JOURNAL of Horticulture, Forestry and Biotechnology
Volume 30(1), 2026, 8-14
https://jhfb.ro/index.php/jhfb
Print ISSN: 2066-1797
Online ISSN: 3045-1876

Molecular screening of allergen-related homologs in commonly used herbs utilizing *ypr-10* and *profilin*-based DNA marker systems

Silvia FARKASOVÁ[1*], Lucia URBANOVÁ[1], Ivana SPEVÁKOVÁ[1], Alžbeta JAUSCHOVÁ[2], Jana ŽIAROVSKÁ[2*]

[1] Research Centre Agrobiotech, Slovak University of Agriculture, Trieda Andreja Hlinku 2, 94976 Nitra, Slovakia, email: lucia.urbanova@uniag.sk
[2] Institute of Plant and Environmental Sciences, Faculty of Agrobiology and Food Resources, Trieda Andreja Hlinku 2, 94976 Nitra, Slovakia, email: xjauschova@uniag.sk

* Corresponding author: silvia.farkasova@uniag.sk, jana.ziarovska@uniag.sk

Manuscript received: 28 October 2025; revised: 03 November 2025; accepted: 12 November 2025

Abstract

Food allergies represent a growing public health concern, and current diagnostic tools often fail to detect sensitizations to minor or less-studied allergens. Although herbs have been used for millennia in traditional medicine and nutrition, their allergenic potential remains insufficiently investigated. Evidence indicates that individuals allergic to birch pollen may experience cross-reactive allergic responses to certain herbs and spices due to shared allergenic proteins. This phenomenon, classified as pollen–plant food allergy syndrome, involves IgE cross-reactivity to homologues of PR-10 proteins, profilins, and nonspecific lipid-transfer proteins.

The aim of the present study was to evaluate the presence of *ypr-10* and *profilin* homologues in the genomes of 11 commonly used European herbs (*Melissa officinalis, Mentha piperita, Tilia cordata, Urtica dioica, Rosa canina, Thymus serpyllum, Plantago lanceolata, Sambucus nigra, Hypericum perforatum, Agrimonia eupatoria, Tanacetum vulgare*) belonging to various plant families.

Two DNA marker-based methodologies, BBAP and PBAP, were applied to identify allergen homologues and assess genetic relatedness among species. All studied herbs were found to contain homologues of the investigated allergen families. Dendrograms based on Jaccard genetic distance indices revealed distinct clustering patterns. Using the PBAP method, two principal clusters were identified, one comprising primarily members of the order *Lamiales* (*Mentha piperita, Thymus serpyllum, Melissa officinalis, Plantago lanceolata*), with the exception of *Agrimonia eupatoria* (*Rosales*). The Jaccard index for this cluster ranged from 0.818 to 0.947. In contrast, dendrograms generated with the BBAP method did not align with taxonomic relationships, however the lowest genetic distance (0.545) was observed between *M. officinalis* and *M. piperita*.

These findings demonstrate that DNA marker systems such as BBAP and PBAP provide cost-effective, rapid tools for screening allergen homologues in medicinal and culinary herbs. This approach may facilitate the identification of potential cross-reactive allergens and improve understanding of herb-related allergic sensitization mechanisms.

Keywords: genetic polymorphism, medicinal plants, panallergens

Introduction

Herbs have been used for millennia, with numerous cultures developing sophisticated traditions surrounding their application. They serve diverse purposes, including the treatment of diseases, the promotion of general well-being, and the enhancement of food flavour or cosmetic formulations. A growing body of research underscores the potential of herbs as valuable sources of bioactive compounds for botanical medicines, functional foods, and dietary supplements, given their demonstrated antioxidant, antimicrobial, and anticancer properties [6]. These therapeutic effects are attributed to a wide range of phytochemicals, such as essential oils, lignans, saponins, polyphenols, phenolic acids, sterols, terpenoids, and polysaccharides [11].

Certain studies have also suggested that some herbs may exert anti-allergic effects. For instance, an herbal blend containing chamomile, saffron, anise, fennel, caraway, liquorice, cardamom, and black seed was found to inhibit histamine release from rat peritoneal mast cells [9]. Nevertheless, herbs may also trigger allergic reactions in sensitive individuals. To date, the allergenic potential of herbs and spices has not been extensively investigated. The most substantial evidence of allergenicity involves species within the *Asteraceae family*, which includes plants such as tansy (*Tanacetum vulgare*) [16].

The Asteraceae family ranks among the largest plant families worldwide, with representatives on nearly every continent. Allergic responses to its members can manifest as eczema, hay fever, asthma, or, in severe cases, anaphylaxis. Considerable cross-reactivity has been observed between food and pollen allergens in individuals sensitised to *Asteraceae* allergens [7]. In Europe, allergies to ragweed and mugwort pollen have become a significant public health concern. Individuals suffering from respiratory allergies to weed pollen may also exhibit hypersensitivity to herbal medicines derived from cross-reactive *Asteraceae* species, including chamomile, marigold, and purple coneflower [23].

The concept of panallergens refers to families of structurally and functionally conserved proteins involved in essential biological processes, which are widely distributed in nature. Plant panallergens possess highly conserved sequence regions and are responsible for many IgE-mediated cross-reactions, even between unrelated sources of pollen and plant-derived foods. Although often classified as minor allergens, sensitisation to panallergens can lead to polysensitisation, complicating diagnosis and treatment [10].

Patients with pollen allergies frequently experience allergic reactions to multiple plant species. Many such reactions are linked to four homologous allergenic structures found in birch pollen. The principal allergenic protein in birch pollen, Bet v 1, shares common epitopes with allergens present in apples, stone fruits, celery, carrots, nuts, and soybeans. Bet v 1-associated food allergens exhibit 35–60% sequence similarity with the PR-10 family of pathogenesis-related proteins [22]. PR-10 proteins are integral to plant defence mechanisms, being induced by infection from viruses, bacteria, or fungi, as well as by abiotic stresses such as cold, drought, salinity, and ultraviolet radiation. Their expression may also be stimulated by phytohormones and physical injury. PR-10 proteins play a vital role in plant growth and development, being constitutively expressed in roots, stems, leaves, flowers, fruits, pollen, and seeds [8].

Profilins are small, ubiquitous eukaryotic proteins with molecular masses ranging between 14 and 17 kDa. They were initially characterized as key regulators of actin polymerization dynamics. The essential roles of profilins in fundamental cellular processes—including proliferation, differentiation, growth, motility, and cytokinesis—are well established [13]. Allergenic forms of profilin occur exclusively in angiosperms. Owing to their high degree of sequence conservation, plant profilins constitute a family of extensively cross-reactive allergens that are widely distributed among both monocotyledonous and dicotyledonous pollens, as well as in various plant-derived foods [17]. Profilin is typically classified as a minor pollen allergen, as sensitization occurs in only approximately 10–20% of individuals with pollen allergies [2, 5].

Testing for plant allergens remains challenging due to the limited availability of standardised patch test materials and the potential for active sensitisation when using whole plants or crude extracts [1]. Currently, allergen extracts are employed for both diagnostic and therapeutic purposes. However, these extracts often comprise mixtures of allergenic and non-allergenic proteins and may lack the specific allergens relevant to an individual patient—particularly minor ones such as panallergens [10].

DNA markers—polymorphic regions of nucleic acids—serve as powerful tools for detecting genetic variability within and among populations, thereby contributing substantially to plant improvement programs. PCR-based marker systems enable the targeted amplification of specific genomic segments, facilitating the identification of polymorphisms generated by point mutations, insertions, or deletions. The application of molecular markers has greatly advanced the understanding of genetic diversity, supported the construction of genetic linkage maps, and accelerated crop enhancement through marker-assisted selection. In addition, these technologies have proven indispensable for germplasm characterization, biodiversity conservation, and phylogenetic studies [4].

This study aimed to detect homologues of *PR-10* and *profilin* allergens in 11 medicinal and culinary herbs commonly used in Europe and to assess their genetic relatedness using BBAP (Bet v 1 based amplicon polymorphism) and PBAP (profilin based amplicon polymorphism) marker systems. The findings were intended to support improved identification of potential cross-reactive panallergens and to enhance understanding of herb-associated sensitization in pollen–plant food allergy syndrome.

Material and Method

The biological material consisted of 12 herbs (Table 1). For the isolation of genomic DNA, fresh plant material was used. The commercial EliGene® Plant DNA Isolation Kit (Elisabeth Pharmacon) was employed for the isolation, adhering to the manufacturer's instructions. The concentration and quality of the isolated DNA were determined spectrophotometrically using a NanoPhotometerTM P360 (Implen).

Table 1. Plant material

Sample	Latin name	Order	Family
1	*Melissa officinalis*	*Lamiales*	*Lamiaceae*
2	*Mentha piperita*	*Lamiales*	*Lamiaceae*
3	*Tilia cordata*	*Malvales*	*Malvaceae*
4	*Urtica dioica*	*Rosales*	*Lamiaceae*
5	*Rosa canina*	*Rosales*	*Rosaceae*
6	*Thymus serpyllum*	*Lamiales*	*Lamiaceae*
7	*Plantago lanceolata*	*Lamiales*	*Plantaginaceae*
8	*Sambucus nigra*	*Dipsacales*	*Adoxaceae*
9	*Hypericum perforatum*	*Malpighiales*	*Hypericaceae*
10	*Agrimonia eupatoria*	*Rosales*	*Rosaceae*
11	*Tanacetum vulgare*	*Asterales*	*Asteraceae*

All PCR reactions were performed on a SureCycler 8800 thermal cycler (Agilent). For the BBAP methodology, a degenerate primer combination was utilised [21], with the primer sequences listed in Table 2. The temperature profile for the reactions was as follows: 95 °C (5 min), followed by 40 cycles of 95 °C (45 s), 55 °C (45 s), and 72 °C (35 s), concluding with a final polymerisation step at 72 °C (10 min). PBAP technique [12] uses 1 pair of primers (Table 2). The PCR protocol was as follows: primary denaturation at 95°C (5 min); 40 cycles of denaturation at 95°C (45 s), primers annealing at 55°C (45 s), polymerization at 72°C (35 s); and final polymerization at 72°C (10 min). The composition of the reaction mixture for both methodologies included EliZyme Robust PCR Master Mix (2X), 400 nM of reverse and forward primers, and 30 ng of DNA. The reactions were carried out in a total volume of 10 µl.

The PCR products were separated on 2% agarose gels stained with the intercalation dye GelRed® Nucleic Acid Gel Stain (Biotinum). The fragments were separated at 120 V for 120 minutes and were subsequently visualised using a BDA digital system 30 transilluminator (Analytik Jena). The electropherogram images obtained were processed using GelAnalyzer software (www.gelanalyzer.com). The amplicons were evaluated using a size standard GeneRuler 100 bp DNA Ladder, Thermo Fisher Scientific®).

Table 2. Primer sequences

Marker technique	Primer name	Primer sequence
PBAP	F forward	ACCGGCCAAGATCTGGTTTT
	R reverse	AGGTAGTCTCCCAACCTCTCC
BBAP	F forward	CCT GGA ACC ATC AAG AAG
	Rd reverse	TTG GTG TGG TA**S** T**K**G CTG

Explanatory note: *S= G/C; K= T/G

According to the data obtained, a binary matrix was created based on the presence or absence of the specified fragments. Distance matrices were subsequently created based on the Jaccard coefficient using R studio with vegan package (2.7.1) [15]. Herb species were clustered using “ward.D” method into individual dendrograms. For statistical calculations the online software iMEC (Online Marker Efficiency Calculator) [3] was employed. Within the iMEC program, the discriminatory power (D%) was determined to represent the probability that two randomly selected individuals would exhibit distinct banding patterns and, therefore, be distinguishable from one another [3]. Additionally, the polymorphism information content (PIC) value was calculated. This parameter reflects the probability that the marker genotype of a given offspring will permit the deduction, in the absence of crossing over, of which of the two marker alleles was inherited from the affected parents [3]. The evaluation of the informativeness level based on the PIC-value was assessed according to the scale of Serrote et al. [18].

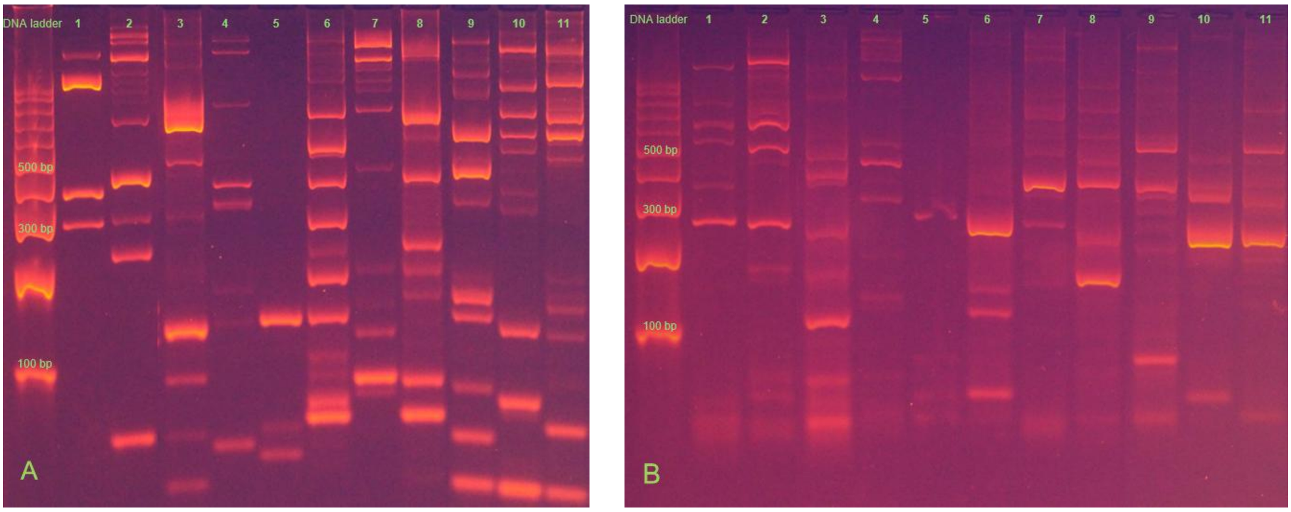
Results and Discussion

Amplification profile of the PBAP method (profilin based amplicon polymorphism)

The PBAP method successfully generated amplicons for all the herbs studied (Figure 1-A), no two identical amplification profiles were observed, resulting in a polymorphism percentage (P %) of 100%. The primer pair produced a total of 87 PCR products. The amplicon sizes ranged from 68 to 1491 base pairs (bp), leading to the creation of 38 loci in total, with an average of 7.91 amplicons per sample (ranging from 3 to 11). The D % was calculated at 95.7 % and the PIC value (Table 3) for the PBAP method was 0.275, which is considered medium to highly informative, for a dominant marker such as PBAP.

Table 3. Statistical information generated for the herbs examined using DNA marker systems BBAP and PBAP

Marker technique	**PIC**	**Amplicon range (bp)**	**Total number of amplicons**	***Number of loci***	**P %**	**D**
PBAP	0.275	68-1491	87	38	100	0.957
BBAP	0.301	76-1239	67	25	100	0.941



Explanatory note: the sample labelling is corresponding with Table 1

Figure 1. PBAP (A) and BBAP (B) amplification profiles

The dendrogram (Figure 2) produced by the PBAP method indicated two principal clusters. One of these groups comprised herbs from the *Lamiales* order (*M. piperita*, *T. serpyllum*, *M. officinalis*, *P. lancelota*), excluding *A. eupatoria*, which is classified under the *Rosales* order (Figure 2). The Jaccard index of genetic distances for this group varied from 0.818 to 0.947. All samples exhibited high indices, with the minimum genetic index recorded at 0.75 for the pairs *H. perforatum - T. vulgare, T. cordata - T. vulgare*, and *S. nigra - H. perforatum*. The three species within the *Rosales* order demonstrated high genetic distance indices: the pair *U. dioica* and *R. canina* recorded an index of 0.9, whereas both *U. dioica* and *A. eupatoria*, as well as *R. canina* and *A. eupatoria*, exhibited an index of 1.0.

Amplification profile of the BBAP method (Bet v 1 based amplicon polymorphism)

The BBAP method also generated amplicons for all the herbs studied (Figure 1-B); the P% was 100%. The degenerated reverse and forward primers produced a total of 67 PCR products. The amplicon sizes ranged from 76 to 1239 bp, leading to the creation of 25 loci in total, with an average of 6.1 amplicons per sample (ranging from 3 to 8). The D% was calculated at 94.1%. The PIC value for the BBAP method was 0.301, which is considered highly informative, being also a dominant marker as BBAP (Table 3).

The BBAP method revealed that the groups within the dendrogram did not correspond with the orders or families of the herbs examined (Figure 3). The lowest recorded genetic distance of 0.545 was noted for the pairs *M. officinalis* and *M. piperita*, which is expected given that both species are categorised within the order *Lamiales* and the family *Lamiaceae*. In contrast, the other members of the *Lamiales* order exhibited greater genetic distances, ranging from 0.636 to 1.0. Notably, the second lowest value of 0.556 was found between *T. vulgare* and *P. lanceolata*, which is intriguing as these species belong to different families and orders. As for the *Rosales* order, the genetic distance between *R. canina* and *U. dioica* was 1.0; between *R. canina* and *A.*

eupatoria, 0.8; and between *U. dioica* and *A. eupatoria*, 0.889. Notably, the same genetic distances were observed for both *M. officinalis* and *M. piperita*, with the same values calculated between these two species and *R. canina* (0.625), *T. serpyllum* (0.909), *H. perforatum* (0.923), *A. eupatoria* (0.9), and *T. vulgare* (0.833). Overall, the values of genetic distances ranged from 0.545 to 1.

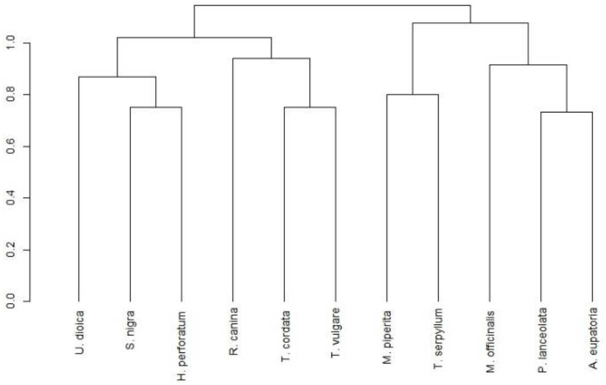


Figure 2. Dendrogram describing the genetic distance of the studied herbs based on *profilin* homologs (PBAP) according to Jaccard coefficient

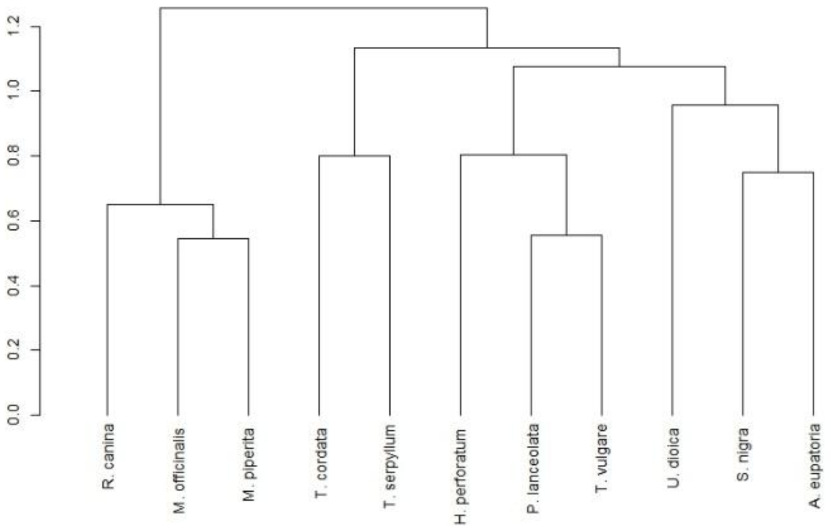


Figure 3. Dendrogram describing the genetic distance of the studied herbs based *ypr-10* homologs according to Jaccard coefficient

For allergen detection, primarily protein-based methodologies are employed, such as the ELISA method; however, it is essential to have prior knowledge of the specific antibodies required for detecting a given protein [19]. Regarding PCR methodologies, real-time PCR is predominantly used for the detection of allergens with known gene sequences [14, 19]. Additionally, DNA marker systems based on allergens can offer a cost-effective and rapid screening approach for identifying allergen homologues, and the results may be further applied in sequencing studies.

The BBAP technique was previously successfully employed for several vegetable species [21]. The PBAP method was first introduced in research focusing on legumes—specifically beans, chickpeas, and lentils—where homologues were successfully amplified across all species studied [12].

The PBAP system, targeting profilin homologues, produced clustering patterns largely consistent with taxonomic relationships, particularly among *Lamiales* species, indicating that profilin sequences are relatively conserved within closely related taxa. Conversely, BBAP analysis, based on PR-10 (*Bet v 1*) homologues, revealed greater variability and clustering independent of taxonomy, reflecting the functional and structural diversification typical of PR-10 gene families involved in plant defense and stress responses [8].

Moderate to high polymorphism information content (PIC = 0.275–0.301) values confirm that both systems are informative and reliable for evaluating genetic relatedness. The universal detection of *PR-10* and profilin homologues across all species supports their widespread distribution in flowering plants and their potential role in IgE-mediated cross-reactivity observed in pollen–plant food allergy syndrome [10, 23].

Conclusions

This study demonstrated the effectiveness of allergen-based DNA marker systems (BBAP and PBAP) in detecting homologues of *PR-10* and *profilin* allergen families across 11 medicinal and culinary herbs commonly used in Europe. Both methodologies successfully amplified target genomic regions in all examined species, generating distinct amplification profiles and a high degree of polymorphism (P = 100%). The PBAP system, targeting profilin homologues, produced a clustering pattern that largely corresponded with taxonomic relationships, particularly within the order *Lamiales*, whereas the BBAP system, based on Bet v 1 (PR-10) homologues, revealed more complex interspecific variation independent of phylogenetic affiliation.

Overall, this work highlights the potential of DNA marker-based approaches as cost-effective and rapid screening tools for the preliminary identification of allergen gene homologues in plant genomes. Their application may enhance the molecular characterization of medicinal and aromatic herbs, inform future sequencing and expression analyses.

Acknowledgements

This publication was supported by the Operational program Integrated Infrastructure within the project: Demand-driven research for the sustainable and innovative food, Drive4SIFood 313011V336 and KEGA 001SPU-4/2025 Internationalization of textbooks with genetic and molecular-security background in the context of activating education.

References

[1] Aberer, W. (2008), *Contact allergy and medicinal herbs*. JDDG: Journal der Deutschen Dermatologischen Gesellschaft, 6(1), pp.15-24. https://doi.org/10.1111/j.1610-0387.2007.06425.x

[2] Alvarado, M. I., Jimeno, L., De La Torre, F., Boissy, P., Rivas, B., Lázaro, M. J., & Barber, D. (2014), *Profilin as a severe food allergen in allergic patients overexposed to grass pollen*. Allergy, 69(12), pp.1610–1616. https://doi.org/10.1111/all.12509

[3] Amiryousefi, A., Hyvönen, J., & Poczai, P. (2018), *iMEC: online marker efficiency calculator.* Applications in Plant Sciences, 6(6), e01159. https://doi.org/10.1002/aps3.1159

[4] Amiteye, S. (2021), *Basic concepts and methodologies of DNA marker systems in plant molecular breeding*. Heliyon, 7(10), e08093. https://doi.org/10.1016/j.heliyon.2021.e08093

[5] 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*. Clinical & Experimental Allergy, 38(6), pp.1033–1037. https://doi.org/10.1111/j.1365-2222.2008.02980.x

[6] Carović-Stanko, K., Petek, M., Grdiša, M., Pintar, J., Bedeković, D., Herak Ćustić, M., & Satovic, Z. (2016), *Medicinal plants of the family Lamiaceae as functional foods – a review*. Czech Journal of Food Sciences, 34(5), pp.377–390. https://doi.org/10.17221/504/2015-CJFS

[7] Denisow-Pietrzyk, M., Pietrzyk, Ł., & Denisow, B. (2019), *Asteraceae species as potential environmental factors of allergy.* Environmental Science and Pollution Research, 26, pp. 6290–6300. https://doi.org/10.1007/s11356-019-04146-w

[8] Fernandes, H., Michalska, K., Sikorski, M., & Jaskolski, M. (2013), *Structural and functional aspects of PR-10 proteins*. The FEBS Journal, 280(5), pp.1169–1199. https://doi.org/10.1111/febs.12114

[9] Haggag, E. G., Abou-Moustafa, M. A., Boucher, W., & Theoharides, T. C. (2003), *The effect of a herbal water-extract on histamine release from mast cells and on allergic asthma*. Journal of Herbal Pharmacotherapy, 3(4), pp.41–54. https://doi.org/10.1080/J157v03n04_03

[10] Hauser, M., Roulias, A., & Ferreira, F. (2010), *Panallergens and their impact on the allergic patient*. Allergy, Asthma & Clinical Immunology, 6, 1. https://doi.org/10.1186/1710-1492-6-1

[11] Ključevšek, T., & Kreft, S. (2025), *Allergic potential of medicinal plants from the Asteraceae family*. Health Science Reports, 8(2), e70398. https://doi.org/10.1002/hsr2.70398

[12] Klongová, L., Kováčik, A., Urbanová, L., Kyseľ, M., Ivanišová, E., & Žiarovská, J. (2021), *Utilization of specific primers in legume allergens based polymorphism screening*. Science, Technology and Innovation, 13(2), pp.12–21.

[13] Krishnan, K., & Moens, P. D. J. (2009), *Structure and functions of profilins*. Biophysical Reviews, 1, pp.71–81. https://doi.org/10.1007/s12551-009-0010-y

[14] Linacero, R., Sanchiz, A., Ballesteros, I., & Cuadrado, C. (2019), *Application of real-time PCR for tree nut allergen detection in processed foods*. Critical Reviews in Food Science and Nutrition*, 60*(7), pp. 1077–1093. https://doi.org/10.1080/10408398.2018.1557103

[15] Oksanen, J., Simpson, G., Blanchet, F. et al. (2025), *vegan: Community Ecology Package.* R package version 2.7-1. https://CRAN.R-project.org/package=vegan

[16] Preda, M., Smolinska, S., & Popescu, F.-D. (2024), *Diagnostic workup in IgE-mediated allergy to Asteraceae weed pollen and herbal medicine products in Europe.* Medicina, 60(9), 1494. https://doi.org/10.3390/medicina60091494

[17] Radauer, C., & Breiteneder, H. (2007), *Evolutionary biology of plant food allergens*. Journal of Allergy and Clinical Immunology, 120(3), pp.518–525. https://doi.org/10.1016/j.jaci.2007.07.024

[18] Serrote, C. M. L., Reiniger, L. R. S., Silva, K. B., Rabaiolli, S. M. S., & Stefanel, C. M. (2020), *Determining the polymorphism information content of a molecular marker.* Gene, 726, 144175. https://doi.org/10.1016/j.gene.2019.144175

[19] Słowianek, M., & Majak, I. (2011), *Methods of allergen detection based on DNA analysis*. Biotechnology and Food Science, *76*(2), pp. 39–44. https://doi.org/10.34658/bfs.2011.75.2.39-44

[20] Török, K., Hajas, L., & Horváth, V. (2015), *Identification of the factors affecting the analytical results of food allergen ELISA methods*. European Food Research and Technology*, 241*, pp. 127–136. https://doi.org/10.1007/s00217-015-2441-y

[21] Urbanová, L., & Žiarovská, J. (2021), *Variability of DNA-based amplicon profiles generated by Bet v 1 homologous among different vegetable species*. Acta Fytotechnica et Zootechnica, 24(1), pp.1–6. https://doi.org/10.15414/afz.2021.24.mi-apa.1-6

[22] Vieths, S., Scheurer, S., & Ballmer-Weber, B. (2002), *Current understanding of cross-reactivity of food allergens and pollen.* Annals of the New York Academy of Sciences, 964(1), pp.47–68. https://doi.org/10.1111/j.1749-6632.2002.tb04132.x

[23] Wagner, W., Buczyłko, K., Wagner, A., Szwed-Kowalska, A., & Stasiak, A. (2023), *Higher risk for sensitization to commonly consumed herbs among adults and youngsters suffering from birch, mugwort or grass pollinosis.* International Journal of Environmental Research and Public Health, 20(1), 33. https://doi.org/10.3390/ijerph20010033