

Allergy to Peanut, Soybean, and other Legumes: Recent Advances in Allergen Characterization, Stability to Processing and IgE Cross-Reactivity

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Abbreviations: **AGEs**, advanced glycation ends; **Ara h**, *Arachis hypogaea*; **Bet v**, *Betula verrucosa*; **DBPCFC**, double-blind placebo-controlled food challenge; **EC**, European Commission; **Gly m**, *Glycine max*; **IUIS**, International Union of Immunological Societies; **LDL**, low-density lipoprotein; **Len c**, *Lens culinaris*; **Lup a**, *Lupinus albus*; **Lup an**, *Lupinus angustifolius*; **ns-LTPs**, non-specific lipid transfer proteins; **OIT**, oral immunotherapy; **Pha v**, *Phaseolus vulgaris* **Pis s**, *Pisum sativum*; **PR**, pathogenesis-related; **SBP**, seed biotinylated protein; **slgE**, specific IgE; **WHO**, World Health Organisation

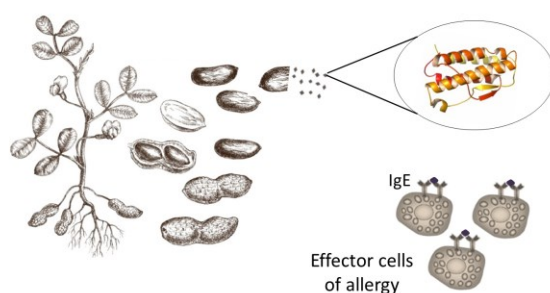
Abstract

Peanut and soybean are members of the *Leguminosae* family. They are two of the eight foods that account for the most significant food allergies in the US and Europe. Allergic reactions to other legume species can be of importance in other regions of the world. The major allergens from peanut and soybean have been extensively analyzed and members of new protein families identified as potential marker allergens for symptom severity. Important recent advances concerning their molecular properties or clinical relevance have been made. Therefore, there is increasing interest in the characterization of allergens from other legume species such as lupine, lentil, chickpea, green bean or pea. As legumes are mainly consumed after thermal processing, knowledge about the effect of such processing on the allergenicity of legumes has increased during the last years. In the present review, we summarize and discuss recent advances in the identification of allergens from peanut, soybean, lupine and other legume species. We provide an overview of the most recently described effects of thermal processing on the allergenic properties of legumes and discuss the potential IgE cross-reactivity among members of the *Leguminosae* family.

We summarize and discuss recent advances in the identification of allergens from peanut, soybean, and other legumes.

We provide an overview of the effects of thermal processing on the allergenic properties of legumes.

We discuss the potential IgE cross-reactivity among members of the *Leguminosae* family.



1 Introduction

Legumes represent 27% of the primary crop production worldwide [1]. Legumes are excellent sources of proteins, water-soluble fibers and numerous micronutrients and phytochemicals. Moreover, they contain low levels of unsaturated fats. Legume consumption is a proven healthy dietary habit. Several studies have suggested links between legume intake and prevention of coronary heart disease and between legume intake and a decrease in total and low-density lipoprotein (LDL) cholesterol [2-6]. However, legumes can also elicit dangerous allergic reactions. Peanut and soybean are two of the eight foods that account for the most significant food allergies in the US and Europe [7, 8]. Furthermore, other legumes, such as lupine, have been increasingly used in Europe in flour form, as an additive for wheat flour [9]. The potential of lupine allergens to elicit allergic reactions has been documented since the early nineties, initially reported as a result of cross-reactivity with peanut allergens but later also described as primary sensitization [10-13]. Allergy to other legume species, such as lentil, green bean, pea and chickpea, can also be important in Mediterranean countries where these foods are a source of fiber and valuable protein [14, 15]. In this review, we present an updated overview of allergy to legumes, with a specific focus on the most recent advances in the characterization of legume allergens, IgE cross-reactivity and stability to thermal processing.

2 Allergen characterization: recent advances

Peanut is the best characterized legume in regard to its allergenic content, with 16 allergens registered by the World Health Organisation/International Union of Immunological Societies

(WHO/IUIS) Allergen Nomenclature Subcommittee (Table 1). In the case of soybean, the number of characterized allergens is relatively limited, with 8 allergens officially registered (Table 1). There is also an increasing interest in the characterization of allergens from other legume species such as lupine. Lentil, chickpea, pea, or green bean have been described to be allergic elicitors mainly in Mediterranean countries and India, and several of their allergens have been characterized (Table 2).

Legume allergens can be grouped into a few protein families and superfamilies. Cupin superfamily proteins share a conserved β -barrel structural core domain, which can be single or double. The seed storage proteins 7S and 11S globulins belong to the cupin superfamily. The prolamin superfamily includes proteins with conserved cysteines at specific positions, sharing a common tridimensional structure with four α -helices stabilized by disulfide bonds. The seed storage proteins 2S albumins and non-specific lipid transfer proteins (ns-LTPs) are included in this superfamily. The profilins and Bet v 1-like superfamilies group food allergens associated with pollen allergy. Defensins, involved in plant defense against pathogens, and oleosins, structural proteins of intracellular lipid storage organelles, are also families to which legume allergens belong [16].

2.1 Prolamin superfamily

Peanut and soybean allergens from the prolamin superfamily

Six peanut allergens belong to the prolamin superfamily, among which Ara h 2, Ara h 6, and Ara h 7 are 2S albumins and Ara h 9, Ara h 16, and Ara h 17 are ns-LTPs. The importance of specific IgE (sIgE) against Ara h 2 as a predictor of clinical reactivity to peanut has been extensively demonstrated [17-19]. Post-translational modifications on Ara h 2 have been described to be critical for its allergenic potential [20]. Double-blind, placebo-controlled food challenge (DBPCFC) is currently the gold

standard for food allergy diagnosis, but it is costly and time consuming. Studies on the predictive value of sIgE against individual peanut allergens are important because they might improve the diagnostic accuracy of peanut allergies and reduce the number of DBPCFC required. In that sense, testing sIgE against Ara h 2 in specific allergic populations may reduce the number of oral challenges required for peanut allergy diagnosis in the future [17, 19]. In soybean, the 2S albumin nGly m 8 has also been demonstrated to be an indicator for severe reactions to this legume in both pediatric and adult soybean-allergic populations [21, 22]. The well-conserved tridimensional structure of 2S albumins and their high resistance to heat and digestive enzymes might explain the high association of the allergens belonging to this group with severe anaphylactic reactions.

Lentil, pea, and green bean allergens from the prolamin superfamily

In lentil, an allergen belonging to the prolamin superfamily has been described and named as Len c 3, which is a ns-LTP [23]. In pea, Pis s 3 was isolated and characterized as a ns-LTP [24]. In the case of green bean, several IgE-binding proteins have been detected and partially characterized [25, 26], but only a ns-LTP from this legume has been described as a major allergen in a Spanish green bean-allergic study population. The allergen has been completely characterized and named as Pha v 3 [27]. A ns-LTP from green bean was also found to be recognized by the serum IgE of 5 Italian green bean-allergic patients [28].

2.2 Cupin superfamily

Peanut and soybean allergens from the cupin superfamily

Two allergens from peanut belong to the cupin superfamily, Ara h 1 (7S globulin) and Ara h 3 (11S globulin), which are important major peanut allergens in specific allergic populations. Recently, it has

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been demonstrated that nAra h 1 is able to interact with lipid components. That interaction could contribute to nAra h 1 allergenic potency by conferring resistance to digestive enzymes and interacting with dendritic cells in a way that can favor the generation of Th2 responses [29]. At the IgE epitope level, it has been found that patients with more severe allergic reactions to peanut recognized a higher diversity of IgE epitopes of peanut allergens such as Ara h 1 or Ara h 3 [30]. In soybean, Gly m 5 (7S globulin) and Gly m 6 (11S globulin) are two soybean allergens that belong to the cupin superfamily. In a European study population of adults and children allergic to soybean, 43% had sIgE to nGly m 5 and 36% to rGly m 6 [31]. However, when the patients were classified according to their symptoms, 86% of the subjects with anaphylaxis were sensitized to nGly m 5 and rGly m 6, and only 33% of the subjects with mild subjective symptoms had sIgE to these allergens [31]. nGly m 5 and nGly m 6 were also associated with severe allergic reactions to soybean in children from Japan [32], and experiments carried out in mouse models have noted the severe allergenic potential of both allergens [33]. However, some reports have demonstrated that the sensitization to nGly m 5 and nGly m 6 can be an indicator of severe allergic reactions to soybean in pediatric soybean-allergic populations [32]. In studies where adult patients allergic to soybean were considered, there was no association of nGly m 5 and nGly m 6 sensitization with severe reactions to the legume [34, 35].

Lupine, lentil, pea, and chickpea allergens from the cupin superfamily

Only two lupine allergens have been officially registered so far, one of them belonging to the cupin superfamily. The allergen α -conglutin (7S globulin) was characterized in 2008 as a major allergen in *Lupinus angustifolius*, the most important lupine species grown in Australia. The allergen was named as Lup an 1 [36]. In lentil, of the three allergens registered so far, one belongs to the cupin superfamily, which is the 7S globulin Len c 1. The number and intensity of Len c 1 epitopes, located

in the C-terminal region, positively correlated with the IgE levels against lentil and the clinical characteristics of the lentil-allergic patients [37]. In pea, Sanchez-Monge et al. showed, using sera from 18 patients with pea allergy, that mature pea vicilin (47 kDa) and its proteolytic fragment of 32 kDa were major allergens (named as Pis s 1). A second major allergen, a convicilin with a molecular weight of 63 kDa, was characterized in this study and named as Pis s 2 [38]. In the case of chickpea, there are no allergens officially registered so far, although some reports have characterized a few IgE-binding proteins from this legume. A vicilin (7S globulin) and the basic subunit of legumin (11S globulin) from chickpea have been recognized as major chickpea allergens since they were recognized by IgE in the sera from 16 pediatric patients with chickpea allergy [39].

2.3 Profilin and Bet v 1-like superfamilies

Peanut, soybean, and lupine allergens from the profilin and Bet v 1-like superfamilies

In peanut, Ara h 5 is a profilin and a pan-allergen. Ara h 8 is a homologue to the major birch pollen allergen Bet v 1 and belongs to the Bet v 1-like superfamily. Both allergens share the structural particularity of a so-called “hydrophobic pocket”, which allows them to associate with lipids [40]. In soybean, Gly m 3 belongs to the profilin superfamily and Gly m 4 to the Bet v 1-like superfamily. In a European study population of adults and children allergic to soybean, 92% of the patients with mild symptoms had sIgE to the birch pollen-related Gly m 4 [31]. Sensitization to Gly m 4, however, has been associated with severe allergic symptoms to soybean, as shown in study populations from Europe and Asia [41, 42]. A recent study has investigated the effect of allergen immunotherapy (AIT) on birch pollen-related soy allergy using a hypoallergenic variant of rBet v 1 (rBet v 1-FV). It is known that Gly m 4 and Bet v 1 share important sequence and structure similarities. The study investigated changes in soybean allergy symptoms, induced by AIT with rBet v 1-FV, by DBPCFC using challenge

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meals standardized by Gly m 4 content [43]. Clinical assessment revealed a tendency in favor of the active group, but it did not reach statistical significance, which is most likely because only 56% of the desired level of patients could be recruited [44]. In the case of lupine, in 2017, the lupine profilin Lup a 5 was registered, which is highly cross-reactive to other profilins (e.g., Ara h 5) and which is recognized by the sera of both lupine- and peanut-allergic patients (Jappe and co-workers; www.allergome.org).

2.4 Defensins

Peanut defensins allergens

Defensins are known to be important for plant defense against pathogens. In 2012, two new peanut allergens were identified during the process of Ara h 8 purification for studies related to lipid association and heat/digestive resistance [40, 45] and were accepted by the WHO/IUIS Allergen Nomenclature Subcommittee. The novel low-molecular-weight peanut allergens were categorized as defensins and named as Ara h 12 and Ara h 13. The allergens reacted particularly with the IgE of patients with severe peanut allergy. Ara h 12 and Ara h 13 showed a dose-dependent anti-fungal activity; however, the allergens did not show anti-bacterial activity [45].

2.5 Oleosins

Peanut and soybean oleosins allergens

During the identification of peanut allergens associated with lipids, Schwager et al. developed a novel strategy for the isolation and purification of peanut oil proteins, present only in the lipid

fraction. For the first time, the eight known peanut oleosins were purified simultaneously, and their IgE-binding capacity was demonstrated [46]. Thus, oleosins have been officially accepted as new allergens by the WHO/IUIS Allergen Nomenclature Subcommittee and were denominated as Ara h 14 and Ara h 15 [46] in addition to the already documented Ara h 10 and Ara h 11 (www.allergome.org). Only recently did the same research group demonstrate that IgE to peanut oleosins is associated with severe allergic reactions [47]. Oleosins have also been identified in soybean, but their allergenic potential has not been demonstrated so far [48, 49].

3 Patterns of allergic sensitization

Legume allergy differs clinically and immunologically in different areas of the world probably due to the differences in pollen exposure and different culinary traditions. One of the best characterized legumes in this matter is peanut. rAra h 8 and rAra h 9 have been demonstrated to be major allergens in central/western and southern Europe, respectively [50], whereas peanut-allergic patients from New York included in a study were sensitized to rAra h 1, rAra h 2, and rAra h 3 in high proportion (56.7% to 90%) and had severe symptoms. Swedish peanut-allergic patients, however, were sensitized to these allergens in a lower proportion than North American patients but in a higher proportion than Spanish patients, who showed high sensitization to rAra h 9. Spanish patients typically developed peanut allergy after becoming allergic to other foods derived from plants, such as peach [51]. This observation has been made in both pediatric and adults patients allergic to peanut from Spain [51, 52]. In fact, it has been found in study populations from Spain that being allergic to peach might be a risk factor for becoming allergic to peanut [52]. The highest sensitization rate to the Bet v 1 homologue rAra h 8, as well as to rBet v 1, was found in Swedish patients, which reflects the high birch pollen exposure in that country [51]. The pattern of sensitization to peanut

allergens has been shown to be not only dependent on the geographical area but also age dependent. Several studies have provided evidence that sensitization to major storage proteins from peanut is higher in children compared to adults. A recent Spanish study showed that sIgE against rAra h 2 decreased with increasing age, while the contrary was found for rAra h 9 [53]. In line with this observation, a European multicenter study showed that sensitization to rAra h 1, rAra h 2 and rAra h 3 was frequently acquired in childhood and rarely in adolescence and adulthood. Sensitization to rAra h 8 and rAra h 9, however, seems to be linked to late-onset peanut allergy [50]. These results have been confirmed in a Norwegian study population of peanut-sensitized patients [54].

4 Effects of processing on the allergenicity of peanut, soybean and other legumes

Legumes are subjected to different thermal treatments according to consumption traditions in different areas of the world. Thermal processing may also be applied to improve legume suitability for specific applications. Some legumes are subjected to boiling or pressure cooker boiling before consumption [55], whereas others are subjected to thermal treatments such as roasting and frying. Thermal processing has been shown to have the capacity to modify the allergenicity of legumes, but their effect may depend on the different conditions of the treatments applied or the specific allergenic features of the legume species, among other properties.

4.1 Effects of boiling

Boiling is widely used to process certain legumes, such as lentils, chickpeas, or green beans, before consumption. Peanuts are consumed boiled in certain countries, such as China, and in some southern U.S. states such as Florida, Georgia, and Alabama, while this kind of treatment is quite

uncommon for peanut processing in other areas of the world [56]. However, boiling has been extensively studied in peanut due to the increased evidence that this treatment can alter peanut allergenicity [56-58]. In this respect, several reports have demonstrated that boiling decreases the IgE-binding capacity of peanut. The decrease was found to be caused by a loss of low-molecular-weight allergens, such as Ara h 2, Ara h 6 or Ara h 7, from the peanuts during boiling, with a transfer of these allergens to the boiling water [57, 58]. Changes in protein structure or aggregation have also been speculated to contribute to the observed effect [59]. Recently, using a murine model, it has been found that boiling decreased the allergenicity of Ara h 2 due to structural modification that can affect IgE-binding epitopes [60]. Such structural modifications in Ara h 2 after boiling were confirmed by other authors [61]. Additionally, allergens such as Ara h 1 have also been shown to have lower IgE-binding capacity after boiling [62]. In studies of skin prick tests, boiling decreased positive reactions to peanut compared to unprocessed peanut [63]. The reduced allergenic content of boiled peanut might be an advantage for the reduction of adverse events that oral immunotherapy can produce. In that respect, a recent study has shown that extended boiling (up to 12 hours) led to a 19-fold reduction of the IgE-binding capacity of peanut, whereas increasing fragmented peptides are retained in the boiled peanut and have the ability to activate antigen-specific T cells. Therefore, boiled peanut might be an interesting candidate for oral immunotherapy in patients with determined peanut sensitization due to the reduced IgE reactivity of some of their allergens and the retention of the capacity for T cell stimulation, which is essential for the success of oral immunotherapy (OIT) [64].

Interestingly, boiling can also affect the allergenicity of other members of the *Leguminosae* family. Lentils, chickpeas, green beans, or peas are consumed boiled; therefore, the assessment of their allergenicity should be performed with boiled legumes. Some studies have investigated the effect of different boiling times on the allergenicity of these legumes. Cuadrado et al. subjected lentils and

chickpeas to boiling for 15, 30, or 60 minutes and analyzed the IgE-binding protein profiles from the two legumes using sera from patients with specific IgE to lentil and chickpea. Little alteration of the IgE-binding protein profiles in the 15 minute-boiled samples compared to raw samples was observed. Sixty minutes of boiling altered the intensity of certain IgE-reactive proteins to some extent. However, numerous heat-stable IgE-binding proteins were still found using individual sera after such treatments, highlighting the thermal resistance of lentil and chickpea allergens to boiling [65]. The stability of lentil and chickpea allergens to this treatment was also confirmed in other studies [66]. Green bean allergens also showed high stability to boiling or even increased allergenicity compared to raw beans. The ns-LTP of green bean, Pha v 3, has been found to be stable to boiling processing [28]. Kasera et al. demonstrated that a purified allergen of 31 kDa from green bean was stable to boiling for 60 minutes [25]. Similar heat stability to boiling has also been shown for pea allergens in several studies [38, 24].

4.2 Effects of roasting and frying

The effects of roasting and frying have been mainly studied in peanut, as these are common treatments applied to peanut for human consumption. It has been largely demonstrated that roasting has the capacity to increase the IgE-binding properties of peanut allergens [67, 56, 57]. The experiments showed a 90-fold increase of the IgE-binding capacity of roasted peanut compared to raw peanut. Purified Ara h 1 and Ara h 2 allergens subjected to the Maillard reaction, a chemical reaction during roasting that causes chemical modifications of amino groups of the protein by reducing sugars, bound increased levels of IgE. The allergens were also more resistant to gastrointestinal enzymes and heat than the untreated allergens [67]. Roasting increases the trypsin inhibitor function of Ara h 2, which in turn increases the protection against digestive enzymes and

thereby enhances allergenic properties of roasted peanut [68]. In a more recent study, it was found that Ara h 1 is not denatured by roasting peanuts; however, roasting induced chemical modifications to the protein surface, which might be responsible for the enhanced IgE-binding properties of roasted Ara h 1 [69]. Ara h 8 was also found to be affected by roasting, increasing its IgE-binding properties and stability to heat and enzymatic digestion. During roasting, lipid oxidation might occur as another effect. Ara h 8 can bind lipids, and such an interaction seems to be a key factor for a diminished gastric and pancreatic digestibility [40]. Furthermore, it has been found that progressive roasting decreased protein solubility. The insoluble proteins would survive initial phases of digestion in the stomach but would be progressively resolubilized by a combination of acidic pH and enzymatic activity of amylase and pepsin. This would provide a source of peanut allergens for the gastrointestinal mucosal immune system [70]. Roasting and frying led to the formation of high molecular weight protein complexes, which supported the induction of specific IgE to native Ara h 6 in a murine model [71]. Most recently, it was demonstrated that, in contrast to oleosins isolated from roasted peanuts, IgE bound to a considerably lesser extent to oleosins obtained from raw peanuts or was almost undetectable in the serum of individuals who suffer from severe allergic symptoms [47].

Recent studies have highlighted the necessity not only to evaluate the IgE-binding capacity of allergens using assays, such as western blotting or ELISA, in processed food but also to study the IgE cross-linking and degranulation capacity of these allergens on effector cells of allergies. These experiments would add further information to the effects of roasting on the immunological activity of peanut allergens. In that respect, it has been found that heated Ara h 1 in the presence or absence of glucose had an increased degranulating capacity, which is an effect that was not observed with Ara h 2 [72]. Variable responses of basophils challenged with roasted peanut were observed and were dependent on the individual peanut-allergic patient tested [73]. These studies

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underlined the necessity to confirm the effects of IgE binding with the help of more physiologically relevant assays, such as basophil activation tests (BATs), since the former cannot predict the ability of allergens to trigger activation and degranulation of effector cells. Furthermore, recent experiments in animal models have demonstrated that roasted peanut enhanced allergenic sensitization across mucosal and cutaneous routes compared to raw peanut. Advanced glycation end products (AGEs), which are produced during the Maillard reaction, were supposed to be responsible for this effect via an increased presentation through AGE receptors on antigen presenting cells [74]. Therefore, roasting (or the glycosylations and lipid oxidations occurring during the process) is able not only to increase the IgE-binding and IgE cross-linking capacity of some peanut allergens but also to influence the induction of Th2 skewing in the initial phases of allergenic sensitization [75, 76]. This process may be facilitated by alterations of the skin barrier function in patients at risk such as patients with atopic dermatitis [77].

The effects of frying on peanut have been less intensively analyzed. Initial reports suggested that frying was able to decrease the IgE-binding capacity of peanut [56]. However, other studies have not confirmed a decrease in allergenicity after frying both *in vitro* and *in vivo* [78, 63, 79].

The different effects of boiling, roasting, and frying on peanut allergenicity can be explained in part by the differences in treatment conditions. Roasting can reach temperatures of 170 °C, while frying and boiling reach temperatures of 140 °C and 100 °C, respectively. Moreover, roasting occurs under dry conditions, while boiling and frying occur under liquid conditions [59]. These different conditions can contribute to the chemical modifications described for each treatment with a direct impact on the allergenicity of peanut.

5 IgE cross-reactivity of peanut, soybean, lupine and other legumes

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Although extensive serologic IgE cross-reactivity among legume species has been found using *in vitro* methods, the rate of clinically relevant cross-reactivity to multiple legumes has been reported to be low [80]. For example, clinically relevant cross-reactivity to at least one legume was observed in 7.9% of 317 children with peanut allergy. Interestingly, atopic dermatitis was the leading risk factor for clinically relevant cross-reactivity in peanut-allergic children [81]. IgE cross-reactivity between certain legume species seems to be of higher clinical relevance than others. For instance, several studies demonstrated a clinical association between peanut and lupine allergies. The studies differed regarding their design, diagnostic criteria or study populations analyzed, but they all reported a tendency for a clinically relevant IgE cross-reactivity between both legumes. A sensitization rate to lupine of 44% was found in a series of peanut-allergic patients in France. However, clinically relevant allergy to lupine was found only in 28% of the patients [10]. In a peanut-allergic study population in the UK, a small but significant number of children with clinically relevant allergy to lupine was shown (4%); however, the sensitization rate to lupine was higher (34%) [82]. Two of 12 (17%) peanut-allergic children in an Italian study had clinical symptoms to lupine-enriched pasta in a DBPCFC [83]. Cross-reactivity has been reported to be mediated by vicilins (7S globulins), 43 kDa [10]. Peeters et al. showed IgE reactivity to lupine proteins of 14, 24, 30 or 66 kDa in patients with both peanut and lupine allergies [84]. Lupine β -conglutin and γ -conglutin were involved in the *in vitro* and *in vivo* cross-reactivity between both legumes [85]. In another study, the rate of clinically relevant sensitization to lupine in peanut-allergic patients from the Netherlands was 35% [86]. However, without any biomarker (marker allergen), there is no possibility to predict the risk for severe lupine allergy in peanut-allergic patients.

Some studies have also demonstrated clinically relevant IgE cross-reactivity between lupine and other legumes such as pea or lentil [87]. Furthermore, it has been shown that lupine can act as a *de novo* sensitizer, independent of its IgE cross-reactivity with peanut or other legumes [84, 11].

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Clinically relevant IgE cross-reactivity between peanut and soybean has been demonstrated as well. However, the rates vary and depend on the study. Bernhisel-Broadbent & Sampson found that 6.5% of the peanut-allergic children included in their study had soybean allergy [88]. Skolnick et al. found that 15% of peanut-allergic children were also allergic to soybean [89]. Peanut allergy was found in a high proportion in a study population of soybean-allergic children in the US [90]. The differences in the rates can be attributed to factors such as differences in the diagnostic criteria used or study populations analyzed. At a molecular level, amino acid sequence comparison, IgE epitope mapping, and molecular modeling studies have demonstrated similarities between peanut and soybean allergens, such as Ara h 1, Ara h 3, and Ara h 8 with Gly m 5, Gly m 6, and Gly m 4, respectively (Table 3), which can explain the IgE cross-reactivity between peanut and soybean [91, 92]. Twenty-one of 38 peanut sensitized patients (55%) were also sensitized to pea, and 29% had a positive history to pea [86]. Table 3 summarizes cross-reactivity on the basis of known peanut, soybean and lupine allergens.

Other members of the *Leguminosae* family, such as lentil, chickpea, and pea, especially relevant in the Mediterranean area, have been demonstrated to display clinically significant IgE cross-reactivity among them [93, 94].

6 Conclusions

Increased knowledge related to the identification of new allergens from legume species and the characterization of their distinctive features at a molecular level has been gained during the last years. Knowledge about common and unique characteristics of legume allergens has provided valuable information related to their allergenic potential in different allergic study populations from

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distinct areas of the world. The ongoing effort in the characterization of legume allergens, their sensitization patterns, IgE cross-reactivity and the effects of thermal processing will profoundly contribute to the improvement of the diagnostic accuracy of legume allergies and the putative development of (immuno)therapeutic approaches. Future research will hopefully advance the full characterization of legume allergens and of allergic adverse reactions that these nutrient-rich foods are capable of eliciting.

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Conflict of interest

B.C.: declares no conflict of interest

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Table 1. List of allergens from peanut and soybean registered by WHO/IUIS Allergen Nomenclature Subcommittee.

	Protein superfamily	Protein family / common name	Other names	Molecular weight (kDa)
Peanut (<i>Arachis hypogaea</i>)				
Ara h 1	cupin	7S globulin	vicilin-type	64
Ara h 2	prolamin	2S albumin	conglutin	17, 19
Ara h 3	cupin	11S globulin	legumin-type, glycinin	61
Ara h 4**				
Ara h 5	profilin	profilin		14
Ara h 6	prolamin	2S albumin	conglutin	15
Ara h 7	prolamin	2S albumin	conglutin	16
Ara h 8	Bet v 1-like	Bet v 1 family		17
Ara h 9	prolamin	ns-LTP		9
Ara h 10	glycosyl transferase GT-C	oleosin		16
Ara h 11	glycosyl transferase GT-C	oleosin		14
Ara h 12	scorpion toxin-like knottin	defensin		5
Ara h 13	Scorpion toxin-like knottin	defensin		5
Ara h 14	glycosyl transferase GT-C	oleosin		17.5
Ara h 15	glycosyl transferase GT-C	oleosin		17
Ara h 16	prolamin	ns-LTP		8.5
Ara h 17	prolamin	ns-LTP		11
Soybean (<i>Glycine max</i>)				
Gly m 1	prolamin	hydrophobic soybean hull protein		8.3
Gly m 2	scorpion toxin-like knottin	defensin		8
Gly m 3	profilin	profilin		14
Gly m 4	Bet v 1-like	Bet v 1 family		17
Gly m 5	cupin	7S globulin	β -conglycinin, vicilin	53-76
Gly m 6	cupin	11 globulin	glycinin, legumin	52-61
Gly m 7		seed biotinylated protein		76
Gly m 8	prolamin	2S albumin		14

Table 2. List of allergens from lupine, lentil, pea, and green bean registered by WHO/TUIS Allergen Nomenclature Subcommittee.

	Protein superfamily	Protein family / common name	Other names	Molecular weight (kDa)*
Lupine (<i>Lupinus angustifolius</i>)				
Lup an 1	cupin	7S globulin	β -conglutin, vicilin	20-80
(Lupinus albus)				
Lup a 5	profilin	profilin		14
Lentil (<i>Lens culinaris</i>)				
Len c 1	cupin	7S globulin	vicilin	47
Len c 2		seed-specific biotinylated protein		66
Len c 3	prolamin	ns-LTP		9
Pea (<i>Pisum sativum</i>)				
Pis s 1	cupin	7S globulin	vicilin	44
Pis s 2	cupin	7S globulin	convicilin	63
Pis s 3	prolamin	ns-LTP		9.5
Green bean (<i>Phaseolus vulgaris</i>)				
Pha v 3	prolamin	ns-LTP		8.8-9

Table 3. Cross-reactivity on the basis of peanut, soybean and lupine allergens.

Plant food allergens	Peanut allergens <i>Arachis hypogaea</i> <u>Ara h</u>		Soy bean allergens <i>Glycine max</i> <u>Gly m</u>		Lupine allergens <i>Lupinus angustifolius</i> <u>Lup an</u> <i>Lupinus albus</i> <u>Lup a</u> <i>Lupinus luteus</i> <u>Lup lut</u>	
	Allergenic molecule	MW [kDa]	Allergenic molecule	MW [kDa]	Allergenic molecule	MW [kDa]
Vicilin-like storage protein	Ara h 1	64	Gly m 5	53-76	Precursor of β -Conglutin Lup an 1	20-80
Conglutin-like protein	Ara h 2	17 19	Gly m 8	14	δ -Conglutin	2 4
Glycinin (Legumin)	Ara h 3/4	61	Gly m 6	52-61	α -Conglutin	43
Profilin	Ara h 5	14	Gly m 3	14	Lup a 5	14
Conglutin-like protein	Ara h 6	15	2S albumin	14	unknown	14
Conglutin-like protein	Ara h 7	16	2S albumin	14	unknown	14
PR-10 protein (Bet v 1 super family)	Ara h 8	17	Gly m 4	17	Protein PR-10 <i>Lupinus albus</i>	16.5
Lipid Transfer Protein	Ara h 9 Ara h 16, Ara h 17	9 7-11	Gly m 1	8,3	unknown	?
Oleosin	Ara h 10, 11, 14, 15	15-18	Oleosin	16; 18; 24	unknown	?
Defensin	Ara h 12, 13	5	Gly m 2	8	unknown	?

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