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Storage molecules from tree nuts, seeds and legumes: relationships and amino acid identity among homologue molecules

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Summary

The families of seed storage proteins, together with profilins, oil-bodies-associated oleosins, and pathogenesis-related (PR) proteins like PR-10 (Bet v 1-like), PR-12 (defensins) and PR-14 (non-specific lipid transfer protein), are the main causes of IgE sensitization to tree nuts, legumes and seeds. All these allergens, with the exclusion of profilins and of PR-10, are heat-stable and possibly responsible for fatal or almost fatal adverse reactions to such foods. In this short review, we will discuss the relationship and amino acid identities among some of the seed storage homologue molecules identified to date from tree nuts, seeds and legumes.

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Tree nuts, seeds and legumes storage proteins

The families of seed storage proteins, together with profilins, oil-bodies-associated oleosins, and pathogenesis-related (PR) proteins like PR-10 (Bet v 1-like), PR-12 (defensins) and PR-14 (non-specific lipid transfer protein), are the main causes of IgE sensitization to tree nuts, legumes and seeds (1). All these allergens, with the exclusion of profilins and of PR-10, are heat-stable, and possibly responsible for fatal or almost fatal adverse reaction to such foods.

In this short review, we will discuss the relationship and amino acid identities among some of the seed storage homologue molecules identified to date from tree nuts, seeds and legumes (figure 1), and choosing those registered in the WHO/International Union of Immunological Societies Allergen Nomen-

clature Subcommittee (<http://www.allergen.org>) database (2), belonging to the following biological sources:

1. tree nuts (coconut, *Cocos nucifera*; Brazilian walnut, *Bertholletia excelsa*; hazelnut, *Corylus avellana*; walnut, *Juglans regia*; black walnut, *Juglans nigra*; pecan nut, *Carya illinoensis*; pine nut, *Pinus pinea*; almond, *Prunus dulcis*; cashew, *Anacardium occidentale*; pistachio, *Pistacia vera*);
2. seeds (sunflower seed, *Helianthus annuus*; mustard, *Sinapis alba*; turnip, *Brassica rapa*; rape seed, *Brassica napus*; Indian mustard, *Brassica juncea*; buckwheat, *Fagopyrum esculentum*; Siberian wheat, *Fagopyrum tataricum*; sesame, *Sesamum indicum*; ricinus, *Ricinus communis*); and
3. legumes (peanut, *Arachis hypogaea*; lentil, *Lens culinaris*; pea, *Pisum sativum*; bean, *Phaseolus vulgaris*; lupine, *Lupinus angustifolius*; soy, *Glycine max*; mung bean, *Vigna radiata*).

Figure 1 - Phylogenetic tree showing the relationships of tree nuts, seeds and legumes sources containing the storage molecules identified as allergen to date.

Seed storage proteins

Storage proteins in plant seeds represent the reserve of amino acids and ions utilized by the plant as source of nutrients during germination and seedling growth. Up to a quarter of the dry weight of any single seed encompasses such proteins. Seed storage proteins encompass prolamins and cupins (3).

The prolamine superfamily includes cereal prolamins and α -Amylase inhibitors, 2s albumins, and nsLTPs.

2s albumins are water-soluble molecules, whose MW is approximately 10-16 kDa. 2s albumins are firstly synthesized as single-chain proteins, and then cleaved into two subunits, linked by 4 to 5 disulfide bonds to form a stable and compact α -helical molecule (4). 2s albumins serve not only as storage proteins, but can also play a defensive role against fungal attack (5).

The cupin superfamily comprises a large family of proteins named after their common conserved β -barrel structure (*cupa* from the Latin term meaning small barrel) probably originated from a prokaryotic precursor (6). The cupin superfamily includes the 7S vicilin-type globulins and the 11S legumin-type globulins, classified on the basis of their sedimentation coefficient (7). The vicilins (7s globulin) represent up to 80% of total proteins in seeds of leguminous and non-leguminous plants, include three subunits with a MW of about 40-80 kDa, and show a cumulative molecular mass of 150-190 kDa (8).

The legumins (11s globulins) are hexameric proteins comprising two associated trimers of around 40-50 kDa MW. Both 7S

globulin and 11S globulin allergens share a similar cupin structure, but have distinct IgE-binding epitopes (9).

These globulins act both as important nutrients, providing amino acids during the germination process, but are also involved in the defense process against fungi and insects (10).

Plant pathogenesis-related (PR) proteins

Plants have inducible defense systems that are stimulated upon attack of multiple pathogens such as viruses, bacteria, fungi, etc. (5). Among such defense mechanisms there are the PR proteins, which include a large number of families with components differing in incidence, appearance and biological activities (11). Some of these families are also important allergens, possibly causing IgE sensitization in predisposed individuals. In tree nuts, seeds and legumes, clinically relevant PR proteins causing IgE sensitization are represented by PR-10 (Bet v 1-Like), PR-12 (defensin) and PR-14 (ns LTP) proteins.

PR-10s are cross-reactive molecules of 16-18 kDa, belonging to the Bet v 1 family (12), sharing a common tertiary structure with seven-stranded anti-parallel β -sheets and three α -helices (13, 14). The amount of these proteins in seeds, nuts, and legumes is influenced by both a-biotic and biotic stresses (15,16). PR-10s homologs are widely distributed in the plant kingdom (17). Such thermo-labile molecules, localized homogeneously throughout peel and pulp (18,19), can provoke oral allergy syndrome after ingestion of raw fruits or vegetables (20). PR-10

proteins are found in tree nuts, seeds and legumes from *Fagales* order (Cor a 1.0401 from hazelnut and Jug r 5 from walnut), and *Fabales* order (Ara h 8 from peanut, Gly m 4 from soybean, and Vig r 1 from mung bean) (**table Ia** and **c**). The steadiness to moderate heating of Gly m 4 gives reason for the reported severe reactions to cooked soybeans (21). An heterogeneous PR-10 food allergens IgE recognition profile was recently found in an Italian Bet v 1 free area, and Ara h 8, Cor a 1.0401, and Gly m 4 IgE reactivity was significantly associated with a history of OAS occurrence (22).

PR-14 allergens, the small, highly conserved, non-specific lipid transfer proteins (nsLTP) (23), are mainly concentrated in the skin of *Rosaceae* fruits (24), and have a very high resistance to gastrointestinal proteolysis, and high temperature exposure (25). nsLTPs belong in the vast majority of cases to the nsLTP1 subfamily (9 kDa), but allergens from the nsLTP2 subfamily (7 kD), as Ara h 16 from peanut, have also been described (26). A broad degree of IgE cross-recognition between the individual nsLTP components has been observed (27), and sensitization to nsLTPs is frequently associated with systemic, even anaphylactic, reactions. Hence, nsLTPs are the most frequent sensitizer in Italian subjects with food-dependent exercise-induced anaphylaxis (28). The recognition at the same time in the same patient of PR-10, PR-14 and profilin allergens is associated with a significantly lower risk of severe adverse reaction to food (29,30). Ara h 9 from peanut represents an important cause of adverse reaction to food in southern Europe (31,32), and Jug r 3 from walnut is the molecule most frequently recognized in patients scoring negative for Pru p 3 in Italy (30). PR-14 proteins are found in tree nuts, seeds and legumes from *Fagales* order (Cor a 8 from hazelnut (33), Ara h 9 from peanut (31,34,35), Jug r 3 from walnut (36)), *Rosales* order (Pru du 3 from almond (37)), *Brassicales* order (Sin a 3 from mustard (38) and Bra r 3 from turnip (39)), *Asterales* order (Hel a 3 from sunflower seed) (40), and *Fabales* order (Ara h 9, Ara h 16 and Ara h 17 from peanut, Len c 3 from lentil (41), Pis s 3 from pea (42), Pha v 3 from bean (43), and Gly m 1 from soybean (44)) (**table I**).

Defensins are low-molecular-weight amphiphilic cationic proteins belonging to the pathogenesis-related protein family 12, and sharing common structural characteristics (45). Such molecules can be found in lipophilic extracts of peanut (Ara h 12 and 13) (46) and soybean (Gly m 2), but associated, in this case, with asthmatic symptoms occurring in workers exposed to soy (47) (**table IIc**).

Oleosins

Oleosins are proteins of around 16-24 kDa of MW, forming the structure of the plant lipid storage bodies called "oil bodies" (OB) (48), involved in severe systemic adverse reactions (49). The main characteristic of oleosins is their extreme hydropho-

bicity and, therefore, their scarce solubility once placed outside of their natural oil environment. As a consequence, such proteins are virtually lacking in defatted diagnostic commercial products for both in vitro and in vivo testing, thus resulting in a poor detection of IgE antibodies to such relevant allergens (50). Oleosins from peanut (49,51), sesame seed (52) and hazelnuts (50) have been described to date. As shown in **table IIId** a likely cross-reactivity is recorded between oleosins from different sources (53), thus representing a possible cause of reactivity towards not botanically related sources. Nevertheless, the clinical relevance and IgE cross-reactivity of these allergens are not fully defined, therefore requiring more studies.

Relationships and identity degree between homologue proteins

In **table I**, the list of the allergenic molecules currently registered in WHO/IUIS database and expressed in tree nuts (**a**), seeds (**b**) and legumes (**c**) is shown. With regard to legumes, all the allergens belong to the same plant order: the *Fabales*. Observing the tables as a whole, there are molecules from some biological sources missing, but this observation, more than a real lack in the respective source, is probably due to the fact that many of these allergens have not been yet discovered.

In **table II** the amino acid identities expressed as percentages among 2s albumins (**a**), vicilins (**b**), legumins (**c**) and oleosins (**d**) are shown.

The primary sequence correspondence among different molecules from nuts, seeds and legumes can be described as amino acid identity percentage. The sequence identity found comparing the different allergen families expressed by nuts, seeds and legumes is mainly associated with their botanical relationship, as in the case of cashew and pistachio, both belonging to *Sapindales* order or the tree nuts walnut, hazelnut and pecan, all from *Fagales* order (9). Accordingly, comparing the sequences of the 2s albumins (Ana o 3 vs Pis v 1), 7s globulins (Ana o 1 vs Pis v 3) and 11s globulins (Ana o 2 vs Pis v 5) from cashew and pistachio, high degree of amino acid identities can be observed (**table IIa-c**). Similarly, in the case of *Fagales* order, the 2s albumins from hazelnut (Cor a 14), walnut (Jug r 1), black walnut (Jug n 1) and pecan nut (Car i 1) ranged between 62 and 86% of amino acid identities, the 11s globulins (Cor a 9, Jug r 4, Jug n 4 and Car i 4) had from 71 to 94% of identities, and the vicilins from walnut (Jug r 2), black walnut (Jug n 2) and pecan (Car i 2), but not the 7s globulin from hazelnut (Cor a 11), showed from 58 to 78% of identities.

Comparable observation can be achieved also comparing the 2s albumins from *Brassicales* (Sin a 1 from mustard, Bra r 1 from turnip, Bra n 1 from rapeseed, and Bra j 1 from Indian mustard) (**table IIa**). Interestingly, very high levels of sequence identity (90.1%) can be observed comparing the vicilin from pea (Pis s 1)

Table I - a, tree nuts allergens (WHO/IUIS allergen nomenclature); **b**, seed allergens (WHO/IUIS allergen nomenclature); **c**, legume allergens (WHO/IUIS allergen nomenclature).

a	Storage proteins			PR-proteins		Oleosin	Profilin	Order
	<i>2s albumins</i>	<i>Vicillins</i>	<i>Legumins</i>	<i>PR10</i>	<i>nsLTP (PR14)</i>			
Coconut <i>Cocos nucifera</i>		Coc n 1						Arecales
Brazilian walnut <i>Bertholletia excelsa</i>	Ber e 1		Ber e 2					Ericales
Hazelnut <i>Corylus avellana</i>	Cor a 14	Cor a 11	Cor a 9	Cor a 1	Cor a 8	Cor a 12 Cor a 13	Cor a 2	Fagales
Walnut <i>Juglans regia</i>	Jug r 1	Jug r 2 Jug r 6	Jug r 4	Jug r 5	Jug r 3			
Black Walnut <i>Juglans nigra</i>	Jug n 1	Jug n 2	Jug n 4					
Pecan nut <i>Carya illinoensis</i>	Car i 1	Car i 2	Car i 4					
Pine nut <i>Pinus pinea</i>	Pin p 1							Pinales
Almond <i>Prunus dulcis</i>			Pru du 6		Pru du 3		Pru du 4	Rosales
Cashew <i>Anacardium occidentale</i>	Ana o 3	Ana o 1	Ana o 2					Sapindales
Pistachio <i>Pistacia vera</i>	Pis v 1	Pis v 3	Pis v 2 Pis v 5					

b	Storage proteins			PR-proteins		Oleosin	Profilin	Order
	<i>2s albumins</i>	<i>Vicillins</i>	<i>Legumins</i>	<i>PR10</i>	<i>nsLTP (PR14)</i>			
Sunflower seed <i>Helianthus annuus</i>					Hel a 3		Hel a 2	Asterales
Mustard <i>Sinapis alba</i>	Sin a 1		Sin a 2		Sin a 3		Sin a 4	Brassicales
Turnip <i>Brassica rapa</i>	Bra r 1				Bra r 3		Bra r 8	
Rapeseed <i>Brassica napus</i>	Bra n 1						Bra n 8	
Indian mustard <i>Brassica juncea</i>	Bra j 1						Bra j 8	
Buckwheat <i>Fagopyrum esculentum</i>	Fag e 2	Fag e 3						Caryo- phyllales
Siberian Wheat <i>Fagopyrum tataricum</i>	Fag t 2							
Sesame <i>Sesamum indicum</i>	Ses i 1 Ses i 2	Ses i 3	Ses i 6 Ses i 7			Ses i 4 Ses i 5		Lamiales
Ricinus <i>Ricinus communis</i>	Ric c 1							Malpighiales

c	Storage proteins			PR-proteins			Oleosin	Profilin	Order
	<i>2s albumins</i>	<i>Vicillins</i>	<i>Legumins</i>	<i>Defensins (PR12)</i>	<i>PR10</i>	<i>nsLTP (PR14)</i>			
Peanut <i>Arachis hypogaea</i>	Ara h 2 Ara h 6 Ara h 7	Ara h 1	Ara h 3	Ara h 12 Ara h 13	Ara h 8	Ara h 9 Ara h 16 Ara h 17	Ara h 10 Ara h 11 Ara h 14 Ara h 15	Ara h 5	Fabales
Lentil <i>Lens culinaris</i>		Len c 1				Len c 3			
Pea <i>Pisum sativum</i>		Pis s 1 Pis s 2				Pis s 3			
Bean <i>Phaseolus vulgaris</i>						Pha v 3			
Lupine <i>Lupinus angustifolius</i>		Lup an 1						Lup a 5	
Soy <i>Glycine max</i>	Gly m 8	Gly m 5	Gly m 6	Gly m 2	Gly m 4	Gly m 1		Gly m 3	
Mung bean <i>Vigna radiata</i>	Vig r 4	Vig r 2			Vig r 1				

Bold font indicates molecules currently available in the market.

a

Ara h 2	Ara h 6	Ara h 7	Ber e 1	Bra j 1	Bra n 1	Bra r 1	Car i 1	Cor a 14	Fag e 2	Fag t 2	Gly m 8	Jug n 1	Jug r 1	Pis v 1	Ric c 1	Ses i 1	Ses i 2	Sin a 1	
20,9	24,5	22,1	27,2	20,7	20,7	21,9	34	37,6	21,6	22,4	14,6	30,2	31,3	64,4	16,2	32,9	24	16,7	Ana o 3
	50,6	32,8	22,5	16,7	14,8	18,5	19,3	24,6	20,6	21,2	31,5	22,1	22	21,9	14,8	23,9	20,4	15,7	Ara h 2
		34,5	24,8	13,8	14,1	20,5	22,9	31,2	23,7	24,3	30,5	21,8	22,3	24,3	14,9	25,8	23,1	13,9	Ara h 6
			19,3	13,6	13,2	16	19	21,8	18,4	16,2	23,5	21,1	20,5	18,5	12,3	20,7	17,7	11,6	Ara h 7
				16,2	17,4	17,9	36,2	40,5	19,1	20,7	16,6	33,5	37,5	28	14,9	30,8	35,1	14,8	Ber e 1
					88,4	60,6	20,8	19,1	12,8	14,8	11,3	18,2	20,7	21,7	10,9	17,7	15,2	79,3	Bra j 1
						61,6	20,8	18,5	13	14,3	12,1	19,3	20,7	20,8	12,2	17,4	15,5	80,7	Bra n 1
							21,9	19,8	17,1	18,2	14,8	19,8	20,2	25,8	15,8	24,1	20,6	67,7	Bra r 1
								59,7	22,3	22,3	16,6	77,6	86	30,9	17,7	35,6	32,4	19,1	Car i 1
									21,5	20,7	16,2	58,5	61,7	35,8	22,7	39,8	32,7	14,8	Cor a 14
										82,5	21	20,5	22,6	20,7	15,6	21,6	20	12	Fag e 2
											21,4	20,4	22,6	21,3	14,9	20,4	21,9	13,8	Fag t 2
												12,7	15,8	19,5	12,6	16,1	17,7	11,9	Gly m 8
													83,2	29,4	18,1	32,1	28	16,8	Jug n 1
														30,9	18,1	34,4	29	18,4	Jug r 1
															16,6	29,9	23,5	20	Pis v 1
																22,9	14,8	10,1	Ric c 1
																	35,7	17,3	Ses i 1
																		14,1	Ses i 2

b

Table II - Amino acid identities among 2s **a**, 2s albumin; **b**, vicilins (7s Globulin); **c**, legumins (11s Globulin); and **d**, oleosin, expressed as percentages. Bold font indicates values above 50% of sequence identity.

Ara h 1	Car i 2	Cor a 11	Fag e 3	Gly m 5	Jug n 2	Jug r 2	Len c 1	Lup an 1	Pin k 2	Pis s 1	Pis s 2	Pis v 3	Ses i 3	
23,3	24,5	44,8	5,5	23,7	29,8	31,3	22,5	25,6	28,5	23,3	28,9	76,3	42,6	Ana o 1
	24,8	25,3	4,7	38,4	28,3	30	35,8	41,4	21,6	34,5	39,1	24,4	26,6	Ara h 1
		26,6	5,4	22,5	56,1	69,3	19,8	28,5	20	20	25,2	25,9	32,2	Car i 2
			3	25,3	43,4	35,9	30,9	26,2	32,9	31,9	29,1	48,6	41	Cor a 11
				6	5	7	8	4,8	5	7	5	4	5,6	Fag e 3
					29,6	29,2	40,1	43,3	22	40,8	42,9	26,2	26,4	Gly m 5
						78,2	31,9	31,1	33,8	32,6	30,8	33,4	34,4	Jug n 2
							25,7	33,5	28	26,5	31,6	34,8	39	Jug r 2
								38,4	26,4	90,1	48,7	24,8	21,1	Len c 1
									23,7	38,7	44,8	25,5	29,1	Lup an 1
										26,5	23,9	29,5	28,3	Pin k 2
											47,2	25,5	22	Pis s 1
												27,8	27,8	Pis s 2
													44,3	Pis v 3

c

Ara h 3	Ber e 2	Cor a 9	Gly m 6	Jug r 4	Pis v 2	Pis v 5	Pru du 6	Ses i 6	Ses i 7	Sin a 2	
38,6	46,4	49,7	45,2	54	44,8	75,5	40,9	37,5	44,8	41,2	Ana o 2
	33,2	41,4	53,8	41,2	34,3	38,9	37,1	29,8	31,2	27,7	Ara h 3
		47,4	37,5	47,6	44,3	46,3	39,8	38,7	44,7	36,2	Ber e 2
			42,3	71,6	46,7	53,2	48,9	36,5	45,4	40,3	Cor a 9
				44,5	38,2	44,8	37,4	29,9	36,1	32,6	Gly m 6
					46,1	55	48,3	39,2	46,3	38,9	Jug r 4
						48,3	39,2	35,2	45,4	36,8	Pis v 2
							43,5	37,5	44,6	41,9	Pis v 5
								34,5	35,9	40,8	Pru du 6
									35,4	31,5	Ses i 6
										36,4	Ses i 7

d

Ara h 11	Ara h 14	Ara h 15	Cor a 12	Cor a 13	Ses i 4	Ses i 5	
30,1	52,4	29	54,4	28,9	44,3	30,1	Ara h 10
	27,8	40,9	32,7	68	29,7	61,9	Ara h 11
		29,4	44,8	27,2	45,2	30,1	Ara h 14
			29,3	42,1	29,1	42,7	Ara h 15
				32,1	49,7	33,1	Cor a 12
					28,5	73,8	Cor a 13
						29,2	Ses i 4

and lentil (Len c 1), indicating that such molecules can be considered virtually identical (**table IIb**). Likewise, also the 2s albumins from buckwheat (Fag e 2) and Siberian wheat (Fag g 2) show a high degree of sequence identity (82.4%) (**table IIa**).

High primary sequence identity levels can be associated with an IgE co-recognition, but this cannot be considered as a definitive proof of cross-reactivity. Clearly, the higher the identity between two molecules, the higher is the possibility that a cross-reactivity

Table III - Amino acid identities among **a**, PR-14 (ns-LTP), and **b**, PR-10 (Bet v 1-Like) molecules expressed as percentages. Bold font indicates values above 50% of sequence identity.

a							
Cor a 8	Gly m 1	Jug r 3	Len c 3	Pha v 3	Pru du 3	Sin a 3	
50	9	50	58	59	48	49	Ara h 9
	7	57	51	53	54	41	Cor a 8
		7	9	6	8	7	Gly m 1
			53	45	49	40	Jug r 3
				50	48	44	Len c 3
					45	41	Pha v 3
						39	Pru du 3

B					
Cor a 1	Gly m 4	Jug r 5	Vig r 1		
42	70	84	66		Ara h 8
	46	65	39		Cor a 1
		54	75		Gly m 4
			50		Jug r 5

may occur. Nevertheless, also in the case of a low sequence identity, the IgE recognition of less variable (i.e. more conserved) shared epitopes can result in a cross reactivity also among molecules with a very low overall sequence identity but a similar tertiary structure, as in the case of PR-10 or nsLTP proteins (22,30). Moreover, an IgE cross-reactivity among Ara h 1, Ara h 2 and Ara h 3 peanut allergens due to the occurrence of similar surface-exposed sequences has been found in peanut-allergic individuals, indicating that cross reactivity can also be found among un-related molecules belonging to the same biological source (54).

In the case of oleosins, as already mentioned and shown in **table IId**, some cross-reactivities are observed among OB proteins from not botanically related plants, due to the homology of peanut (Ara h 11) and hazelnut (Cor a 13) oleosins, or the sesame (Ses i 5) and the hazelnut (Cor a 13) oleosins (51).

In **table III** the amino acid identity among PR-10 and PR-14 molecules from nuts seeds and legumes is shown, suggestive of the existence of cross-reactivity among not botanically related proteins.

Conclusion

Adverse reaction to seed storage proteins is frequently associated with severe, even anaphylactic reactions. The main diagnostic challenge when facing patients sensitized to such families of proteins is exactly how to manage the possible exposure to foods, possibly containing similar molecules. In the clinical practice, in case of proven anaphylactic reactions due to such molecules, we commonly suggest to carefully avoid the foods that cause the reaction and to carry an auto-injector containing adrenaline. On the other hand, it is not clear how to advise people who are allergic to a given group of allergens, when they approach food containing possibly related molecules. A better knowledge of the phylogenetic and molecular relationship among the distinct biological sources could help in a better and reliable management of patients sensitized to such dangerous food.

Conflict of interest

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