Plant Omics Journal POJ

POJ 4(7):428-434 (2011) ISSN:1836-3644

Genomic identification of putative allergen genes in woodland strawberry (*Fragaria vesca*) and mandarin orange (*Citrus clementina*)

Tae Kyung Hyun[1] and Ju-Sung Kim[2*]

[1]Department of Biochemistry, Gyeongsang National University, Jinju 660-701, Korea
[2]Majors in Plant Resource Sciences and Environment, College of Applied Life Sciences, Jeju National University, Jeju 690-756, Korea

***Corresponding author: aha2011@jejunu.ac.kr**

Abstract

Although many fruits are plant-derived healthy foods, they may harbor a series of allergenic proteins. Based on biochemical and molecular biological approaches, a number of allergens have been identified and characterized in *Rosaceae* family fruits, such as apple and peach. However, our understanding of the allergens from the *Rosoideae* subfamily and the *Rutaceae* family is very limited. Therefore, in this study, we identified gene families, including pathogenesis-related protein 10 (PR-10), non-specific lipid transfer protein (nsLTP), and profilin as allergens in *Fragaria vesca* (woodland strawberry) and *Citrus clementina* (mandarin orange). Using comprehensive analysis, we identified 25 putative allergen genes from woodland strawberry and eight mandarin orange genome sequences. The analysis of functional annotation indicated that these allergens have complex physiological roles that are involved in plant defense and plant development. Although further functional analysis of these allergens though the analysis of cross-reactivity of immunoglobulin E antibody to these allergens will be required, our comparative genomic analysis of the allergen genes and encoded proteins in woodland strawberry and mandarin orange provides a basis for future research on developing low-allergenic cultivars without compromising the natural defense of plants.

Keywords: Comparative genomics analysis, Allergen, *Fragaria vesca*, *Citrus clementina*, Functional annotation.
Abbreviations: PR-10 - Pathogenesis-related protein-10; nsLTP - non-specific lipid transfer protein.

Introduction

Fruits are generally considered to be an important component of nutritious foods for humans. They are rich in vitamins and a rich source of carbohydrates, sugar, and minerals; they also contain a variety of biologically active compounds that can be beneficial to human health. In addition, there is substantial evidence that low intake of fruits and vegetables is a major risk factor for coronary heart disease, cancer, and stroke (World Health Organization, 2002; DiBello et al., 2008). However, several kinds of fruits belong to the most important elicitors of food allergy in adults and adolescents (Vieths et al., 2002; Kiambi et al., 2008; Tehrani et al., 2011;). Food allergy is an adverse reaction to normally harmless substances, such as food proteins (allergens), by the immune system, and it involves immune responses mediated by an increased amount of immunoglobulin E (IgE) or IgG antibodies (Bohle, 2004). Once sensitized, it can become allergic to homologous proteins of other food, including fruits, via cross-reactivity (Vieths et al., 2002; Gao et al., 2005). Food allergens are typically categorized as class I and class II (Jyonouchi, 2008). Class I allergens are water-soluble glycoproteins that are stable to acid and digestive enzymes, and the immune reaction takes place in the gastrointestinal tract. Class II allergens are those that cause oral mucosal irritation through sensitization by homologous components of plant allergens that come into contact with the immune system via inhalation. For example, the inhalation of pollen from several birch trees and grasses is the main cause of primary sensitization in humans (Gao et al., 2005; Bartra et al., 2009). The fruits from the *Rosaceae* family, including peach, almond, cherry (*Prunoideae* subfamily), apple, pear (*Pomoideae* subfamily), blackberry, and strawberry (*Rosoideae* subfamily) have been reported to cause allergic reactions mediated by cross-reactive phenomena linked to birch pollinosis (Scheurer et al., 1997; Pastorello et al., 1999a; Karamloo et al., 2001; Fernandez-Rivas et al., 2006; Zuidmeer et al., 2006). Several allergens from those fruits have been identified that might be responsible for cross-reactivity (Zuidmeer et al., 2006). Based on structural and biochemical analysis, fruit allergens are grouped into a limited number of protein families (Bohle, 2007; Radauer and Breiteneder, 2007). In the *Rosaceae* family, four major allergens have been identified: pathogenesis-related protein (PR-10, birch allergen Bet v1 homologous protein), thaumatin-like protein (TLP, PR-5 protein), non-specific lipid transfer protein (nsLTP, PR-14 protein), and profilin (Breiteneder and Ebner, 2000; Botton et al., 2008). These four allergen families in apple and peach fruits are on the international allergen list (http://www.allergen.org/index.php). Allergic reactions caused by PR-10, the major apple allergen (Mal d 1), are associated with birch pollinosis due to cross-reactivity with Bet v1, whereas nsLTP causes a class I food allergy (Fritsch et al., 1998; Pastorello et al., 1999b; Breiteneder and Ebner, 2000). In apple, variation in quantity of Mal d1 (PR-10 protein) correlates with IgE binding, indicating that different isoforms of Mal d1 exist in a single fruit (Son et al., 1999; Hjerno et al., 2006). In fact, 24 isoforms of Mal d1 have been listed (http://www.allergen.org/index.php), and the existence of allergenic and hypoallergenic isoforms suggests that PR-10 isoforms present different IgE-binding capacity (Friedl-Hajek et al., 1999; Vieths et al., 2002). A large-scale genomic sequence analysis of the *Rosaceae* family revealed a high degree of protein sequence similarity among crop species and contributed to the identification of novel putative fruit allergens, using comparative genomic analysis (Maghuly et al., 2009; Schenk et al., 2009; Lebel et al., 2010). In contrast to other *Rosaceae* fruits, allergens to fruit belonging to the *Rosoideae* subfamily (e.g., strawberry) or the *Rutaceae* family (e.g., orange and mandarin orange) are not fully understood, although hypersensitivity to strawberry and orange is commonly reported (Zuidmeer et al., 2006; Ebo et al., 2007; Glaspole et al., 2007; Asero et al., 2008). So far, three PR-10 proteins (Fra a1e, Fra a2, and Fra a3) from strawberry (Hjerno et al., 2006; Munoz et al., 2010) and IgE-binding profilin and nsLTP have been identified from strawberry, orange, and mandarin orange (Zuidmeer et al., 2006; Ebo et al., 2007; Glaspole et al., 2007; Asero et al., 2008). The low number of allergen isoforms, compared with other fruits, such as apple and peach, might be due to limited genome and EST information in the *Rosoideae* subfamily and the *Rutaceae* family. In this study, by means of *in silico* analysis of the genome sequence, we conducted a genome-wide analysis of allergen gene families and identified woodland strawberry (*Fragaria vesca*) and mandarin orange (*Citrus clementina*) genes potentially encoding PR-10, nsLTP, and profilin. Our genomic and bioinformatic analysis will provide a foundation for further functional analysis of the allergen gene families, and it will be useful for future research on the selection and breeding of low-allergenic cultivars.

Results and discussion

Availability of complete woodland strawberry and mandarin orange genome sequences has made it possible for the first time to identify the putative allergen families in those plant species. In the international allergen database (http://www.allergen.org/index.php), four nsLTPs, a single PR-10, and a single profilin of strawberry (*Fragaria ananassa*) were already on the list, and two nsLTPs, a single germin-like protein, and a single profilin were listed as allergens in sweet orange (*Citrus sinensis*). Although germin-like protein has been reported as one of the allergens in sweet orange (Ahrazem et al., 2006), it was not possible to identify the homologue proteins in the mandarin orange database, due to limited information regarding amino acid sequences of germin-like protein. Therefore, we focused on the identification of three allergen families (PR-10, nsLTP, and profilin) for woodland strawberry and two families (nsLTP and profiling) for mandarin orange. In order to identify the allergen genes, sequences of allergens from strawberry, sweet orange, apple, and peach were analyzed by BLASTp against protein sequences of woodland strawberry and mandarin orange. The redundant sequences were removed according to self BLAST of sequences, followed by manual editing, resulting in a total of 25 putative allergen genes from woodland strawberry and eight from mandarin orange (Table 1). Following the systematic allergen nomenclature (King et al., 1995), three allergen families for woodland strawberry were named: Fra v1 (PR-10), Fra v3 (nsLTP), and Fra v4 (profilin). In addition, Cit c2 and Cit c3 were named to indicate profilin and nsLTP, respectively, for mandarin orange. Each gene family is described in more detail below.

Fra v1 (PR-10) genes

PR-10 proteins are present as a multigene family with low intraspecific variation and higher interspecific variation (Wen et al., 1997; Finkler et al., 2005), and they are identified as intracellular pathogenesis-related proteins, including tree pollen allergens and major food allergens (Wen et al., 1997). According to *Fra a1* cDNA and protein reference sequences derived from strawberry in the GenBank database and international allergen database, we obtained 15 putative *Fra v1* genes from the woodland strawberry genome (Table 1 and Fig. 1A). Most genes of *Fra v1* have an open reading frame (ORF) from 470 to 490 bp encoding a polypeptide of 157-161 amino acids with the common feature of a p-loop motif (GxxGGxGxxK) (Table 1 and Fig. 1B), which is presumed to function as a binding site for nucleotides involved in RNase activity for PR-10 proteins (Liu et al., 2006). As shown in Fig. 1A, ORF from *Fra v1* genes is interrupted by an intron of 119-149 bp at a highly conserved position, whereas nine out of 15 putative *Fra v1* genes are intronless. Similarly, most PR-10, including tree pollen allergens in a number of plant species, share an ORF from 456 to 489 bp, and have a single or no intron at the conserved position (Liu et al., 2006; Chen et al., 2008), indicating that the putative *Fra v1* genes have a conserved gene structure, like the other pollen allergens. Most putative Fra v1 proteins, except Fra v1.12 and Fra v1.13, have a calculated molecular mass ranging from 17.3 to 17.8 kDa and a theoretical pI from 5.0 to 5.7 (Table 1), consistent with usual plant PR-10 features (Liu et al., 2006). To further support that the 15 computationally predicted Fra v1 proteins belong to tree pollen allergens, the presence of Bet_v_1 (PF00407) domain, which is the signature for the pollen allergen (Bet v1) family (Liu et al., 2006), was analyzed using PROSITE (http://www.expasy.org/prosite/) and SMART (http://smart.embl-heidelberg.de/); the results suggested that 15 Fra v1 proteins belong to the pollen allergen family (Fig. 1B). Although Fra v1.12 and Fra v1.13 did not exhibit a conserved gene structure like the other pollen allergens, the presence of Bet_v_1 (PF00407) domain and p-loop motif indicated that those proteins also might act as pollen allergens.

Fra v3 and Cit c3 (nsLTP) genes

Since nsLTPs have been identified as the major allergens in *Rosaceae* fruits, a large LTP family has been described from over 100 potential members in up to 50 different species (Jose-Estanyol et al., 2004; Salcedo et al., 2004). The high resistance of nsLTPs to proteolytic digestion and heat treatment indicates that nsLTP is the allergen that is the primary sensitizer by ingestion (Scheurer et al., 2004; Zuidmeer and van Ree, 2007). In woodland strawberry and mandarin orange genomes, we identified five *Fra v3* and five *Cit c3* genes, which encode a polypeptide of 113 to 164 amino acids (Table 1). Eight of the putative nsLTP genes had two exons and one intron (Fig. 2A). The size of the second exon in these genes was conserved and extremely short (10 bp), although the sizes of the second exons of *Fra v3.04* and *Cit c3.03* were 39 bp and 97 bp, respectively. Such conserved gene structure has also been found in other plant nsLTPs, such as peach and almond (Chen et al., 2008). In addition, these putative nsLTPs, with the exception of Cit c3.04, have a calculated molecular mass ranging from 11.5 to14.8 kDa and a theoretical pI ranging from 8.8 to 9.7 (Table 1). Plant nsLTPs are members of the prolamin superfamily, which are low-molecular weight proteins (below ~15 kDa) and basic polypeptides (pI ~9), and characterized by a conserved pattern of six to eight cysteine residues forming a network of three to

Table 1. Nomenclature of allergen genes in *Fragaria vesca* and *Citrus clementina* genomes.

Allergen	Plant	Gene name	Gene ID	Scaffold (scf) Position	ORF (nt)	AA length (aa)	pI/MW(kDa)
PR-10	*Fragaria vesca*	*Fra v1.01*	gene05123	scf0513154:1206036..1206518	483	160	5.5/17.6
		Fra v1.02	gene07064	scf0513159:2419221..2419703	483	160	5.7/17.5
		Fra v1.03	gene07083	scf0513159:2533779..2534261	483	160	5.2/17.5
		Fra v1.04	gene32299	scf0513098:423355..423837	483	160	5.4/17.5
		Fra v1.05A	gene07086	scf0513159:2540194..2540676	483	160	5.4/17.4
		Fra v1.05B	gene07065	scf0513159:2420786..2421268	483	160	5.2/17.5
		Fra v1.06A	gene07088	scf0513159:2543054..2543536	483	160	5.1/17.5
		Fra v1.06B	gene05122	scf0513154:1204903..1205385	483	160	5.1/17.5
		Fra v1.07	gene04962	scf0513154:1631133..1631615	483	160	5.1/17.5
		Fra v1.08	gene07082	scf0513159:2530739..2531337	480	159	5.6/17.4
		Fra v1.09	gene07085	scf0513159:2538076..2538689	480	159	5.4/17.5
		Fra v1.10	gene07081	scf0513159:2528332..2528966	486	161	5.0/17.8
		Fra v1.11	gene05185	scf0513154:1571373..1571987	474	157	5.5/17.3
		Fra v1.12	gene07084	scf0513159:2535865..2536938	528	175	5.6/19.4
		Fra v1.13	gene07087	scf0513159:2541780..2542862	585	194	6.4/21.7
nsLTP	*Fragaria vesca*	*Fra v3.01*	gene13870	scf0513177:3465880..3466359	354	117	9.3/11.5
		Fra v3.02A	gene13871	scf0513177:3471392..3471866	369	122	9.0/12.3
		Fra v3.02B	gene13390	scf0513190:3051038..3051511	369	122	9.1/12.3
		Fra v3.03	gene13867	scf0513177:3449301..3449733	342	113	8.9/11.8
		Fra v3.04	gene13868	scf0513177:3452538..3453995	438	145	9.0/14.8
	Citrus clementina	*Cit c3.01*	clementine0.9_025808m	scf_2: 2599662..2600423	354	117	8.9/11.9
		Cit c3.02	clementine0.9_032770m	scf_10: 4805337..4805690	354	117	8.8/11.9
		Cit c3.03	clementine0.9_025486m	scf_2: 2568575..2569330	381	126	9.7/13.7
		Cit c3.04	clementine0.9_023997m	scf_2: 2574152..2574923	495	164	8.7/17.5
		Cit c3.05	clementine0.9_025527m	scf_2: 2604682..2605530	378	125	9.0/13.2
Profilin	*Fragaria vesca*	*Fra v4.01*	gene11106	scf0513144:567471..568539	396	131	5.0/14.0
		Fra v4.02	gene09020	scf0513134:2069851..2070771	396	131	4.6/14.1
		Fra v4.03	gene11336	scf0513146:1825438..1826502	402	133	5.2/14.1
		Fra v4.04	gene25705	scf0513110:100888..103002	396	131	5.0/14.0
		Fra v4.05	gene11335	scf0513146:1822788..1823557	396	131	4.6/14.0
	Citrus clementina	*Cit c2.01*	clementine0.9_025205m	scf_58: 249842..251491	402	133	5.4/14.2
		Cit c2.02	clementine0.9_025303m	scf_33:1967653..1970342	396	131	5.0/14.1
		Cit c2.03	clementine0.9_025288m	scf_58: 254707..256661	396	131	4.6/14.1

four disulphide bridges (Salcedo et al., 2004; Zuidmeer and van Ree, 2007). As shown in Fig. 2B, all putative nsLTPs from woodland strawberry and mandarin orange were predicted to contain N-terminal signal peptides, followed by eight well-conserved cysteine residues. Among nsLTP1 family members, two conserved pentapeptides (T/SxxDR/K and PYxIS) have been proposed as important for catalysis or binding (Douliez et al., 2000). The presence of these pentapeptides in Fra v3.01, Fra v3.04, Cit c3.01, Cit c3.03, and Cit c3.04 indicates that these proteins belong to the nsLTP1 family.

Fra v4 and Cit c2 (profilin) genes

Profilin was first identified as an allergen in birch pollen Bet v2, which is a minor allergen (Valenta et al., 1991). Many plant profilins have been found to be highly cross-reactive allergens, due to conserved amino acid sequences and shared IgE-reactive epitopes (Westphal et al., 2004; Sankian et al., 2005). The family of profilins contains conserved protein sequences with molecular masses ranging from 12 to 15 kDa, corresponding to polypeptides of 124-153 amino acids (Vieths et al., 2002). The putative Fra v4 and Cit c2 proteins have three exons encoding a polypeptide of 131 to 133 amino acids, with a calculated molecular mass ranging from 14.0 to14.2 kDa, in agreement with that found in most plant species (Table 1 and Fig. 3A). All known profilin structures exhibit a tertiary structure, with three helices and seven β-strands (Vieths et al., 2002; Krishnan and Moens, 2009). In birch pollen profilins, the seven β-strands appear as two orthogonal β-sheets, with the first sheet formed by β1, β2, β4, β5, and β6 strands, and the second sheet formed by β3 and β4 strands (Fedorov et al., 1997). As shown in Fig. 3B, the putative Fra v4 and Cit c2 proteins exhibited protein structures similar to other plant profilins, whereas Fra v4.03 consisted of three helices, but six β-strands. The different second structures of profilin have also been shown in *Pheum* profilin (Phl p11 allergen), which has a tertiary structure (Asturias et al., 1997). Therefore, we hypothesize that the missing β2 strand in Fra v4.03 does not change the tertiary structure but regulates its stability or ligand-binding activity.

Functional annotation of allergens in woodland strawberry and mandarin orange

Gene ontology (GO), which is a functional gene classification system, was used to classify the functions of the putative allergens from woodland strawberry and mandarin orange. Based on sequence homology, the woodland strawberry allergens were assigned at least one GO term, including eight functional groups, whereas six functional groups were identified from mandarin orange allergens (Fig. 4A and B). Under the biological process category, "defense response" and "response to biotic stimulus" were prominently represented, due to the presence of PR-10 in the woodland strawberry (Fig. 4A). Although the physiological function of PR-10 in higher plants is not fully understood, the induction of PR-10 genes by biotic and abiotic stresses and during plant development has been observed (Koistine et al., 2005; Hanschuh et al., 2007). This finding indicates that PR-10 proteins act not only as allergens, but also as defense molecules produced in response to pathogen attack. nsLTPs (Fra v3 and Cit c3) and profilins (Fra v4 and Cit c2) are related to binding (lipid-binding and actin-binding) in the molecular function category (Fig 4 A and B). In eukaryotes, profilins are identified as being involved in actin polymerization, playing an important role as key regulators of F-actin dynamics (Krishnan and Moens, 2009), whereas nsLTPs facilitate the movement of various polar lipids across membranes (Kader, 1996). Although PR-10, nsLTP, and profilin have been identified as fruit allergens, these findings indicate that they have a major and complex physiological role involved in plant defense, membrane trafficking, and linkage between the microfilament system and signal transduction pathways.

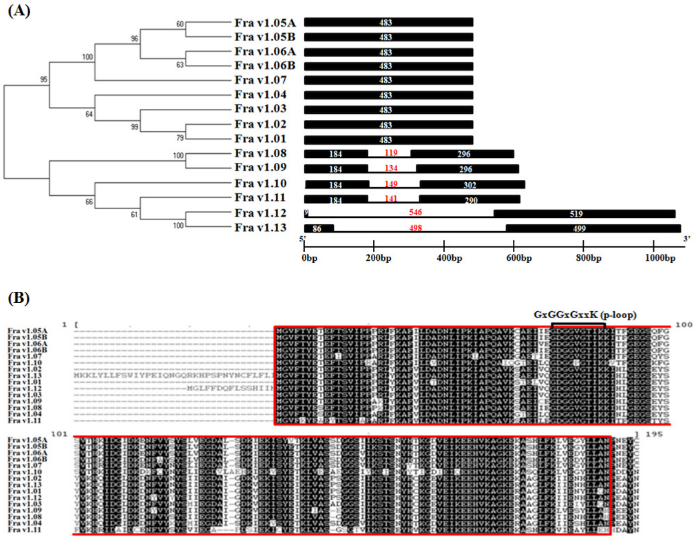

Fig 1. The putative PR-10 family (Fra v1) from the *F. vesca* genome. (A) Phylogenetic tree and gene structures of Fra v1 members. Default values were used except for 100 bootstraps. Exons are drawn as boxes, and connecting lines indicate the positions of introns. Exon and intron sizes are shown by number. (B) Alignment of amino acid sequences of Fra v1 members. Red box indicate the Bet_v_1 (PF00407) domain.

Materials and methods

Sequence and database search for allergens from Fragaria vesca and Citrus clementina

In an attempt to obtain all the allergens, woodland strawberry protein sequences available in the Strawberry Gene Model Bioview server (Hybrid gene models; https://strawberry.plantandfood.co.nz/gbrowse/navbar/strawberry/DownloadData/vescagenemodels2.faa) were downloaded to construct a local protein database. It was comprised of 35,234 sequences. This database and the Plant and Food Research database (Strawberry Gene Model Bioview server, https://strawberry.plantandfood.co.nz/) were searched with allergen query sequences downloaded from NCBI (http://www.ncbi.nlm.nih.gov/) and the International Allergen Database (http://www.allergen.org/index.php).

To identify members of mandarin orange allergens, multiple database searches were performed using the Basic Local Alignment Search tool (BLAST) algorithms BLASTn and BLASTp, available on Phytozome (http://www.phytozome.net/) and the Citrus Genome Database (http://www.citrusgenomedb.org/). We used nucleotide and amino acid sequences of the allergens in *Malus domestica* (apple) and *Prunus persica* (peach) as seed to BLAST all databases. The information regarding the gene structure was obtained from

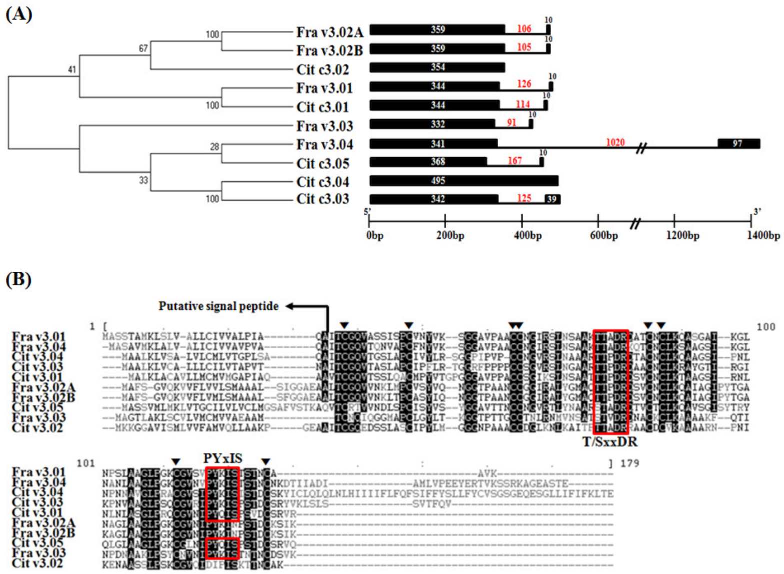

Fig 2. The putative nsLTP family from the *F. vesca* (Fra v3) and *C. clementina* (Cit c3) genomes. (A) Phylogenetic tree and gene structures of Fra v3 and Cit c3 members. (B) Alignment of amino acid sequences of Fra v3 and Cit c3 members. Eight conserved cysteine residues are arrowed.

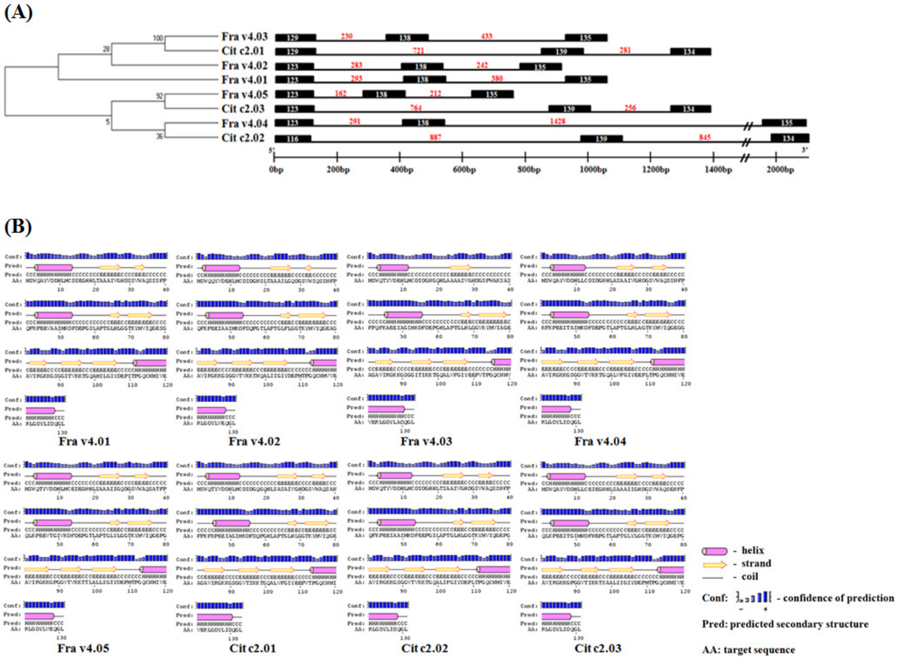

Fig 3. The putative profilin family from the *F. vesca* (Fra v4) and *C. clementina* (Cit c2) genomes. (A) Phylogenetic tree and gene structures of Fra v4 and Cit c2 members. (B) Secondary structure prediction of Fra v4 and Cit c2 members.

the *Fragaria vesca* Genome Browser (https://strawberry.plantandfood.co.nz/cgi-bin/gbrowse/ strawberry_genome/) and *Citrus clementina* GBrowse (http:// www.citrusgenomedb.org/gb/gbrowse/citrus_clementina_v0.9/).

Construction of phylogenetic tree and conserved domain analysis

To construct the phylogenetic trees, the amino acid sequences were aligned using the ClustalW (http://www.clustal.org) algorithm-based AlignX module from MEGA Version 4.0 (http://www.megasoftware.net/mega.html). The alignments were saved and executed by MEGA Version 4.0 to generate a neighbor joining tree with a pairwise deletion option for handling alignment gaps and bootstrapping analysis. To analyze the conserved domain, all the putative allergens were analyzed with the SMART domain search program (http://smart.embl-heidelberg.de/) and PROSITE scanning (http://www.expasy.ch/prosite/). In addition, we predicted

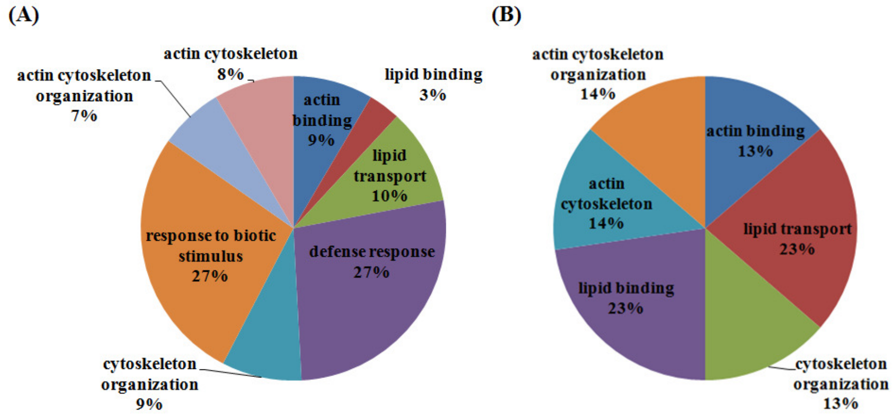

Fig 4. Gene ontology classification of putative allergens from *F. vesca* (A) and *C. clementina* (B).

secondary structures of putative allergens using the Protein Structure Prediction Server (PSIPRED, http://bioinf.cs.ucl.ac.uk/psipred/).

Functional annotation of putative allergens

For functional annotation, the Blast2GO program (Conesa et al., 2005) was used to obtain GO annotation of putative allergens, according to component function, biological process, and cellular component ontologies.

Conclusion

The availability of the genome sequences of *F. vesca* and *C. clementina* allowed us to characterize the gene families of the three major allergens PR-10, nsLTP, and profilin. In addition, the analysis of functional annotation indicated that these allergens play an important role in plant defense and plant development. A further and motivating challenge would be to understand the cross-reactivity of these allergens and control the spatial and temporal expression of allergens without compromising the natural defense of plants.

References

Ahrazem O, Ibanez MD, Lopez-Torrejon G, Sanchez-Monge R, Sastre J, Lombardero M, Barber D, Salcedo G (2006) Orange germin-like glycoprotein Cit s 1: an equivocal allergen. Int Arch Allergy Immunol 139:96-103

Asero R, Monsalve R, Barber D (2008) Profilin sensitization detected in the office by skin prick test: a study of prevalence and clinical relevance of profilin as a plant food allergen. Clin Exp Allergy 38: 1033-1037

Asturias JA, Arilla MC, Bartolome B, Martinez J, Martinez A, Palacios R (1997) Sequence polymorphism and structure analysis of timothy grass pollen profilin allergen (Phl p11). Biochem Biophys Acta 1352: 253-257

Bartra J, Sastre J, del Cuvillo A, Montoro J, Jáuregui I, Dávila I, Ferrer M, Mullol J, Valero A (2009) From pollinosis to digestive allergy. J Investig Allergol Clin Immunol 19: 3-10

Bohle B (2004) T lymphocytes and food allergy. Mol Nutri Food Res 48: 424-433

Bohle B (2007) The impact of pollen-related food allergens on pollen allergy. Allergy 62: 3-10

Botton A, Lezzer P, Dorigoni A, Barcaccia G, Ruperti B, Ramina A (2008) Genetic and environmental factors affecting allergen-related gene expression in apple fruit (*Malus domestica* L. Borkh). J Agri Food Chem 56: 6707-6716

Breiteneder H, Ebner C (2000) Molecular and biochemical classification of plant-derived food allergens. J Allergy Clin Immunol 106: 27-36

Chen W, Zhang S, Illa E, Song L, Wu S, Howad W, Arus P, van de Weg E, Chen K, Gao Z (2008) Genomic characterization of putative allergen genes in peach/almond and their synteny with apple. BMC Genomics 9: 543

Conesa A, Gotz S, Garcia-Gomez JM, Terol J, Talon M, Robles M (2005) Blast2GO: a universal tool for annotation, visualization and analysis in functional genomics research. Bioinformatics 21:3674-3676

DiBello JR, Kraft P, McGarvey ST, Goldberg R, Campos H, Baylin A (2008) Comparison of 3 methods for identifying dietary patterns associated with risk of disease. Am J Epidemiol 168:1433-1443

Douliez JP, Michon T, Elmorjani K, Marion D (2000) Structure, biological and technological functions of lipid transfer proteins and indolines, the major lipid binding proteins from cereal kernels. J Cereal Sci 32: 1-20

Ebo DG, Ahrazem O, Lopez-Torrejon G, Bridts CH, Salcedo G, Stevens WJ (2007) Anaphylaxis from mandarin (*Citrus reticulata*): identification of potential responsible allergens. Int Arch Allergy Immunol 144: 39-43

Fedorov AA, Ball T, Mahoney NM, Valenta R, Almo SC (1997) The molecular basis for allergen cross-reactivity: crystal structure and IgE-epitope mapping of birch pollen profilin. Structure 5: 33-45

Fernandez-Rivas M, Bolhaar S, Gonzalez-Mancebo E, Asero R, van Leeuwen A, Bohle B, Ebner C, Rigby N, Sancho AI, Miles S, Zuidmeer L, Knulst A, Breiteneder H, Mills C, Hoffmann-Sommergruber K, van Ree R (2006) Apple allergy across Europe: How allergen sensitization profiles determine the clinical expression of allergies to plant foods. J Allergy Clin Immunol 118:481-488

Finkler C, Giacomet C, Muschner VC, Salzano FM, Freitas LB (2005) Molecular investigations of pathogenesis-related Bet v 1 homologues in *Passiflora* (Passifloraceae). Genetica 124:117-125

Friedl-Hajek R, Radauer C, O'Riordain G, Hoffmann-Sommergruber K, Leberl K, Scheiner O, Breiteneder H (1999) New Bet v 1 isoforms including a naturally occurring truncated form of the protein derived from Austrian birch pollen. Molecular Immunol 36:639-645

Fritsch R, Bohle B, Vollmann U, Wiedermann U, Jahn-Schmid B, Krebitz M, Breiteneder H, Kraft D, Ebner C (1998) Bet v 1, the major birch pollen allergen, and Mal d 1, the major apple allergen, cross-react at the level of allergen-specific T helper cells. J Allergy Clin Immunol 102: 679-686

Gao ZS, van de Weg WE, Schaart JG, van der Meer IM, Kodde L, Laimer ML, Breiteneder H, Hoffmann-Sommergruber K, Gilissen LJWJ (2005) Linkage map positions and allelic diversity of two Mal d 3 (non-specific lipid transfer protein) genes in the cultivated apple (*Malus domestica*). Theor Appl Genet 110: 479-491

Glaspole IN, de Leon MP, Rolland JM, O'Hehir RE (2007) Anaphylaxis to lemon soap: citrus seed and peanut allergen cross-reactivity. Ann Allergy Asthma Immunol 98: 286-289

Handschuh L, Femiak I, Kasperska A, Figlerowicz M, Sikorski MM (2007) Structural and functional characteristics of two novel members of pathogenesis related multigene family of class 10 from yellow lupine. Acta Bioch Pol 54:783-796

Hjerno K, Alm R, Canback B, Matthiesen R, Trajkovski K, Bjork L, Roepstorff P, Emanuelsson C (2006) Down-regulation of the strawberry Bet v 1-homologous allergen in concert with the flavonoid biosynthesis pathway in colorless strawberry mutant. Proteomics 6: 1574-1587

Jose-Estanyol M, Gomis-Ruth FX, Puigdomenech P (2004) The eight cysteine motif, a versatile structure in plant proteins. Plant Physiol Biochem 42: 355-365

Jyonouchi H (2008) Non-IgE mediated food allergy. Inflamn Allergy Drug Targets 7: 1-8

Kader JC (1996) Lipid transfer proteins in plants, Annu RevPlant Physiol Plant Mol Biol 47: 627-654

Karamloo F, Scheurer S, Wangorsch A, May S, Haustein D, Vieths S (2001) Pyr c 1, the major allergen from pear (*Pyrus communis*), is a new member of the Bet v 1 allergen family. J Chromatogr B Biomed Sci Appl 756: 281-293

Kiambi DK, Fortin M, Stromvick M (2008) Linking transcript profiles to metabolites and metabolic pathways: A systems biology approach to transgene risk assessment. Plant Omics J 1: 26-36

King TP, Hoffman D, Lowenstein H, Marsh DG, Platts TAE, Thomas W (1995) Allergen nomenclature. J Allergy Clin Immunol 96:5-14

Koistinen KM, Soininen P, Vanäläinen TA, Häyrinen J, Laatikainen R, Peräkylä M, Tervahauta AI, Kärenlampi SO (2005) Birch PR-10c interacts with several biologically important ligands. Phytochem 66:2524-2533

Krishnan K, Moens PDJ (2009) Structure and functions of profilins. Biophys Rev 1:71-81

Lebel S, Schellenbaum P, Walter B, Maillot P (2010) Characterisation of the Vitis vinifera PR10 multigene family. BMC Plant Biology 10:184

Liu JJ, Ekramoddoullah AKM (2006) The family 10 of plant pathogenesis-related proteins: Their structure, regulation, and function in response to biotic and abiotic stresses. Physiol Mol Plant Pathol 68:3-13

Maghuly F, Borroto-fernandez EG, Khan MA; Herndl A; Marzban G; Laimer M (2009) Expression of calmodulin and lipid transfer protein genes in *Prunus incisa* x *serrula* under different stress conditions. Tree Physiol 29: 437-444

Munoz C, Hoffmann T, Escobar NM, Ludemann F, Botella MA, Valpuesta V, Schwab W (2010) The strawberry fruit Fra a allergen functions in flavonoid biosynthesis. Mol Plant 3: 113-124

Pastorello EA, Farioli L, Pravettoni V, Ortolani C, Ispano M, Monza M, Baroglio C, Scibola E, Ansaloni R, Incorvaia C, Conti A (1999a) The major allergen of peach (*Prunus persica*) is a lipid transfer protein. J Allergy Clin Immunol 103: 520-526

Pastorello EA, Pravettoni V, Farioli L, Ispano M, Fortunato D, Monza M, Giuffrida MG, Rivolta F, Scibola E, Ansaloni R, Incorvaia C, Conti A, Ortolani C (1999b) Clinical role of a lipid transfer protein that acts as a new apple-specific allergen. J Allergy Clin Immunol 104: 1099-1106

Radauer C, Breiteneder H (2007) Evolutionary biology of plant food allergens. J Allergy Clin Immunol 120: 518-525

Salcedo G, Sanchez-Monge R, Diaz-Perales A, Garcia-Casado G, Barberw D (2004) Plant non-specific lipid transfer proteins as food and pollen allergens. Clin Exp Allergy 34: 1336-1341

Sankian M, Varasteh A, Pazouki N, Mahmoudi M (2005) Sequence homology: A poor predictive value for profilins cross-reactivity. Clin Mol Allergy 3:13

Schenk MF, Cordewener JHG, America AHP, van't Westende WPC, Smulders MJM, Gilissen LJWJ (2009) Characterization of PR-10 genes from eight Betula species and detection of Bet v 1 isoforms in birch pollen. BMC Plant Biology 9:24

Scheurer S, Lauer I, Foetisch K, Moncin MSM, Retzek M, Hartz C, Enrique E, Lidholm J, Cistero-Bahima A, Vieths S (2004) Strong allergenicity of Pru av 3, the lipid transfer protein from cherry, is related to high stability against thermal processing and digestion. J Allergy Clin Immun 114: 900-907

Scheurer S, Metzner K, Haustein D, Vieths S (1997) Molecular cloning, expression and characterization of Pru a 1, the major cherry allergen. Mol Immunol 34: 619-629

Son DY, Scheurer S, Hoffmann A, Haustein D, Vieths S (1999) Pollen related food allergy: cloning and immunological analysis of isoforms and mutants of Mal d 1, the major apple allergen, and Bet v 1, the major birch pollen allergen. Eur J Nutr 38:201-215

Tehrani M, Chandran S, Sharif Hossain ABM, Nasrulhaq-Boyce A (2011) Postharvest physico-chemical and mechanical changes in jambu air (*Syzygium aqueum* Alston) fruits. Aust J Crop Sci. 5(1): 32-38.

Valenta R, Duchene M, Pettenburger K, Sillaber C, Valent P, Bettelheim P, Breitenbach M, Rumpold H, Kraft D, Scheiner O (1991) Identification of profilin as a novel pollen allergen; IgE auto reactivity in sensitized individuals. Science 253:557-560

Vieths S, Scheurer S, Ballmer-Weber B (2002) Current understanding of cross-reactivity of food allergens and pollen. Ann N Y Acad Sci 964: 47-68

Wen J, Vanek-Krebitz M, Hoffmann-Sommergruber K, Scheiner O, Breiteneder H (1997) The potential of Betv1 homologues, a nuclear multigene family as phylogenetic markers in flowering plants. Mol Phylogenet Evol 8:317-333

Westphal S, Kempf W, Foetisch K, Retzek M, Vieths S, Scheurer S (2004) Tomato profilin Lye e 1: IgE cross-reactivity and allergenic potency. Allergy 59:526-32

World Health Organization (2002) The world health report 2002 - Reducing risks, promoting healthy life. Geneva: World Health Organization

Zuidmeer L, Salentijn E, Rivas MF, Mancebo EG, Asero R, Matos CI, Pelgrom KTB, Gilissen LJWJ, van Ree R (2006) The role of profilin and lipid transfer protein in strawberry allergy in the Mediterranean area. Clin Exp Allergy 36: 666-675

Zuidmeer L, van Ree R (2007) Lipid transfer protein allergy: primary food allergy or pollen/food syndrome in some cases. Curr Opin Allergy Clin Immunol 7:269-273