Science, Technology and Innovation
ISSN 2544-9125 ▪ 2021; 12 (1): 12-21
doi: 10.5604/01.3001.0015.5431

STI • 2021;12 (1): 12-21

Utilization of specific primers in legume allergens based polymorphism screening

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Original article

Abstract

Different types of allergies became a part of life of many people around the world. The research activities connecting to allergens are actually not oriented only for protein and immunological interactions, but to the genomic and transcriptomic background of them, too. Analysis and description of genomic variability of allergens in plant food resources will help to manage the allergen based strategies in the future. Here, the bioinformatic approach was used to develop and validate the specific primers for genomic screening of polymorphism of profilins (Profilin Based Amplicon Polymorphism; PBAP) and vicilins (Vicilin Based Amplicon Polymorphism; VBAP) among the legumes. The alignment of existing public databases data for these allergens in the group of legumes was performed. Subsequently, specific primers were designed and their ability to generate polymorphic amplicons were tested for three legumes – bean, lentil and chickpeas. In all cases, amplicons were generated and polymorphism was detected in all three species for profilin as well as for vicilin.

Keywords

- legumes
- profilins
- vicilins
- specific primers
- polymorphism

Authors contributions

A – Conceptualization
B – Methodology
C – Validation
D – Investigation
E – Writing – original draft preperation
F – Writing, reviewing & editing

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Article info

Article history

- Received: 2021-09-30
- Accepted: 2021-11-22
- Published: 2021-11-22

Publisher

University of Applied Sciences in Tarnow
ul. Mickiewicza 8, 33-100 Tarnow, Poland

Financing

This publication was supported by the Operational Program Integrated Infrastructure within the project: Demand-driven Research for the Sustainable and Innovative Food, Drive4SIFood 313011V336, cofinanced by the European Regional Development Fund.

Conflict of interest

None declared.

Introduction

Food allergy has become a part of the life of many people around the world. The incidence of food allergic diseases is increasing. Although the prevalence is unknown, food alergy affects approximately 6-8% of children [1] and 1.5% - 2.5% of adults [2,3]. The principle of food allergy is an adverse reaction to an otherwise harmless food. The immune system responds to one or more proteins in food that are recognized as foreign, and the immune system triggers a response to neutralize them. The onset and extent of an allergic reaction depend on a person's genetics, the allergic condition, and the type and amount of allergen involved [4].

On a worldwide scale, legumes are important crop plants and represent more than 27% of the primary crop production, with grain legumes alone contributing 33% of the dietary protein nitrogen (N) needs of humans [5]. Legumes have a high nutritional value. Their seeds contain 20% to 30% protein [6]. Oil content is more variable from 1% (e.g. lentil) to 50% (e.g. peanut) [7]. However, they are responsible for a high incidence and severity of allergic reactions. Allergenic food proteins have been identified in nearly all of the most important legume crops, including peanut (*Arachis hypogaea*), soybean (*Glycine max*), lentil (*Lens culinaris*), common bean (*Phaseolus vulgaris*), mungbean (*Vigna radiata*), chickpea (*Cicer arietinum*), and pea (*Pisum sativum*) (Table 2). Unfortunately, there are no estimates of the prevalence of other legume allergies [8].

The prevalence of allergy to legume is variable and depends on the geographical location and the types of legumes. Higher legume consumption is responsible for increased sensitization [4]. The majority of legume allergens belong to mostly four protein families and superfamilies: cupins, prolamins, profilins, and pathogenesis-related proteins [9].

Profilins are small 14- to 17-kDa cytosolic proteins expressed in all eukaryotic cells [10]. The major role of profilin in plant cells is the rapid reorganization of microfilaments during processes like cytokinesis, cytoplasmic streaming, cell elongation, and growth of pollen tubes and root hairs [11–12]. Plant profilin was discovered by Valenta et al. [13] as an allergen source in birch pollen. Since then, the structure, physical and chemical properties, and functions of plant profilins have been widely and deeply studied and important progress has been made. Currently, about more than 400 profilin proteins from plants are available at NCBI (National Center for Biotechnology Information) gene database [14].

Vicilins belong to the superfamily of cupins, it is a functionally diverse superfamily of globular proteins with the so-called cupin domain, including seed storage proteins. Vicilins (7 / 8S globulins) are trimeric proteins with a molecular weight from 150 to 190 kDa. The molecular weight of the subunit is approximately 40-80 kDa [15].

This study focuses on designation and validation of specific primers for the genomic screening of polymorphism of profilins (Profilin Based Amplicon Polymorphism; PBAB) and vicilins (Vicilin Based Amplicon Polymorphism; VBAB) among the legumes and tests the ability to generate a polymorphic amplicon for selected legumes.

Materials and Methods

Bioinformatics and in silico methods

Basic bioinformatic screening of available known nucleotide sequences was performed in NCBI and AllerBase databases. Subsequently, conserved parts of profilin and vicilin sequences were obtained by the BLAST algorithm in NCBI [16] and primers were designed by Primer3 software.

Plant material

The biological material of 3 species of legumes – bean, chickpea and lentil was used in the study. The plants for genomic analyzes of polymorphism profilins (Profilin Based Amplicon Polymorphism; PBAB) and vicilins (Vicilin Based Amplicon Polymorphism; VBAB) were grown in the *in vitro* conditions. The leaves were harvested from five weeks old plants and frozen immediately. The plants were stored at −50°C until further use.

DNA extraction and PCR reaction

Total genomic DNA was isolated by GeneJET™ Plant Genomic DNA Purification Mini Kit (Thermo Scientific) following the manufacturer´s instructions. NanoPhotometer™ (IMPLEN) was used for quality and quantity checking of the extracted DNA.

The bioinformatics screening was performed to find the conserved parts of nucleotide sequences stored in the National Center for Biotechnology Information (NCBI) database for the genes of profilin and vicilin and for the designation of specific primers (Table 1). No specific data are stored for the profilins of legumes, that is why the *Rosaceae* species was used for finding the conserved parts of the profilins and the main comparison

was performed to the profiling sequences of apple. In the case of vicilin, the pea vicilin gene (accession code X14076.1) was used to be compared with other genes of legume vicilins stored in the NCBI database.

Table 1. The primers used in the study

	Nucleotide sequence (5´→ 3´)	
Primer name	Forward	Reverse
Profilin	ACCGGCCAAGA-TCTGGTTTT	AGGTAGTCTCC-CAACCTCTCC
Vicilin	AGGGATCTTT-ATTGTTGCCA	TCATTTCTTT-GACCCACAAG

PCR reactions were performed in a 10 µl reaction mixture with EliZyme HS Robust MIX with 30 ng of DNA and 400 nM of specific primers. Amplification was performed in Biometra TProfessional Thermocycler under the following conditions: 95°C 5 min (95°C 45 s; 55°C 45 s; 72°C 35 s) 40x; 72°C 10 min.

Data analysis

PCR products separation was performed in 3% agarose gel in 1xTBE buffer stained with GelRed™ (Biotium) by agarose electrophoresis. The amplicons were visualized by BDAdigital system 30 (Analytik Jena). Profiles of PBAB and VBAB were analysed by software GelAnalyzer (www.gelanalyzer.com).

Results and discussion

Analysis of actual available data for profilin and vicilin sequences in selected legumes

A different situation exists for the knowledge about the individual allergens in plant species. In many cases, even the clinically most relevant allergenic molecules are not characterized on both levels, proteomic and genomic ones. For legumes, the most information is available for the model species and clinically the most relevant – peanut and soybean (Tables 2 and 3).

The symptoms of an allergic reaction associated with the consumption of legumes are similar to other food allergens and are more or less similar for all legumes. People sensitive to these allergens can have mild to severe symptoms such as oral allergies, angioedema, hives, rhinitis, asthma, but also anaphylaxis, and in rare cases, death. Inhalation of vapor, powder, or flour from some legumes can create respiratory problems such as asthma and hypersensitivity pneumonitis [4]. In Spain, legumes are the fifth most common cause of food allergy, especially among children [17]. The estimated prevalence of lupine allergy in Europe and the Mediterranean ranges from 1.6% to 4.1% [18, 19]. The incidence and prevalence of peanut allergy are increasing every year. In 2018, approximately 2,2% of children and adolescents in the USA had peanut allergies [20]. Peanut allergy is one of the most common legume allergies [21]. Even a small amount of peanuts can set off an allergic reaction in sensitive individuals [22]. Adult patients with peanut allergy have been shown to have frequently clinically relevant sensitization to lupines, peas, and soybeans [23]. The best-identified allergens in peanuts include 7S globulin (vicilin) Ara h1 [24] and profilin Ara h5 [25]. Thermal processing of peanuts significantly reduced the allergenicity due to changes in the structure of the proteins when heated [26].

Among legumes, lentils seem to be one of the most common foods associated with allergic reactions in the Mediterranean and many Asian countries, especially among children [42]. Allergy signs and symptoms after ingestion of uncooked and cooked lentils can include oropharyngeal symptoms and acute urticaria, followed by anaphylaxis [43]. Several cases of anaphylaxis caused by inhaling vapors from cooking or eating lentils have rarely been described [44]. Thermal processing of lentils does not affect the change in immunoreactivity [33]. Allergenicity is significantly reduced for up to 30 minutes by autoclaving at 2.56 atm [45]. The main allergen in lentils is vicilin Len c 1 [33]. Len c 1 is 90% homologous to vicilin in pea Pis s 1 [37], and to more than 50% homologous to the major allergen in Ara h 1 peanuts [46] and the beta subunit of soy conglycinin [47]. Only Len c 1, Ara h 1 and Pis s 1 share common epitopes and therefore may elicit a cross-reaction [48].

The main allergen of lupine is the allergen Lup an 1 belonging to the family 7S vicilin like globulin [34] and Lup a vicilin [35]. Lupine allergy can result from a cross-reaction in people allergic to another member of the legume family, especially peanuts [18], in some cases resulting from primary sensitization [49]. Results [50] suggest that only autoclaving at 138 °C has a significant effect on the integrity and structure of allergens, thereby reducing the overall allergenicity.

The main pea allergens, wich belongs to the 7S globulin family, are named Pis s 1 and Pis s 2 [37]. Pis s 1 has

Table 2. Well-known, identified, and characterized profilins and vicilins from different legumes

S. No	Allergen	Protein family	References
		Peanut (*Arachis hypogea*)	
1	Ara h1	Vicilin (7S vicilin like globulin)	Burks et al. 1991 [24]
2	Ara h5	Profilin	Kleber-Janke et al. 2001 [25]
		Soybean (*Glycine max*)	
1	Gly m3	Profilin	Ogawa et al. 2000 [27]
2	Gly m Bd28K	Cupin (7S vicilin like globulin)	Gonzalez et al. 1992 [28]
3	Gly m Bd 60 K	Cupin(7S vicilin like globulin)	Codina et al. 1999 [29]
4	Gly m 5	Cupin (7S globulin)	Holzhauser et al. 2009, Ogawa et al. 1995 [30, 31]
		Mung beans (*Vigna radiata*)	
1	Vig r 5 (75)	Profilin	Mittag et al. 2005 [32]
		Lentil (*Lens culinaris*)	
1	Len c1	Vicilin	Sanchez-Monge et al. 2000 [33]
		Lupin (*Lupinus angustifolius*)	
1	Lup an1	Conglutin beta (7S seed storage globulin ,vicilin)	Goggin et al. 2008 [34]
2	Lup a vicilin	Cupin (7S globulin)	Peeters et al. 2007 [35]
		White lupin (*Lupinus albus*)	
1	Lup a γ-conglutin (80)	Cupin (7S globulin)	Magni et al. 2005 [36]
2	Lup a vicilin (81)	Cupin (7S globulin)	Peeters et al. 2007 [35]
		Pea (*Pisum sativum*)	
1	Pis s 1	Vicilin	Sanchez- Monge et al. 2004 [37]
2	Pis s 2	Convicilin	Sanchez- Monge et al. 2004 [37]
3	Pis s 5	Profilin	van Ree et al. 1992 [38]
		Locust tree (*Robinia pseudoacacia*)	
1	Rob p 2 (82)	Profilins	Compés et al. 2006 [39]
		Chickpea (*Cicer arietinum*)	
1	Cic a VCL	7S Vicilin-like Globulins	Bar-El Dadon et al. 2013 [40]
		French bean (*Phaseolus vulgaris*)	
1	Pha v PSL	Vicilin	Rougé et al. 2011 [41]

a high degree of homology with vicilin Len c 1 (~90%) [41] and with Pis s 2 (~70%) [51]. Pis s 2, identified as convicillin, recognizes a higher percentage of individual sera from allergic patients and therefore appears to be the major allergen in peas [37].

Chickpea allergy is very specific, rare, and most often associated with lentil allergy [52]. A putative allergen in chickpeas is vicilin Cic a VCL and likely cross-reacts with the allergens of pea and lentils [40].

First, the *in silico* prediction was performed for the designed primers by using the available sequences of profilin and vicilin of legumes (Table 3).

Table 3. *In silico* prediction of generating amplicons in PCR based on actual data in NCBI for viciline

Species	Accession number of vicilin	F primer	R primer	Predicted polymorphic
Chickpea (*Cicer arietinum*)	X51908.1	Yes	Yes	Yes
French bean (*Phaseolus vulgaris*)	X03004.1	Yes	No	?
Lentil (*Lens culinaris*)	AJ551424.1	Yes	Yes	Yes

The sequences of the designed specific primers for vicilin were used to recognize the site of the corresponding template sequences actually available in the public databases. Each primer was examined using the BLAST algorithm for the sequences listed in Table 2. The specificity and efficiency of primers were verified further in PCR using the extracted DNA of selected legume species. Up to date, no profilin sequences of nucleic acids are available in the public databases, that is why this part of validation was performed directly by PCR. Based on the in silico primer binding, a polymorphism was predicted for species of *Cicer arietinum* and *Lens culinaris*. In the case of *Phaseolus vulgaris*, the prediction of polymorphism is questionable due to any founded binding site of the reverse primer.

Characteristic of amplified fragments

The transferability of designed specific primers for profiling and vicilin to PCR amplification was performed further. The analysis for three selected species – chickpea, bean and lentil, were performed.

Completely different amplicon profiles of PBAP were obtained for the analysed species (table 4) where the amplicons were generated within the range of 46 up to the 669 bp and all of the three species can be differentiated by this marker. Evaluation of genetic diversity and an understanding of the genetic relationships in the germplasm collection provide useful information to address breeding programmes and also confirm the usefulness of diverse germplasm in crop breeding programs. Chickpea has a narrow genetic background [53] and looking for desirable traits is fundamental for varietal improvement in different breeding strategies.

Table 4. Characteristics of obtained fragment length in selected legumes for profilin specific primers

Species	Length of amplified fragments by PBAP [bp]
Chickpea (*Cicer arietinum*)	117, 76, 46
French bean (*Phaseolus vulgaris*)	669, 530, 335, 135
Lentil (*Lens culinaris*)	192, 120, 89

The PBAP marker system seems to be generating a limited number of amplicons for legume species, as three different amplicons were obtained for chickpea and lentil and four amplicons were obtained (see Figure 1).

In the case of VBAP, similar results were obtained and different amplicon profiles were generated for the analysed species (Table 5), too. Here, the amplicons were obtained within the range of 83 up to 253 bp and all of the three species were differentiated by this marker.

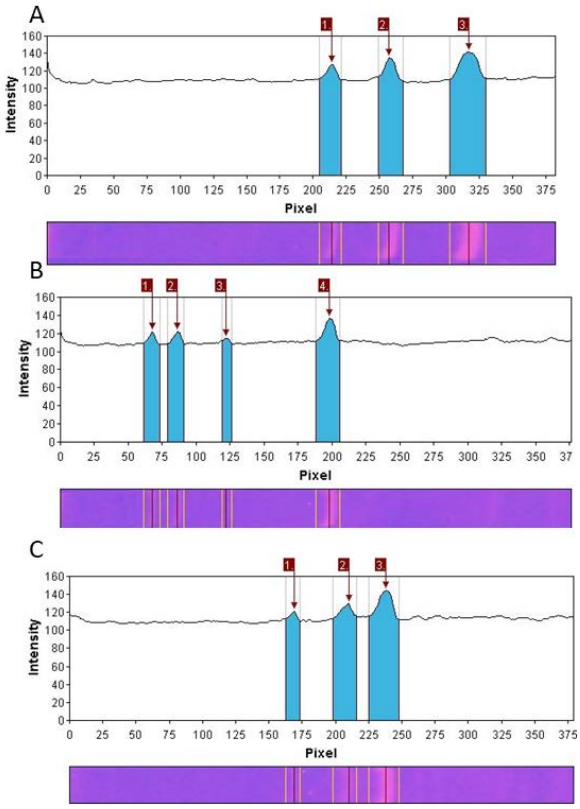

Figure 1. Fragment analysis of amplicons generated by PBAP for chickpea (A), bean (B) and lentil (C) evaluated with software GelAnalyzer

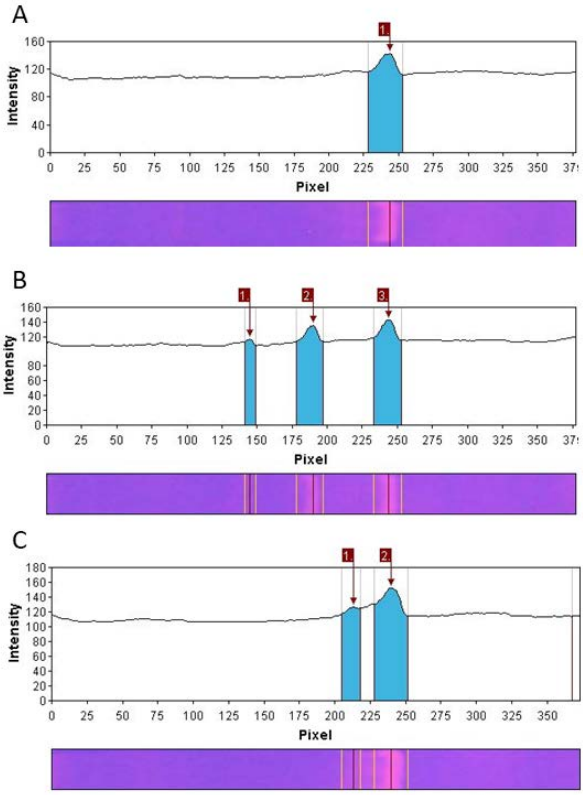

Figure 2. Fragment analysis of amplicons generated by VBAP for chickpea (A), bean (B) and lentil (C) evaluated with software GelAnalyzer

Table 5. Characteristics of obtained fragment length in selected legumes for vicilin specific primers

Species	Length of amplified fragments by VBAP [bp]
Chickpea (*Cicer arietinum*)	83
French bean (*Phaseolus vulgaris*)	253, 148, 83
Lentil (*Lens culinaris*)	112, 84

Here again, only a limited number of amplicons is generated by VBAP marker system for analysed legume species (Figure 2) and only one amplicon was amplified in the case of chickpea.

Both of the markers used in this study were proved to generate the polymorphism among legume species and we suppose they will provide valuable data for the analysis of the legume germplasm variability concerned specifically to coding regions of allergens.

Coding regions are normally used to describe the variability of legumes. DNA fingerprinting database was produced using three different PCR-based molecular marker systems (SSR, SCoT, and CDDP) for 48 chickpea genotypes collected from different sources. The results indicated that primers obtained from different regions of genomic DNA successfully amplified genotype DNAs. All of the three molecular marker sets were able to distinguish and identify each of the 48 chickpea genotypes. Salient features of fingerprinting database obtained using SSR, SCoT, and CDDP markers are given below [54].

Different tools were used to determine the genetic relationship between members of *Fabaceae* as a morphological marker and protein electrophoresis. But the relation at some plants needs additional techniques such as molecular markers like random amplified polymorphic DNA (RAPD) [55] and SCoT primers. SCoT markers provide easier development of species-specific primers than SSR [56], lower cost than AFLP [56] and higher reproducibility than RAPD [57]. In some cases, it is recommended that molecular markers such as SCoT should be employed to complement the findings of the protein electrophoresis study [55]. *Phaseolus vulgare*

was found alone within legume protein genetic diversity [58]. The relation between *Phaseolus vulgare* and *Cicer arietinum* was reported to be the same [59]. Also, the present results are supported by the rbcL data [60, 61]. Therefore, the results of the reported study show that SCoT markers are efficient in assessing the genetic diversity among *Fabaceae*.

Conclusions

Here, new types of DNA-based marker techniques for the analyse of *Fabaceae* members are reported – Profilin Based Amplicon Polymorphism (PBAP) and Vicilin Based Amplicon Polymorphism (VBAP). Proteins homologous to main allergens are abundant in the plant kingdom, and the homology of their genes provides a sufficient base for the functionality of DNA markers based on their sequences. The functionality of the PBAP and VBAP were proved for different legume species and was resulted in universal marker systems.

References

[1] Sampson HA. Clinical practice. Peanut allergy. New England Journal of Medicine 2002;346(17):1294–1299. doi: https://doi.org/10.1056/NEJMcp012667.

[2] Young E, Stoneham MD, Petruckevitch A, Barton J, Rona R. A population study of food intolerance. Lancet. 1994;343(8906):1127–1129. doi: https://doi.org/10.1016/s0140-6736(94)90234-8.

[3] Jansen JJ, Kardinaal AF, Huijbers G, Vlieg-Boerstra BJ, Martens BP, Ockhuizen T. Prevalence of food allergy and intolerance in the adult Dutch population. Journal of Allergy and Clinical Immunology. 1994;93(2):446–456. doi: https://doi.org/10.1016/0091-6749(94)90353-0.

[4] Verma AK, Kumar S, Das M, Dwivedi PD. A comprehensive review of legume allergy. Clinical Reviews in Allergy and Immunology. 2013;45(1):30–46. doi: https://doi.org/10.1007/s12016-012-8310-6.

[5] Vance CP, Graham PH, Allan DL. Biological nitrogen fixation. Phosphorus: A critical future need. In: Pedrosa FO, Hungria M, Yates MG, Newton WE, editors. Nitrogen Fixation: From Molecules to Crop Productivity. Dordrecht: Kluwer Academic Publishers; 2000. p. 506–514.

[6] Duranti M, Gius C. Legume seeds: protein content and nutritional value. Field Crops Research. 1997;53:31–45. doi: https://doi.org/10.1016/S0378-4290(97)00021-X.

[7] Werner D. Production and biological nitrogen fixation of tropical legumes. In: Werner D, Newton WE, editors. Nitrogen Fixation in Agriculture, Forestry, Ecology, and the Environment. Dordrecht: Springer; 2005. p. 1–13. doi: https://doi.org/10.1007/1-4020-3544-6_1.

[8] Riascos JJ, Weissinger AK, Weissinger SM, Burks AW. Hypoallergenic legume crops and food allergy: Factors affecting feasibility and risk. Journal of Agricultural and Food Chemistry. 2010;58(1):20–27. doi: https://doi.org/10.1021/jf902526y.

[9] Jenkins JA, Griffiths-Jones S, Shewry PR, Breiteneder H, Mills EN. Structural relatedness of plant food allergens with specific reference to cross-reactive allergens: an in silico analysis. Journal of Allergy and Clinical Immunology. 2005;115(1):163–170. doi: https://doi.org/10.1016/j.jaci.2004.10.026.

[10] Carlsson L, Nyström LE, Sundkvist I, Markey F, Lindberg U. Actin polymerizability is influenced by profilin, a low molecular weight protein in non-muscle cells. Journal of Molecular Biology. 1977;115(3):465–483. doi: https://doi.org/10.1016/0022-2836(77)90166-8.

[11] Cuesta-Herranz J, Pastor C, Figueredo E, Vidarte L, De las Heras M, Durán C, Fernández-Caldas E, de Miguel J, Vivanco F. Identification of Cucumisin (Cuc m 1), a subtilisin-like endopeptidase, as the major allergen of melon fruit. Clinical and Experimental Allergy. 2003;33(6):827–833. doi: https://doi.org/10.1046/j.1365-2222.2003.01680.x.

[12] Valster AH, Pierson ES, Valenta R, Hepler PK, Emons A. Probing the plant actin cytoskeleton during cytokinesis and interphase by profilin microinjection. Plant Cell. 1997;9(10):1815–1824. doi: https://doi.org/10.1105/tpc.9.10.1815.

[13] Valenta R, Duchêne M, Pettenburger K, Sillaber C, Valent P, Bettelheim P, Breitenbach M, Rumpold H, Kraft D, Scheiner O. Identification of profilin as a novel pollen allergen; IgE autoreactivity in sensitized individuals. Science. 1991;253(5019):557–560. doi: https://doi.org/10.1126/science.1857985.

[14] Jimenez-Lopez JC, Morales S, Castro AJ, Volkmann D, Rodríguez-García MI, Alché JD. Characterization of profilin polymorphism in pollen with a focus on multifunctionality. PLoS One. 2012;7(2):e30878. doi: https://doi.org/10.1371/journal.pone.0030878.

[15] Shewry PR, Napier JA, Tatham AS. Seed storage proteins: structures and biosynthesis. Plant Cell. 1995;7(7):945–956. doi: https://doi.org/10.1105/tpc.7.7.945.

[16] Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. Journal of Molecular Biology. 1990;215(3):403–410. doi: https://doi.org/10.1016/S0022-2836(05)80360-2.

[17] Martínez San Ireneo M, Ibáñez MD, Fernández-Caldas E, Carnés J. In vitro and in vivo cross-reactivity studies of legume allergy in a Mediterranean population. International Archives of Allergy and Immunology. 2008;147(3):222–230. doi: https://doi.org/10.1159/000142045.

[18] Moneret-Vautrin DA, Guérin L, Kanny G, Flabbee J, Frémont S, Morisset M. Cross-allergenicity of peanut and lupine: The risk of lupine allergy in patients allergic to peanuts. Journal of Allergy and Clinical Immunology. 1999;104(4 Pt 1):883–888. doi: https://doi.org/10.1016/s0091-6749(99)70303-9.

[19] Hieta N, Hasan T, Mäkinen-Kiljunen S, Lammintausta K. Lupin allergy and lupin sensitization among patients with suspected food allergy. Annals of Allergy, Asthma and Immunology. 2009;103(3):233–237. doi: https://doi.org/10.1016/S1081-1206(10)60187-1.

[20] Liberman J, Sblett J, Ali Y, Haselkorn T, Damle V, Chidambaram A, Rosen K, Mahr T. Increased incidence and prevalence of peanut allergy in children and adolescents in the United States. Annals of Allergy, Asthma & Immunology. 2018;121(5):13. doi: https://doi.org/10.1016/j.anai.2018.09.039.

[21] Hourihane JO, Aiken R, Briggs R, Gudgeon LA, Grimshaw KE, DunnGalvin A, Roberts SR. The impact of government advice to pregnant mothers regarding peanut avoidance on the prevalence of peanut allergy in United Kingdom children at school entry. Journal of Allergy and Clinical Immunology. 2007;119(5):1197–1202. doi: https://doi.org/10.1016/j.jaci.2006.12.670.

[22] Taylor SL, Hefle SL, Bindslev-Jensen C, Bock SA, Burks AW Jr, Christie L, Hill DJ, Host A, Hourihane JO, Lack G, Metcalfe DD, Moneret-Vautrin DA, Vadas PA, Rance F, Skrypec DJ, Trautman TA, Yman IM, Zeiger RS. Factors affecting the determination of threshold doses for allergenic foods: How much is too much? Journal of Allergy and Clinical Immunology. 2002;109(1):24–30. doi: https://doi.org/10.1067/mai.2002.120564.

[23] Peeters KA, Koppelman SJ, Penninks AH, Lebens A, Bruijnzeel-Koomen CA, Hefle SL, Taylor SL, van Hoffen E, Knulst AC. Clinical relevance of sensitization to lupine in peanut-sensitized adults. Allergy. 2009;64(4):549–555. doi: https://doi.org/10.1111/j.1398-9995.2008.01818.x.

[24] Burks AW, Williams LW, Helm RM, Connaughton C, Cockrell G, O'Brien T. Identification of a major peanut allergen, Ara h I, in patients with atopic dermatitis and positive peanut challenges. Journal of Allergy and Clinical Immunology. 1991;88(2):172–9. doi: https://doi.org/10.1016/0091-6749(91)90325-i.

[25] Kleber-Janke T, Crameri R, Scheurer S, Vieths S, Becker WM. Patient-tailored cloning of allergens by phage display: peanut (Arachis hypogaea) profilin, a food allergen derived from a rare mRNA. Journal of Chromatography, B: Biomedical Sciences and Applications. 2001;756(1–2):295–305. doi: https://doi.org/10.1016/s0378-4347(01)00088-3.

[26] Beyer K, Morrow E, Li XM, Bardina L, Bannon GA, Burks AW, Sampson HA. Effects of cooking methods on peanut allergenicity. Journal of Allergy and Clinical Immunology. 2001;107(6):1077–1081. doi: https://doi.org/10.1067/mai.2001.115480.

[27] Ogawa A, Samoto M, Takahashi K. Soybean allergens and hypoallergenic soybean products. Journal of Nutritional Science and Vitaminology (Tokyo). 2000;46(6):271–279. doi: https://doi.org/10.3177/jnsv.46.271.

[28] González R, Polo F, Zapatero L, Caravaca F, Carreira J. Purification and characterization of major inhalant allergens from soybean hulls. Clinical and Experimental Allergy. 1992;22(8):748–755. doi: https://doi.org/10.1111/j.1365-2222.1992.tb02814.x.

[29] Codina R, Lockey RF, Fernández-Caldas E, Rama R. Identification of the soybean hull allergens responsible for the Barcelona asthma outbreaks. International Archives of Allergy and Immunology. 1999;119(1):69–71. doi: https://doi.org/10.1159/000024178.

[30] Holzhauser T, Wackermann O, Ballmer-Weber BK, Bindslev-Jensen C, Scibilia J, Perono-Garoffo L, Utsumi S, Poulsen LK, Vieths S. Soybean (Glycine max) allergy in Europe: Gly m 5 (beta-conglycinin) and Gly m 6 (glycinin) are potential diagnostic markers for severe allergic reactions to soy. Journal of Allergy and Clinical Immunology. 2009;123(2):452–458. doi: https://doi.org/10.1016/j.jaci.2008.09.034.

[31] Ogawa T, Bando N, Tsuji H, Nishikawa K, Kitamura K. Alpha-subunit of beta-conglycinin, an allergenic protein recognized by IgE antibodies of soybean-sensitive patients with atopic dermatitis. Bioscience, Biotechnology, and Biochemistry. 1995;59(5):831–833. doi: https://doi.org/10.1271/bbb.59.831.

[32] Mittag D, Vieths S, Vogel L, Wagner-Loew D, Starke A, Hunziker P, Becker WM, Ballmer-Weber BK. Birch pollen-related food allergy to legumes: identification and characterization of the Bet v 1 homologue in mungbean (Vigna radiata), Vig r 1. Clinical adn Experimental Allergy. 2005;35(8):1049–1055. doi: https://doi.org/10.1111/j.1365-2222.2005.02309.x.

[33] Sánchez-Monge R, Pascual CY, Díaz-Perales A, Fernández-Crespo J, Martín-Esteban M, Salcedo G. Isolation and characterization of relevant allergens from boiled lentils. Journal of Allergy and Clinical Immunology. 2000;106(5):955–961. doi: https://doi.org/10.1067/mai.2000.109912.

[34] Goggin DE, Mir G, Smith WB, Stuckey M, Smith PM. Proteomic analysis of lupin seed proteins to identify conglutin Beta as an allergen, Lup an 1. Journal of Agricultural and Food Chemistry. 2008;56(15):6370–6377. doi: https://doi.org/10.1021/jf800840u.

[35] Peeters KA, Nordlee JA, Penninks AH, Chen L, Goodman RE, Bruijnzeel-Koomen CA, Hefle SL, Taylor SL, Knulst AC. Lupine allergy: not simply cross-reactivity with peanut or soy. Journal of Allergy and Clinical Immunology. 2007;120(3):647–53. doi: https://doi.org/10.1016/j.jaci.2007.05.032.

[36] Magni C, Herndl A, Sironi E, Scarafoni A, Ballabio C, Restani P, Bernardini R, Novembre E, Vierucci A, Duranti M. One- and two-dimensional electrophoretic identification of IgE-binding polypeptides of Lupinus albus and other legume seeds. Journal of Agricultural and Food Chemistry. 2005;53(11):4567–4571. doi: https://doi.org/10.1021/jf0500785.

[37] Sanchez-Monge R, Lopez-Torrejón G, Pascual CY, Varela J, Martin-Esteban M, Salcedo G. Vicilin and convicilin are potential major allergens from pea. Clinical and Experimental Allergy. 2004;34(11):1747–1753. doi: https://doi.org/10.1111/j.1365-2222.2004.02085.x.

[38] van Ree R, Voitenko V, van Leeuwen WA, Aalberse RC. Profilin is a cross-reactive allergen in pollen and vegetable foods. International Archives of Allergy and Immunology. 1992;98(2):97–104. doi: https://doi.org/10.1159/000236171.

[39] Compés E, Hernández E, Quirce S, Palomares O, Rodríguez R, Cuesta J, Sastre J, Villalba M. Hypersensitivity to black locust (Robinia pseudoacacia) pollen: "Allergy mirages". Annals of Allergy Asthma and Immunology. 2006;96(4):586–92. doi: https://doi.org/10.1016/S1081-1206(10)63554-5.

[40] Bar-El Dadon S, Pascual CY, Eshel D, Teper-Bamnolker P, Ibáñez MD, Reifen R. Vicilin and the basic subunit of legumin are putative chickpea allergens. Food Chemistry. 2013;138(1):13–8. doi: https://doi.org/10.1016/j.foodchem.2012.10.031.

[41] Rougé P, Culerrier R, Thibau F, Didier A, Barre A. A case of severe anaphylaxis to kidney bean: phaseolin (vicilin) and PHA (lectin) identified as putative allergens. Allergy. 2011;66(2):301–302. doi: https://doi.org/10.1111/j.1398-9995.2010.02466.x.

[42] López-Torrejón G, Salcedo G, Martín-Esteban M, Díaz-Perales A, Pascual CY, Sánchez-Monge R. Len c 1, a major allergen and vicilin from lentil seeds: protein isolation and cDNA cloning. Journal of Allergy and Clinical Immunology. 2003;112(6):1208–1215. doi: https://doi.org/10.1016/j.jaci.2003.08.035.

[43] Martínez San Ireneo M, Ibáñez MD, Sánchez JJ, Carnés J, Fernández-Caldas E. Clinical features of legume allergy in children from a Mediterranean area. Annales of Allergy, Asthma and Immunology. 2008;101(2):179–184. doi: https://doi.org/10.1016/s1081-1206(10)60207-4.

[44] Martin JA, Compaired JA, de la Hoz B, Quirce S, Alonso MD, Igea JM, Losada E. Bronchial asthma induced by chick pea and lentil. Allergy. 1992;47(2 Pt 2):185–187. doi: https://doi.org/10.1111/j.1398-9995.1992.tb00962.x.

[45] Cuadrado C, Cabanillas B, Pedrosa MM, Varela A, Guillamón E, Muzquiz M, Crespo JF, Rodriguez J, Burbano C. Influence of thermal processing on IgE reactivity to lentil and chickpea proteins. Molecular Nutrition and Food Research. 2009;53(11):1462–1468. doi: https://doi.org/10.1002/mnfr.200800485.

[46] Shin DS, Compadre CM, Maleki SJ, Kopper RA, Sampson H, Huang SK, Burks AW, Bannon GA. Biochemical and structural analysis of the IgE binding sites on ara h1, an abundant and highly allergenic peanut protein. Journal of Biological Chemistry. 1998;273(22):13753–13759. doi: https://doi.org/10.1074/jbc.273.22.13753.

[47] Ogawa T, Bando N, Tsuji H, Nishikawa K, Kitamura K. Alpha-subunit of beta-conglycinin, an allergenic protein recognized by IgE antibodies of soybean-sensitive patients with atopic dermatitis. Bioscience, Biotechnology, and Biochemistry. 1995;59(5):831–833. doi: https://doi.org/10.1271/bbb.59.831.

[48] Barre A, Borges JP, Rougé P. Molecular modelling of the major peanut allergen Ara h 1 and other homotrimeric allergens of the cupin superfamily: A structural basis for their IgE-binding cross-reactivity. Biochimie. 2005;87(6):499–506. doi: https://doi.org/10.1016/j.biochi.2005.02.011.

[49] Smith WB, Gillis D, Kette FE. Lupin: A new hidden food allergen. Medical Journal of Australia. 2004;181(4):219–220. doi: https://doi.org/10.5694/j.1326-5377.2004.tb06242.x.

[50] Alvarez-Alvarez J, Guillamón E, Crespo JF, Cuadrado C, Burbano C, Rodríguez J, Fernández C, Muzquiz M. Effects of extrusion, boiling, autoclaving, and microwave heating on lupine allergenicity. Journal of Agricultural and Food Chemistry. 2005;53(4):1294–1298. doi: https://doi.org/10.1021/jf0490145.

[51] Croy RR, Gatehouse JA, Tyler M, Boulter D. The purification and characterization of a third storage protein (convicilin) from the seeds of pea (Pisum sativum L.). Biochemical Journal. 1980;191(2):509–516. doi: https://doi.org/10.1042/bj1910509.

[52] Jukanti AK, Gaur PM, Gowda CL, Chibbar RN. Nutritional quality and health benefits of chickpea (Cicer arietinum L.): A review. British Journal of Nutrition. 2012;108(Suppl 1):S11–26. doi: https://doi.org/10.1017/S0007114512000797.

[53] Nguyen TT, Taylor PWJ, Redden RJ, Ford R. Genetic diversity estimates in Cicer using AFLP analysis. Plant Breeding. 2008;123(2):173–179. doi: https://doi.org/10.1046/j.1439-0523.2003.00942.x.

[54] Hajibarat Z, Saidi A, Hajibarat Z, Talebi R. Characterization of genetic diversity in chickpea using SSR markers, Start Codon Targeted Polymorphism (SCoT) and Conserved DNA-Derived Polymorphism (CDDP). Physiology and molecular Biology of Plants. 2015;21(3):365–73. doi: https://doi.org/10.1007/s12298-015-0306-2.

[55] Alege GO, Abu Ngozi E, Sunday CE. Seed protein electrophoresis of some members of the family fabaceae. African Journal of Biotechnology. 2014;13(36):3730–3735. doi: https://doi.org/10.5897/AJB2014.13715.

[56] Jiang LF, Qi X, Zhang XQ, Huang LK, Ma X, Xie WG. Analysis of diversity and relationships among orchardgrass

(Dactylis glomerata L.) accessions using start codon-targeted markers. Genetics and Molecular Research. 2014;13(2):4406–4418. doi: https://doi.org/10.4238/2014.June.11.4.

[57] Xiong F, Zhong R, Han Z, Jiang J, He L, Zhuang W, Tang R. Start codon targeted polymorphism for evaluation of functional genetic variation and relationships in cultivated peanut (Arachis hypogaea L.) genotypes. Molecular Biology Reports. 2011;38(5):3487–3494. doi: https://doi.org/10.1007/s11033-010-0459-6.

[58] Valizadeh M. Seed storage protein of grain legumes grown in Iran using SDS-PAGE. Journal of Agricultural Science and Technology. 2001;3(4):287–292.

[59] Mondal AK, Mondal S. Circumscription of the families within Leguminales as determined by cladistic analysis based on seed protein. African Journal of Biotechnology. 2011;10(15):2850–2856. doi: https://doi.org/10.5897/AJB10.206.

[60] Doyle JJ, Chappill JA, Bailey CD, Kajita T. Towards a comprehensive phylogeny of legumes: evidence from rbcL sequences and non-molecular data. In: Herendeen PS, Bruneau A, editors. Advances in Legume Systematics. Part 9. Royal Botanic Gardens, Kew; 2000. p. 1–20.

[61] Wojciechowski MF, Lavin M, Sanderson MJ. A phylogeny of legumes (Leguminosae) based on analysis of the plastid matK gene resolves many well-supported subclades within the family. American Journal of Botany. 2004;91(11):1846–1862. doi: https://doi.org/10.3732/ajb.91.11.1846.