. Journal of the Science of Food and Agriculture **75**:359-370.

[51]Jensen-Jarolim, E., Grimm, R., Wüthrich, B., Ebner, H., Scheiner, O., Kraft, D. and Ebner, C. 1998a. Allergens in pepper and paprika: Immunologic investigations of the celery-birch-mugwort-spice syndrome. Allergy **53**:36-41.

[52]Jensen-Jarolim, E., Santner, B., Leitner, A., Grimm, R., Scheiner, O., Ebner, C. and Breiteneder, H. 1998b. Bell peppers (*Capsicum annuum*) express allergens (profilin, pathogenesis-related protein P23 and Bet v1) depending on the horticultural strain. International Archives of Allergy and Immunology **116**:103-109.

[53]Jordan-Wagner, D.L., Whisman, B. A. and Goetz, D.W. 1993. Cross-allergenicity among celery, cucumber, carrot and watermelon. Annals of Allergy **71**:70-79.

[54]Kader, J. C. 1996. Lipid transfer proteins in plants. Annual Review of Plant Physiology and Plant Molecular Biology **47**:627-654.

[55]Kimber, I., Kerkvliet, N. I., Taylor, S. L., Astwood, J.D., Sarlo, K. and Dearman, R. J. 1999. Toxicology of protein allergenicity: prediction and characterization. Toxicol. Sci. **48**:157-162.

[56]Knox, R. B. and Suphioglu, C. 1996. Pollen allergen: development and function. Sexual Plant Reproduction **9**:318-323.

[57]Kovar, D. R., Drobak, B. K. and Staiger, C. J. 2000. Maize profilin isoforms are functionally distinct. Plant Cell **12**:583-598.

[58]Kurosaki, F., Itoh, M., Yamada, M. and Nishi, A. 1991. 6-Hydroxymellein synthetase as a multifunctional enzyme complex in elicitor treated carrot extracts. FEBS Letters **288**:219-221.

[59]Larkö, O., Lindstedt, G., Lundberg, P. A. and Mobacken, H. 1983. Biochemical and clinical studies in a case of contact urticaria to potato. Contact Dermatitis **9**:108-114.

[60]LaRosa, P. C., Singh, N. K., Hasegawa, P. M. and Bressan, R. A. 1986. Stable NaCl tolerance of tobacco cells is associated with enhanced accumulation of osmotin. Plant Physiology **91**:855-861.

[61]Laskowski, M. and Kato, I. 1980. Protein inhibitors of proteinases. Annual Review of Biochemistry **49**:593-626.

[62]Legrand, M., Fritig, B. and Hirth, L. 1976. Enzymes of the phenylpropanoid pathway and the necrotic reaction of hypersensitive tobacco to tobacco mosaic virus. Phytochemistry **15**:1353-1359.

[63]Lehrer, S. B., Horner, W. E. and Reese, G. 1996. Why are some proteins allergenic? Implications for biotechnology. Crit. Rev. Food Sci. Nutr. **36**:533-564.

[64]Leubner-Metzger, G. and Meins, F. Jr. 1999. Functions and regulation of plant β-1,3-glucanases (PR-2). In: Datta, S. K. and Muthukrishnan, S. (eds). Pathogenesis-related proteins in plants. CRC Press, Boca Raton, pp. 49-76.

[65]Liener, I. E. 1986. Trypsin inhibitors: Concern for human nutrition or not? Journal of Nutrition **116**:920-923.

[66]Linthorst, H. J. M., Danbash, N., Brederode, F. T., van Kann, J. A. L., de Wit P. J. G. M. and Bol, J. F. 1991. Tobacco and tomato PR proteins homologous to win and prohevein lack the hevein domain. Molecular Plant-Microbe Interactions **4**:586-592.

[67]Lleonhart, R., Cistero, A., Carreira, J., Batista, A. and Moscoso del Prado, J. 1992. Food allergy: identification of the major IgE-binding component of peach (*Prunus persica*). Annals of Allergy **69**:128-130.

[68]Lotan, T., Ori, N. and Fluhr, R. 1989. Pathogenesis-related proteins are developmentally regulated in tobacco flowers. Plant Cell **1**:881-887.

[69]Lu, G., Liang, H. and Lu, Z. 1992. Metabolism of ribonucleic acid and protein in ripening apple fruits. Journal of Plant Physiology **139**: 569-573.

[70]Malehorn, D. E., Borgmeyer, J. R., Smith, C. E. and Shah, D. M. 1994. Characterization and expression of an antifungal zeamatin-like protein (Zlp) gene from *Zea mays*. Plant Physiology **106**:1471-1481.

[71]Matton, D. P., Bell, B. and Brisson, N. 1990. Nucleotide sequence of a pathogenesis-related gene of potato. Plant Molecular Biology **14**:863-865.

[72]Mauch, F. and Staehelin, A. 1989. Functional implications of the subcellular localization of ethylene-induced chitinase and β-1,3-glucanase in bean leaves. Plant Cell **1**:447-457.

[73]Metcalfe, D.D. 1998. Food allergy. Prim. Care **25**:819-829.

[74]Metcalfe, D.D., Astwood, J. D., Townsend, R., Sampson, H. A., Taylor, S. L. and Fuchs, R. L. 1996. Assessment of the allergenic potential of foods derived from genetically engineered crop plants. Crit. Rev. Food Sci. Nutr. **36**:165-186.

[75]Metraux, J. P., Burkadt, W., Moyer, M., Dichner, S., Middlesteadt, W., Payne, G., Cames, M. and Ryals, J. 1989. Isolation of a complementary DNA encoding a chitinase with structural homology to a bifunctional lysozyme/chitinase. Proceedings of the National Academy of Sciences of the United States of America **86**:869-900.

[76]Midoh, N. and Iwata, M. 1996. Cloning and characterization of a probenazole-inducible gene for an intracellular pathogenesis-related protein in rice. Plant Cell Physiology **37**:9-18.

[77]Mittermann, I., Swoboda, I., Pierson, E., Eller, N., Kraft, D., Valenta, R. and Heberle-Bors, E. 1995. Molecular cloning and characterization of profilin from tobacco (*Nicotiana tabacum*): increased profilin expression during pollen maturation. Plant Molecular Biology **27**:137-146.

[78]Molina, A., Diaz, I., Vasil, I. K., Carbonero, P. and Carcia-Olmedo, F. 1996. Two cold-inducible genes encoding lipid transfer protein LTP4 from barley show differential responses to bacterial pathogens. Molecular & General Genetics **252**:162-168.

[79]Moneo, I., Gomez, M., Sanchez-Monge, R., Alday, E., Heras, M. de las, Esteban, I., Bootello, A., Salcedo, G. and de las Heras, M. 1999. Lack of crossreaction with Bet v 1 in patients sensitized to Dau c 1, a carrot allergen. Annals of Allergy, Asthma, and Immunology **83**:71-75.

[80]Moneret-Vautrin, D. A. 2000. Evaluation of the allergic risk of transgenic foods from vegetable origin. Comptes Rendus de l'Academie d'Agriculture de France **86**(6):77-85.

[81]Moorhead, G. G. and Plaxton, W. C. 1990. Purification and characterization of cytosolic aldolase from carrot storage root. Biochemical Journal **269**:133-139.

[82]Moiseyev, G. P., Fedoreyeva, L. I., Zhuravlev, Y. N., Yasnetskaya, E., Jekel, P. A. and Beintema, J. J. 1997. Primary structures of two ribonucleases from ginseng calluses. New members of the PR-10 family of intracellular pathogenesis-related proteins. FEBS-Letters **407**: 207-210.

[83]Moroz, L. A. and Yang, W. H. 1980. Kunizt soybean trypsin inhibitor, a specific allergen in food anaphylaxis. N. Engl. J. Med. **302**: 1126-1128.

[84]Möller, C. 1989. Effect of pollen immunotherapy on food hypersensitivity in children with birch pollinosis. Annals of Allergy **62**: 343-345.

[85]Nater, J. P. and Zwartz, J. A. 1967. Atopic allergic reactions due to raw potato. Journal of Allergy **40**:202-206.

[86]Neuhaus, J. M., Sticher, L., Meins, F. Jr. and Boller, T. A. 1991. A short C-terminal sequence is necessary and sufficient for the targeting of chitinases to the plant vacuole. Proceedings of the National Academy of Sciences of the United States of America **88**:10362-10366.

[87]Ohme-Takagi, M. and Shinsi, H. 1995. Ethylene-inducible DNA, binding proteins that interact with an ethylene-responsive element. Plant Cell **7**:173-182.

[88]Przymusiñski, R. and Gwózdz, E. A. 1999. Heavy metal-induced polypeptides in lupin roots are similar to pathogenesis-related proteins. Journal of Plant Physiology **154**(5-6): 703-708.

[89]Pühringer, H., Moll, D., Hoffmann-Sommergruber, K., Watillon, B., Katinger, H. and Laimer da Camara Machado, M. 2000. The promoter of apple *Ypr 10* gene, encoding the major allergen Mal d 1, stress- and

pathogen-inducible. Plant Science **152**: 35-50.
[90]Quirce, S., Dìez Gòmez, M. L., Hinojosa, M., Cuevas, M., Ureña, V., Rivas, M. F., Puyana, J., Cuesta, J. and Losada, E. 1989. Housewives with raw potato-induced bronchial asthma. Allergy **44**:532-536.
[91]Roberts, W. K. and Selitrennikoff, C. P. 1990. Zeamatin, an antifungal protein from maize with membrane-permeabilizing activity. Journal of General Microbiology **136**:1771-1778.
[92]Rohac, M., Birkner, T., Reimitzer, I., Bohle, B., Steiner, R., Breitenbach, M., Kraft, D., Scheiner, O., Gabl, F. and Rumpold, H. 1991. The immunological relationship of epitopes on major tree pollen allergens. Molecular Immunology **28**:897-906.
[93]Samac, D. A. and Shah, D. M. 1994. Effect of chitinase antisense RNA expression on disease susceptibility of *Arabidopsis* plants. Plant Molecular Biology **25**:587-596.
[94]Sampson, H. A. 1999. Food allergy. Part I. Immunopathogenesis and clinical disorders. Journal of Allergy and Clinical Immunology **103**: 717-728.
[95]Sanchez-Monge, R., Blanco, C., Diaz-Perales, A., Collada, C., Carillo, T. and Aragoncillo, C. 1999. Isolation and characterization of major banana allergens: Identification as fruit class I chitinases. Clin. Exp. Allergy **29**:673-680.
[96]Scandalios, J. G. 1993. Oxygen stress and superoxide dismutase. Plant Physiology **101**:7-12.
[97]Schlesinger, M. J., Aliperti, G. and Kelley, P. M. 1982. The response of cells to heat shock. Trends in Biochemical Science **7**:222-225.
[98]Schmelzer, E., Kruger-Lebus, S. and Hahlbrock, K. 1989. Temporal and spatial patterns of gene expression around sites of attempted fungal infection in parsley leaves. Plant Cell **1**:993-1001.
[99]Schoning, B., Ziegler, W. H., Vieths, S. and Baltes, W. 1996. Apple allergy: the cDNA sequence of the major allergen of apple, determined by performing PCR with a primer based on the N-terminal amino acid sequence, is highly homologous to the sequence of the major birch pollen allergen. Journal of the Science of Food and Agriculture **71**: 475-482.
[100]Seppälä, U. 2001. Characterization of potato allergens. Publications of the National Public Health Istitute A 1/2001. Helsinki. 66 p.
[101]Seppälä, U., Alenius, H., Turjanmaa, K., Reunala, T., Palosuo, T. and Kalkkinen, N. 1999. Identification of patatin as a novel allergen for children with positive skin prick test responses to raw potato. Journal of Allergy and Clinical Immunology **103**:165-171.
[102]Sikorski, M. M., Biesiadka, J., Kasperska, A. E., Kopcinska, J., Lotocka, B., Golinowski, W. and Legocki, A. B. 1999. Expression of genes encoding PR10 class pathogenesis-related proteins is inhibited in yellow lupine root nodules. Plant Science Limerick **149**(2):125-137.
[103]Sommsich, I. E., Schmelzer, E., Kawalleck, P. and Hahlbrock, K. 1988. Gene structure and in situ transcript localization of pathogenesis related protein 1 in parsley. Molecular & General Genetics **213**: 93-98.
[104]Son, D. Y., Scheurer, S., Hoffmann, A., Haustein, D. and Vieths, S. 1999. Pollen-related food allergy: cloning and immunological analysis of isoforms and mutants of Mal d1, the major apple allergen, and Bet v 1, the major birch pollen allergen. European Journal of Nutrition **38**:201-215.
[105]Staiger, C. J., Goodbody, K. C., Hussey, P. J., Valenta, R., Drobak, B. K. and Lloyd, C. W. 1993. The profilin multigene family of maize: differential expression of three isoforms. Plant Journal **4**:631-641.
[106]Stanford, A., Bevan, M. and Northcote, D. 1989. Differential expression within family of novel wound induced genes in potato. Molecular & General Genetics **215**:200-208.
[107]Stanley, J. S. and Bannon, G. A. 1999. Biochemistry of food allergens. Clin. Rev. Allergy Immunol. **17**:279-291.
[108]Stintzi, A., Heitz, V., Prasad, S., Wiedemann-Merdinoglu, S., Kauffmann, S., Geofroy, P., Legrand, M. and Fritig, B. 1993. Plant 'pathogenesis-related' proteins and their role in defence against pathogens. Biochimie **75**:687-706.
[109]Sunderasan, E., Hamzah, S., Hamid, S., Ward, M. A., Yeang, H. Y. and Cardosa, M. J. 1996. Latex B-serum β-1,3-glucanase (Hev b 2) and a component of the microhelix (Hev b 4) are major latex allergens. J. Nat. Rub. Res. **10**:82-99.
[110]Swoboda, I., Hoffmann-Sommergruber, K., O'Riordain, G., Schneiner, O., Heberle-Bors, E. and Vicente, O. 1996. Bet v 1 proteins, the major birch pollen allergens and members of a family of conserved pathogenesis-related proteins, show ribonuclease activity in vitro. Physiologia Plantarum **96**:433-438.
[111]Swoboda, I., Schneiner, O., Heberle-Bors, E. and Vicente, O. 1995. cDNA cloning and characterization of three genes in the Bet v 1 gene family that encode pathogenesis-related proteins. Plant, Cell and Environment **18**:865-874.
[112]Thiel, Cl. 1988. Nahrungsmittelallergien bei Pollenallergikern (sogenannte pollenassoziierte Nahrungsmittelallergien). Allergologie **11**:397-410.
[113]Tierney, M. L., Iechert, J. and Pluymers, D. 1988. Analysis of the expression of extensin and p33-related cell wall proteins in carrot and soybean. Molecular & General Genetics **211**:393-399.
[114]Valenta, R., Duchêne, M., Pettenburger, K., Sillaber, Ch., Valent, P., Bettelheim, M., Breitenbach, H., Rumbold, H., Kraft, D. and Schneider, O. 1991. Identification of profilin as a novel pollen allergen: IgE autoreactivity in sensitized individuals. Science **253**:557-560.
[115]Valenta, R., Lidholm, J., Niederberger, V., Hayek, B., Kraft, D. and Grönlund, H. 1999. The recombinant allergen-based concept of component-resolved diagnostics and immunotherapy (CRD and CRIT). Clin. Exp. Allergy **29**:896-904.
[116]Van Damme, E. J. M., Charels, D., Roy, S., Tierens, K., Barre, A., Martins, J. C., Rouge, P., Van Leuven, F., Does, M. and Peumans, W. 1999. A gene encoding a hevein-like protein from elderberry fruits is homologous to PR-4 and class V chitinase genes. Plant Physiology **119**:1547-1556.
[117]Van Loon, L. C. 1985. Pathogenesis-related proteins. Plant Molecular Biology **4**:111-116.
[118]Van Loon, L. C. and Antoniw, J. F. 1982. Comparison of the effects of salicylic acid and ethephon with virus-induced hypersensitivity and acquired resistance in tobacco. Netherland Journal of Plant Pathology **88**:237-256.
[119]Van Loon, L. C. and Van Kammern, A. 1970. Polyacrylamide disc electrophoresis of the soluble leaf proteins from *Nicotiana tabacum* var Samsun and Samsun NN II: changes in leaf protein constitution after infection with tobacco mosaic virus. Virology **40**:199-211.
[120]Van Loon, L. C. and Van Strien, E. A. 1999. The families of pathogenesis-related proteins, their activities, and comparative analysis of PR-1 type proteins. Phys. Mol. Plant Pathol. **55**:85-87.
[121]Vieths, S., Schöning, B. and Jankiewicz, A. 1993. Occurrence of IgE binding allergens during ripening of apple fruits. Food & Agricultural Immunology **5**: 93-105.
[122]Vieths, S., Jankiewicz, A., Schöning, B. and Aulepp, H. 1994. Apple allergy: the IgE-binding potency of apple strains is related to the occurrence of the 18 kDa allergen. Allergy **49**:262-271.
[123]Walter, M. H., Liu, J. W., Grans, C., Lamb, C. and Hess, D. 1990. Bean pathogenesis-related (PR) proteins deduced from elicitor-induced transcripts are members of a new class of conserved PR proteins including pollen allergens. Molecular & General Genetics **222**: 353-360.
[124]Wahl, R., Lau, S., Maasch, H. J. and Wahn, U. 1990. IgE-mediated allergic reactions to potatoes. International Archives of Allergy and Applied Immunology **92**:164-168.
[125]Warner, S. A. J. , Gill, A. and Draper, J. 1994. The developmental expression of the asparagus intracellular PR protein (AoPr1) gene correlates with sites of phenylpropanoid biosynthesis. Plant Journal **6**:31-43.
[126]Warner, S. A. J., Scott, R. and Draper, J. 1992. Characterization of a wound-induced transcript from the monocot asparagus that shares similarity with a class of intracellular pathogenesis-related (PR9) proteins. Plant Molecular Biology **19**:55-561.
[127]Wen, J., Vanek-Krebitz, M., Hoffmann-Sommergruber, K., Scheiner,

O. and Breiteneder, H. 1997. The potential of Bet v 1 homologous, a nuclear multigene family, as phylogenetic markers in flowering plants. Mol. Phyl. Evol. **8**:317-333.

[128]Wilson, I, B. H. and Altmann, F. 1998. Structural analysis of N-glycans from allergenic grass, ragweed and tree pollens: core α(1,3)-linked fucose and xylose present in all pollen examined. Glycoconj. J. **15**: 1055-1070.

[129]Xu, Y., Chang, P. F. L., Narasimhan, M. L., Raghothama, K. G., Hasegawa, P. M. and Bressan, R. 1994. Plant defence genes are synergistically induced by ethylene and methyl jasmonate. Plant Cell **6**:1077-1085.

[130]Yamamoto, M., Torikai, S. and Oeda, K. 1997. A major root protein of carrots with high homology to intracellular pathogenesis-related (PR) proteins and pollen allergens. Plant Cell Physiology **38**:1080-1086.

[131]Zhu, B., Chen, T. H. and Li, P. H. 1996. Analysis of late-blight disease resistance and freezing tolerance in transgenic potato plants expressing sense and antisense genes for an osmotin-like protein. Planta **198**:70-75.

[132]Vigers, A. J., Wiedemann, S., Roberts, W. K., Legrand, M., Selitrennikoff, C. P. and Fritig, B. 1992. Thaumatin-like pathogenesis-related proteins are antifungal. Plant Science Limerick 83:155-161.

[133]Datta, S. K. and Muthukrishnan, S. (eds) 1999. Pathogenesis-related proteins in plants. CRC Press, Boca Raton, Florida, USA. 291 p.