Agrobiodiversity for Improving Nutrition, Health and Life Quality

Research Article OPEN ACCESS

In silico Identification of Sequential Similarities of Selected Lipid Transfer Proteins

Lucia Hovaňáková[1], Lucia Klongová[2], Jana Žiarovská*[1]

[1]Slovak University of Agriculture in Nitra, Faculty of Agrobiology and Food Resources, Institute of Plant and Environmental Sciences, Nitra, Slovak Republic
[2]Slovak University of Agriculture in Nitra, Department of Agrobiology, Research Centre Agrobiotech, Slovak Republic

Lucia Hovaňáková: https://orcid.org/0000-0002-6466-4645
Lucia Klongová: https://orcid.org/0000-0003-1111-7709
Jana Žiarovská: https://orcid.org/0000-0002-0005-9729

Article Details:
Received: 2023-12-05
Accepted: 2024-04-30
Available online: 2024-05-31

DOI: https://doi.org/10.15414/ainhlq.2024.0001

Nonspecific lipid transfer proteins (nsLTP) are common allergens discovered and described 40 years ago. They are present in many plant species and protect plants from stressors such as heat and drought. Studies on the peach nsLTP, Pru p 3, show that nsLTP are very cross-reactive through presented IgE epitopes shared by nsLTP from other botanically related fruits. These allergens are to varying degrees resistant to heat and digestion, and sensitization may occur through the oral, cutaneous, or inhaled routes. In the last years, several web tools for the prediction of allergenicity of new molecules based on their homology with known allergens have been created, and have established guidelines to assess potential allergenicity of proteins through bioinformatics. Here, Allerbase and NCBI Protein BLAST were applied to characterize the protein sequences of nsLTPs of plant species – *Malus domestica* Borkh., *Prunus persica* (L.) Batsch., *Daucus carota* (L.), *Vitis vinifera* (L.) and *Solanum lycopersicum* (L.). Obtained primary data were evaluated by the algorithm NCBI BLAST (Basic Local Alignment Search Tools). This tool allows searching similar protein sequences, in this case, was used Protein BLAST for comparing protein sequences. Most of the analysed sequences displayed a high probability of being allergenic according to the high sequencing identity and sharing IgE epitopes to each other. The highest sequencing identity in preselected plant species was presented between Pru p 3 (isoform Pru p 3.0102) and Mal d 3 (isoform Mal d 3.0101), 91.32% and they share the same epitope, NNA. The lowest sequencing homology was identified between Mal d 3.0203 and Dau c LTP, 43.10% and they did not share any epitope to each other.

Keywords: nsLTP, *in silico* analyses, IgE epitope, sequence identity, homology

***Corresponding Author:** Jana Žiarovská, Faculty of Agrobiology and Food Resource, Institute of Plant and Environmental Sciences, Slovak University of Agriculture in Nitra, Tr. Andreja Hlinku 2, 949 76, Nitra, Slovak Republic
jana.ziarovska@uniag.sk

Introduction

Non-specific lipid transfer proteins (LTP), 7–9 kDa proteins present in high concentration (around 4% of the total soluble proteins) in higher plants (Kader, 1996; Yeast and Rose, 2008), are one of the most common causes of primary food allergy in the Mediterranean area, where it induced the largest number of food-dependent anaphylactic reactions. A gradient exists in the prevalence of LTP sensitization between northern and southern regions (Asero et al., 2009). This peculiar geographical distribution is still poorly described, the hypotheses of primary sensitization are probably associated with genetic factors, abundance of cultivation/maintenance, number of harvest (the harvest in the Mediterranean can take up to 8–12 months), and year-round consumption. However, the main factor is associated with the prevalence of flowering trees to which the population is exposed. The pollen in these trees has cross allergens with LTPs. Observation of data suggests, that the presence of birch pollen protects against the symptoms of LTP allergy; the higher the levels of birch pollen, the lower the prevalence of LTP in different parts of the world (Vereda et al., 2011).

Thanks to the structural homology among LTPs from botanically unrelated species and their widespread distribution throughout the plant kingdom, patients may become sensitized after ingesting a large array of plant foods. LTPs are pepsin and thermal- resistant, they remain in an unmodified form in the intestinal tract, which is the essential condition to induce systemic reaction (Asero et al., 2002).

Most LTPs are located in the superficial layers of fruits (peach, apple), and some are present in higher concentrations in seeds, which are eaten with pulp (kiwi, tomato). Other seeds, like almonds, have no significant amount of LTP, but they contain a peel that is frequently eaten along with the seed and probably contains it (Pravettoni et al., 2009; Bernadri et al., 2011; Giangrieco et al., 2015).

LTP families are divided into two subfamilies, which are different in their molecular mass, LTP 1–9 kDa – which includes the majority of LTP proteins, and LTP 2–7 kDa which includes a much more limited number of representatives.

These subfamilies share a general molecular structure but indicate low sequence similarity – about 30% sequence identity. Peach is the most probable cause of LTP sensitization in an endemic area and according to Pastorello et al. (2013) a peach contains most of LTP epitopes. Based on sequence identity between LTPs, after peach, the most offending foods belong to the Rosaceae family (apple, pear, apricot, plum, almond, etc.), followed by hazelnut, walnut, and peanut. Asero et al. (2013) reported a study of LTP-safe plant foods with a conclusion that plant foods like carrots, bananas, melons and potatoes were clinically tolerated by all LTP-allergic patients studied previously (Asero, 2007). In melon, LTP are located on the surface and may induce contact dermatitis after handling (Gandolfo-Cano, 2014). LTPs isolated from banana can cause adverse reaction in a selected paediatric population (Palacin et al., 2011). Allergic reaction to other plant foods such as cereals, tomato, onion, fennel, celery, broccoli, and saffron are rarer and prevail in patients with very elevated IgE level to Pru p 3 (Pastorello, 2013).

Here, *in silico* approach was used to identify conserved parts in the aminoacid sequences of lipid transfer proteins in selected plant species to be able to select the regions suitable for the design of degenerate primers as a base for a new DNA marker technique.

Material and methodology

Biological material

A total of five plant species were selected – *Malus domestica* Borkh., *Prunus persica* (L.) Batsch., *Daucus carota* (L.), *Vitis vinifera* (L.) and *Solanum lycopersicum* (L.) (Table 1) for sequentional characteristics of nsLTP.

All the species were selected based on our previous studies where homologous sequences of allergen coding genes were *in silico* evaluated and a functional DNA marker techniques were developed for this genes (Žiarovská and Urbanová, 2022; Čerteková et al., 2023).

Applied algorithms

First, identification of LTP proteins was performed for selected plant species. Allerbase was used to search for allergens and their isoforms (Table 1).

Selected primary sequences data were evaluated by the algorithm NCBI BLAST (Basic local Alignment Search Tools) (Altschul et al., 1990; Altschul et al., 1997) for cross-aligning of the obtained sequences for individual intraspecific variability of isoform as well as for interspecific variability among selected plants. Output data were shown in a defined table with indicators such as sequences, alignment, and score.

The main parameters evaluated were query cover and percentage of identity. The query cover is a number that describes how much of the query sequence was

Table 1 Plant species selected for bioinformatic analysis

Species	Isoform of allergen	NCBI Protein (GenPept) accession code
Prunus persica	Pru p 3.0101	P81402
	Pru p 3.0102	CAB96876
Malus domestica	Mal d 3.0101	AAT80633
	Mal d 3.0101	AAT80634
	Mal d 3.0101	AAT80635
	Mal d 3.0101	AAT80636
	Mal d 3.0101	AAT80637
	Mal d 3.0102	AAT80649
	Mal d 3.0201	AAT80650
	Mal d 3.0201	AAT80653
	Mal d 3.0201	AAT80654
	Mal d 3.0201	AAT80655
	Mal d 3.0201	AAT80656
	Mal d 3.0202	AAT80663
	Mal d 3.0202	AAT80664
	Mal d 3.0203	AAT80665
Daucus carota	Dau c LTP	AAB96834
Vitis ***vinifera***	Vit v 1.0101	AAO33394
Solanum lycopersicum	Sola l 3.0101	AAB42069

covered by the target sequence. The percent identity is a number that describes how similar the query sequence is to the target sequence (how many characters in each sequence are identical).

In the obtained amino acid sequences IgE epitopes were marked and were obtained their sharing with each other.

Statistical analysis

Results processing and evaluation was handled with the basic software device Microsoft Office Excel 2007 and Microsoft Office Word 2007 (WINDOWS 10 Home). Free-available databases NCBI GenBank and Allerbase served as material resources and also free-available tools NCBI BLAST among selected sequences.

Results and discussion

Pru p 3, the peach allergen, is a non-specific lipid transfer protein type 1 known as a precursor for other nsLTP-related sensitizations (Pravettoni et al., 2009; Bernadri et al., 2011; Asero et al., 2007, 2013; Pastorello, 2013; Gandalfo-Cano, 2014; Giangrieco et al., 2015). Peach LTP operates as an archetype of the LTP family due to its broad distribution among the plant species. It mostly cross-reacts with most members of the Rosaceae family, such as apple, cherry, plum, apricot, and with other families such as hazelnut, peanut, tomato, onion, and carrot. Pru p 3 acts as a marker allergen for severe systemic symptoms and it is a diagnostic indicator for detecting allergy to the Rosaceae family. Peach LTPs are expressed as two isoforms: LTP 1 – mostly found in pollinated flowers and LTP 2 – usually found in the ovary (Allergen Encyclopedia, 2022). In peach Pru p 3 six amino acid sequences were identified, which were bound IgE antibodies – known as epitopes (Table 2).

Due to the sharing of LTP homologous IgE-epitopes in botanically unrelated species, cross-reactivity to one another can occur (Asero et al., 2002; Sharma, Vitte, 2004). Pru p 3 is referred to as plant panallergen as it has widespread distribution among plant-foods and pollens. The difference between the two isoforms of Pru p 3 is only 2.2% and they are varied in two epitopes. Pru p 3.101 contains epitopes in 72–91 sequence positions (violet in Figure 1) and are different from each other in 76. amino sequence positions. Epitop of Pru p 3.101 is situated in 78. –80. sequences position (Figure 1) and is different from another isoform in 76. sequence position.

Pru p 3 shows a significantly similar identity to apple LTP – Mal d 3 – from 76,92% to 91,32%. Pru p 3 contains apple LTP epitope – NNA from 64. sequence position (Figure 2).

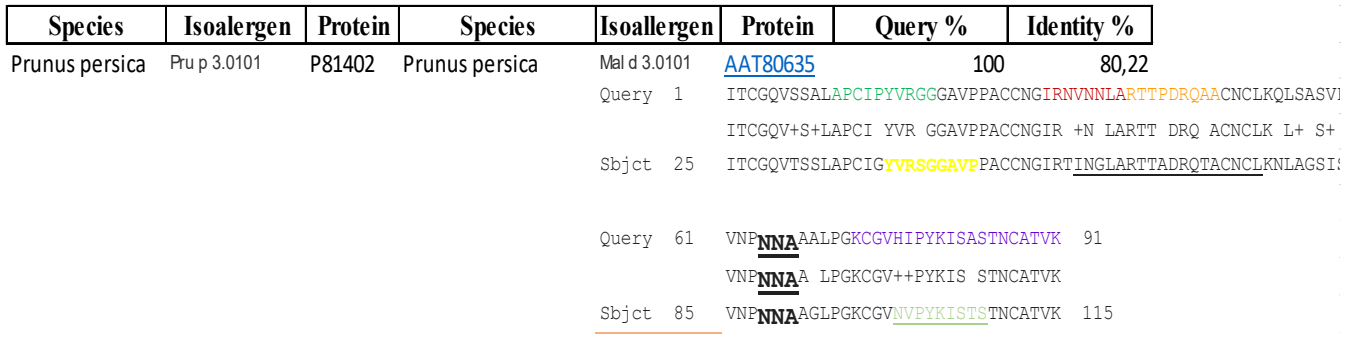

Figure 1 The epitopes of Pru p 3 isoforms and their query cover and identity
Standard IUPAC codes are used for aminoacids

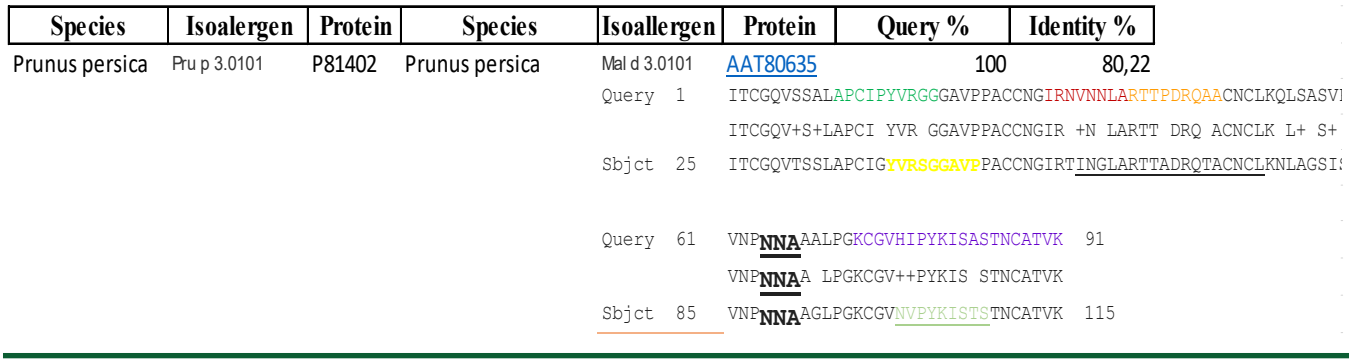

Figure 2 Epitopes of Pru p 3.0101 and Mal d 3.0101
Standart IUPAC codes are used for aminoacids

Pru p 3 shares 62.92% sequence identity with the allergen of *Vitis vinifera* Vit v 1. Unrelated species such as carrots, amino sequence identity is between 55.06–56.18%. They share the same epitope in structures – NNA. The lowest similarity species is between peach and tomato allergen Sola l 3 – 49.45% but it is still enough for cross-reactivity to occur. Mal d 3 is a non-specific lipid transfer protein (nsLTP) and is known as an important pan-allergen of apple (*Malus domestica*) (Sancho et al., 2006). The most reported cases of apple allergy are in European countries but especially in the Mediterranean regions. Mal d 3 is concentrated in the epidermis of the apple, but it depends on the type of cultivated apple, storage after harvest, position of the tree, and apple maturity. Apple LTP is a highly thermostable protein, and it is resistant to pepsin digestion (Allergen Encyclopedia, 2022). Sancho et al. (2006) state that the concentration of Mal d 3 is higher in apple grown in the shady site compared to sunny sites and in fully mature fruit ready for consumption. Mal d 3 occurs in two isoforms – Mal d 3.01 and Mal d 3.02, in which the sequence identity is between 85.22 to 98.26%. Mal d 3 sensitization can double as a marker for primary sensitization to apple

Table 2 LTP epitopes and cross-reactive allergens of Pru p 3 by Allerbase database

Allergen	Cross reactive allergen	Isoallergen	Locus	Epitope	Type of epitope	Aminoacid position
Pru p 3	Mal d 3	Pru p 3.0101	P81402	APCIPYVRGG	sequentional/linear	11–20
	Pru ar 3	–	–	IRNVNNLARTTPDRQ	sequentional/linear	31–45
	Zea m 14	–	–	GKCGVSIPYK	sequentional/linear	71–80
	Ory s LTP	–	–	RTTPDRQAA	sequentional/linear	39–47
	Pru d 3	–	–	RNVNNLARTT	sequentional/linear	32–41
	Vit v 1	–	–	KCGVHIPYKISASTNC	–	–
	Jug r 3	Pru p 3.0102	CAB96876	ATVK	sequentional/linear	72–91

Note: Standard IUPAC codes are used for aminoacids

Table 3 The query cover and sequence identity between Mal d 3.0101 and its isoforms and other LTP allergens

Isoallergen	Protein	Isoallergen	Protein	Query (%)	Identity (%)
Mal d 3.0101	AAT80633	Mal d 3.0101	AAT80634	100	100
		Mal d 3.0101	AAT80635	100	100
		Mal d 3.0101	AAT80636	100	100
		Mal d 3.0101	AAT80637	100	100
		Mal d 3.0102	AAT80649	100	98.26
		Mal d 3.0201	AAT80650	100	86.96
		Mal d 3.0201	AAT80653	100	86.96
		Mal d 3.0201	AAT80654	100	86.96
		Mal d 3.0201	AAT80655	100	86.96
		Mal d 3.0201	AAT80656	100	86.96
		Mal d 3.0202	AAT80663	100	85.22
		Mal d 3.0202	AAT80664	100	85.22
		Mal d 3.0203	AAT80665	100	85.22
		Dau c LTP	AAB96834	99	49.14
		Vit v 1.0101	AAO33394	99	55.08
		Sola l 3.0101	AAB42069	81	53.19

Note: Standard IUPAC codes are used for aminoacids

allergy and can differentiate true apple allergy from birch-apple syndrome. Extensive cross-reactivity may be observed between Mal d 3 and LTP-containing foods (Gomez et al., 2014; Chebib et al., 2022). The highest amino sequence identity observed in species has Mal d 3 with Pru p 3 – 76.92–91.32% (Table 3).

The similarity between Mal d 3.01 and the LTP of *Vitis vinifera* L. is 50.05% and with Mal d 3.02 it is 55.93%. Sola l 3 shares with Mal d 3 identity equal to 52.66% and with Mal d 3.02 about 51.06%.

In Mal d 3, four epitopes are identified. The epitope NNA shares with all of its isoforms and also with Pru p 3 and Dau c LTP. The epitope YVRSGGAVP is currently only in isoform Mal d 3.0101. Place for binding with IgE antibodies – NVPYKSTS is present in every apple isoform LTP.

Daucus carota is one of the most important and popular widely cultivated root vegetables. Carrot sensitization is found wide across Europe and in the USA, Mexico, and some countries of Asia. Ingestion of carrot can trigger pollen-food-allergy syndrome associated with birch pollens. Dau c 1 was reported as a major allergen which is homologous to Bet v 1, the birch pollen allergen. According to Uter et al. (2017), systemic reaction was developed in about 50% of carrot-allergic patients. Dau c LTP shares the same epitopes as Pru p 3 and Mal d 3 but has yet not found a negative influence on the formation of allergic reactions. These allergens share sequence identity from 43.97–56.18%. With Vit v 1 similarity in sequence equals to 50.46% and with Sola l 3 to 55.56% (Figure 3).

Vitis vinifera is one of the oldest cultivated plants (Grassi, Arroyo-Garcia, 2020; Guzmán-Ardiles et al., 2023). It is grown especially around the Mediterranean, but it is consumed all over the world also in the form of juice or wine. Some patients who are allergic to grapes can tolerate wine. Vit v 1 is the main allergen of vitis vinifera with sensibilization from 70 to 100% in grape-allergic individuals (Allergen Encyclopedia, 2022). Vit v 1 shares the highest sequence homology with Pru p 3 – 62.92%. With Mal d 3 the sequence identity is

Table 4 LTP epitopes and cross-reactive allergens of Mal d 3 by Allerbase database

Allergen	Cross reactive allergen	Isoallergen	Locus	Epitope	Type of epitope	Aminoacid position
Mal d 3	Pru p 3	Mal d 3.0101	AAT80633	YVRSGGAVP	sequentional/linear	40–48
	Fra a 3	Mal d 3.0101	AAT80634	INGLARTTADRQTACI	sequentional/linear	58–75
	Pru p 3	Mal d 3.0101	AAT80635	NNA	sequentional/linear	88–90
	-	Mal d 3.0101	AAT80636	NVPYKISTS	sequentional/linear	100–108

	Pru p 3.0101	Pru p 3.0102	Mal d 3.0101	Mal d 3.0101	Mal d 3.0101	Mal d 3.0101	Mal d 3.0101	Mal d 3.0102	Mal d 3.0201	Mal d 3.0201	Mal d 3.0201	Mal d 3.0201	Mal d 3.0201	Mal d 3.0202	Mal d 3.0202	Mal d 3.0203	Dau c LTP	Vit v 1.0101	Sola l 3.0101
Pru p 3.0101	x	97.80	80.22	80.22	80.22	80.22	80.22	80.22	78.02	78.02	78.02	78.02	78.02	76.92	76.92	76.92	56.18	62.92	49.45
Pru p 3.0102	97.80	x	81.32	91.32	81.32	81.32	81.32	81.32	78.02	78.02	78.02	76.92	78.02	76.92	76.92	76.92	55.06	62.92	49.45
Mal d 3.0101	80.22	81.32	x	100.00	100.00	100.00	100.00	98.26	86.96	86.96	86.96	86.96	86.96	85.22	85.22	85.22	49.14	55.08	53.19
Mal d 3.0101	80.22	91.32	100.00	x	100.00	100.00	100.00	98.26	86.96	86.96	86.96	86.96	86.96	85.22	85.22	85.22	49.14	55.08	53.19
Mal d 3.0101	80.22	81.32	100.00	100.00	x	100.00	100.00	98.26	86.96	86.96	86.96	86.96	86.96	85.22	85.22	85.22	49.14	55.08	53.19
Mal d 3.0101	80.22	81.32	100.00	100.00	100.00	x	100.00	98.26	86.96	86.96	86.96	86.96	86.96	85.22	85.22	85.22	49.14	55.08	53.19
Mal d 3.0101	80.22	81.32	100.00	100.00	100.00	100.00	x	98.26	86.96	86.96	86.96	86.96	86.96	85.22	85.22	85.22	49.14	55.08	53.19
Mal d 3.0102	80.22	81.32	98.26	98.26	98.26	98.26	98.26	x	85.22	85.22	85.22	85.22	85.22	83.48	83.48	83.48	48.28	55.93	52.13
Mal d 3.0201	78.02	78.02	86.96	86.96	86.96	86.96	86.96	85.22	x	100.00	100.00	100.00	100.00	98.26	98.26	98.26	43.97	55.93	51.06
Mal d 3.0201	78.02	78.02	86.96	86.96	86.96	86.96	86.96	85,22	100.00	x	100.00	100.00	100.00	98.26	98.26	98.26	43.97	55.93	51.06
Mal d 3.0201	78.02	78.02	86.96	86.96	86.96	86.96	86,96	85,22	100.00	100.00	x	100.00	100.00	98.26	98.26	98.26	43.97	55.93	51.06
Mal d 3.0201	78.02	76.92	86.96	86.96	86.96	86.96	86.96	85.22	100.00	100.00	100.00	x	100.00	98.26	98.26	98.26	43.97	55.39	51.06
Mal d 3.0201	78.02	78.02	86,96	86.96	86.96	86.96	86.96	85.22	100.00	100.00	100.00	100.00	x	98.26	98.26	98.26	43.97	55.93	51.06
Mal d 3.0202	76.92	76.92	85.22	85.22	85.22	85.22	85.22	83.48	98.26	98.26	98.26	98.26	98.26	x	100.00	98.26	43.97	54.24	51.06
Mal d 3.0202	76.92	76.92	85.22	85.22	85.22	85.22	85.22	83.48	98.26	98.26	98.26	98.26	98.26	100,00	x	98.26	43.97	54.24	51.06
Mal d 3.0203	76.92	76.92	85.22	85.22	85.22	85.22	85.22	83.48	98.26	98.26	98.26	98.26	98.26	98,26	98.26	x	43.10	54.24	50.00
Dau c LTP	56.18	55.06	49.14	49.14	49.14	49.14	49.14	48.28	43.97	43.97	43.97	43.97	43.97	43,97	43.97	43.10	x	50.46	55.56
Vit v 1.0101	62.92	62.92	55.08	55.08	55.08	55.08	55.08	55.93	55.93	55.93	55.93	55.39	55.93	54,24	54.24	54.24	50.46	x	56.04
Sola l 3.0101	49.45	49.45	53.19	53.19	53.19	53.19	53.19	52.13	51.06	51.06	51.06	51.06	51.06	5106	51.06	50.00	55.56	56.04	x

Figure 3 The sequence identity of nsLTP among analysed species

on average 55.23% similar to each other. Homology between Vit v 1 and Dau c LTP is equal to 50.5% and in Sola 3 it is a little bit higher – 56.40% (Figure 3). In the allergen, no epitopes have been identified.

Tomatoes are one of the most important popular vegetables which, are cultivated all over the world (Bai, Lindhout, 2007; Klee, Resende, 2020). however tomato-allergic individuals are not reported frequently (Allergen Encyclopedia, 2022). The highest homology in sequence shares with grape LTP protein – 56.04%, with Dau c LTP is identity equal to 55.56% and the lowest identity is with Pru p 3 – 49.45% (Figure 3). Sol l 3 does not contain any of the above-mentioned epitopes.

Plant allergens are typical with their great variability at the proteomic and genomic sequences and the multilevel analysis of these sequences is an actual part of research activities because of the raising number of food allergies worldwide. Previously, *in silico* approach was used for profilins and oleosins (Kyseľ et al., 2022) and the transfer of the *in silico* based predictions to an DNA marker techniques were applied for different plant species in the case of the analysis of Bet v 1 sequences as well as profilins (Speváková et al., 2021; Urbanová and Žiarovská, 2021; Kováčik et al., 2024).

Conclusion

Investigating the structure of nsLTP is of great importance due to the high degree of cross-reactivity between nsLTP from different plant species. These allergens, which are presented in fruits, vegetables, or nuts, are able to sensitize through oral, continuous, and respiratory routes. Here, the descriptions of sequence similarity was performed for selected plant species and the identification of conserved parts was done. These will provide a background for the development of DNA based marker technique, such as this approach was successful previously.

Conflict of interest

The authors have no conflicts of interest to declare.

Ethical statement

This article does not contain any studies that would require an ethical statement.

Funding

The work was financially supported under the Operational Programme Integrated Infrastructure for the project Long-term strategic research of prevention, intervention and mechanisms of obesity and its comorbidities, IMTS: 313011V344, co-financed by

the European Regional Development Fund (50%) and by the Operational Program Integrated Infrastructure within the project: Demand-driven Research for the Sustainable and Innovative Food, Drive4SIFood 313011V336, co-financed by the European Regional Development Fund (50%).

References

Allergen Encyclopedia, 2022. Online. [cit. 05. 04. 2022]. URL: https://www.thermofisher.com/diagnostic-education/hcp/wo/en/resource-center/allergen-encyclopedia.html

Altschul, S.F., Gish, W., Miller, W., Myers, E.W., & Lipman, D.J. 1990. Basic local alignment search tool. In *Journal of Molecular Biology*, 215(3), 403–410. https://doi.org/10.1016/S0022-2836(05)80360-2

Altschul, S.F., Madden, T.L., Schaffer, A.A., Zhang, J., Zhang, Z., Miller, W., & Lipman, D.J. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. In *Nucleic Acids Research*, 25(17), 3389–3402. https://doi.org/10.1093/nar/25.17.3389

Asero, R., Antonicelli, L., Arena, A., Bommarito, B., Caruso, M., Crivellaro, M., De Carli, E., Della Torre, F., Della Torre, E., Heffler, F., Lodi Rizzini, R., Longo, G., Manzotti, M., Marcotulli, A., Melchiorre, P., Minale, P., Morandi, B., Moreni, A., Moschella, F., Murzilli, F., Nebiolo, M., Poppa, S., Randazzo, G., Rossi, G., & Senna, G.E. 2009. EpidemAAITO: features of food allergy in Italian adults attending allergy clinics: a multicentre study. In *Clinical & Experimental Allergy*, 39(4), 547–555. https://doi.org/10.1111/j.1365-2222.2008.03167.x

Asero, R., Antonicelli, L., Arena, A., Bommarito, L., Caruso, B., Colombo, G., Crivellaro, M., De Carli, M., Della Torre, E., Della Torre, F., Heffler, E., Lodi Rizzini, F., Longo, R., Manzotti, G., Marcotulli, M., Melchiorre, A., Minale, P., Morandi, P., Moreni, B., Moschella, A., Murzilli, F., Nebiolo, F., Poppa, M., Randazzo, S., Rossi, G., & Senna, G.E. 2009. Causes of food-induced anaphylaxis in Italian adults: a multicentre study. In *International Archives of Allergy and Immunology*, 150(3), 271–277. https://doi.org/10.1159/000222679

Asero, R., Mistrello, G., Roncarolo, D., & Amato, S. 2007. Detection of some safe plant-derived foods for LTP-allergic patients. In *International Archives of Allergy and Immunology*, 144(1), 57–63. https://doi.org/10.1159/000102615

Asero, R., Mistrello, G., Roncarolo, D., Amato, S., Caldironi, G., Barocci, F., & van Ree, R. 2002. Immunological cross-eactivity between lipid transfer proteins from botanically unrelated plantderived foods: a clinical study. In Allergy, 57(10), 900–906. https://doi.org/10.1034/j.1398-9995.2002.t01-1-23541.x

Bai, Y., & Lindhout, P. 2007. Domestication and breeding of tomatoes: what have we gained and what can we gain in the future? In *Annals of Botany*, 100(5), 1085–1094. https://doi.org/10.1093/aob/mcm150

Bernardi, M.L., Giangrieco, I., Camardella, L., Ferrara, R., Palazzo, P., Panico, M.R., Crescenzo, R., Carratore, V., Zennaro, D., Liso, M., Santoro, M., Zuzzi, S., Tamburrini, M., Ciardiello, M.A., & Mari, A.2011. Allergenic lipid transfer proteins from plant-derived foods do not immunologically and clinically behave homogeneously: the kiwifruit LTP as a model. In *PLoS ONE*, 6(11), e27856. https://doi.org/10.1371/journal.pone.0027856

Čerteková, S., Kováčik, A., Klongová, L., & Žiarovská, J. 2023. Utilization of plant profilins as DNA markers. In *Acta Fytot Zoot*, 26(3), 324–331. https://doi.org/10.15414/afz.2023.26.03.324-331

Chebib, S., Meng, Ch., Ludwig, Ch., Bergmann, K.Ch., Becker, S., Dierend, W., & Schwab, W. 2022. Identification of allergenomic signatures in allergic and well-tolerated apple genotypes using LC-MS/MS. In *Food Chemistry: Molecular Sciences*, 4, 100111. https://doi.org/10.1016/j.fochms.2022.100111

Gandolfo-Cano, M., Bartra, J., Gonzalez-Mancebo, E., Feo-Brito, F., Gómez, E., Bartolomé, B., Muñoz-García, E., Sanz Maroto, A., Vivanco, F., Cuesta-Herranz, J., & Pastor-Vargas, C. 2014. Molecular characterization of contact urticaria in patients with melon allergy. In *British Journal of Dermatology,* 170(3), 651–656. https://doi.org/10.1111/bjd.12701

Giangrieco, I., Alessandri, C., Rafaiani, C., Santoro, M., Zuzzi, S., Tuppo, L., Tamburrini, M., D'Avino, R., Ciardiello, M.A., & Mari, A. 2015. Structural features, IgE binding and preliminary clinical findings of the 7 kDa Lipid Transfer Protein from tomato seeds. In *Molecular Immunology*, 66(2), 154–163. https://doi.org/10.1016/j.molimm.2015.02.025

Gomez, F., Aranda, A., Campo, P., Diaz-Perales, A., Blanca-Lopez, N., Perkins, J., Garrido, M., Blanca, M., Mayorga, C., & Torres, M.J. 2014. High prevalence of lipid transfer protein sensitization in apple allergic patients with systemic symptoms. In *PLoS One*, 9(9), e107304. https://doi.org/10.1371/journal.pone.0107304

Grassi, F., & Arroyo-Garcia, R. 2020. Editorial: Origins and domestication of the grape. In *Frontiers in Plant Science*, 11, 176. https://doi.org/10.3389/fpls.2020.01176

Guzmán-Ardiles, R.E., Pegoraro, C., da Maia, L.C., & Costa de Oliveira, A. 2023. Genetic changes in the genus *Vitis* and the domestication of vine. In *Frontiers in Plant Science*, 13, 1019311. https://doi.org/10.3389/fpls.2022.1019311

Kader, J-C. 1996. Lipid-transfer protein in plants. In *Annual Review of Plant Physiology and Plant Molecular Biology*, 47, 627–654. https://doi.org/10.1146/annurev.arplant.47.1.627

Klee, H.J, & Resende, M.F.R. 2020. Plant domestication: Reconstructing the route to modern tomatoes. In *Current Biology*, 30, 340–361. https://doi.org/10.1016/j.cub.2020.02.072

Kováčik, A., Žiarovská, J., & Urbanová, L. 2024. Variability of allergen-based length polymorphism of *Glycine max* L. varieties. In *Biology and Life Sciences Forum*, 30(1), 20. https://doi.org/10.3390/IOCAG2023-16879

Kyseľ, M., Farkasová, S., Štefúnová, V., Zeleňáková, L., & Žiarovská, J. 2022. *In silico* approach in the analysis of allergenic profilins and oleosins of *Amaranthus* spp. In *Journal of Hygienic Engineering and Design*, 41, 84–89.

Palacin, A., Quirce, S., Sanchez-Monge, R., Bobolea, I., Diaz-Perales, A., Martin-Muñoz, F., Pascual, C., & Salcedo, G. 2011. Sensitization profiles to purified plant food allergens among pediatric patients with allergy to banana. In *Pediatric Allergy and Immunology*, 22(2), 186–195. https://doi.org/10.1111/j.1399-3038.2010.01125.x

Pastorello, E.A., Farioli, L., Stafylaraki, C., Scibilia, J., Giuffrida, M.G., Mascheri, A., Piantanida, M., Baro, C., Primavesi, L., Nichelatti, M., Schroeder, J.W., Pravettoni, V.2013. Fennel allergy is a lipidtransfer protein (LTP)-related food hypersensitivity associated with peach allergy. In *Journal of Agricultural and Food Chemistry*, 61(3), 740–746. https://doi.org/10.1021/jf303291k

Pastorello, E.A., Scibilia, J., Farioli, L., Primavesi, L., Giuffrida, M.G., Mascheri, A., Piantanida, M., Mirone, C., Stafylaraki, C.H., Violetta, M.R., Nichelatti, M., Preziosi, D., Losappio, L., & Pravettoni, V. 2013. Rice allergy demonstrated by double-blind placebo-controlled food challenge in peach-allergic patients is related to lipid transfer protein reactivity. In *International Archives of Allergy and Immunology*, 161(3), 265–273. https://doi.org/10.1159/000345974

Pravettoni, V., Primavesi, L., Farioli, L., Brenna, O.V., Pompei, C., Conti, A., Scibilia, J., Piantanida, M., Mascheri, A., & Pastorello, E.A. 2009. Tomato allergy: detection of IgE-binding lipid transfer proteins in tomato derivatives and in fresh tomato peel, pulp, and seeds. In *Journal of Agricultural and Food Chemistry*, 57(22), 10749–10754. https://doi.org/10.1021/jf9022367

Sancho, A.I., Foxall, R., Rigby, N.M., Browne, T., Zuidmeer, L., van Ree, R., Waldron, K.W., & Clare Mills, E.N. 2006. Maturity and storage influence on the apple (*Malus domestica*) allergen Mal d 3, a nonspecific lipid transfer protein. In *Journal of Agricultural and Food Chemistry*, 54(14), 5098–5104. https://doi.org/10.1021/jf0530446

Sharma, V., & Vitte, J. 2024. A systematic review of allergen cross-reactivity: Translating basic concepts into clinical relevance. In Journal of allergy and clinical immunology, 3 (2), 100230. https://doi.org/10.1016/j.jacig.2024.100230

Speváková, I., Urbanová, L., Kyseľ, M., Bilčíková, J., Farkasová, S.,&ŽiarovskáJ.2021.BBAPamplificationprofilesofapple varieties. In *Science, Technologies, Innovations*, 12(1), 1–6. https://doi.org/10.5604/01.3001.0015.5233

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

Uter, W., Amario-Hita, J.C., Balato, A., Ballmer-Weber, B., Bauer, A., Belloni Fortina, A., Bircher, A., Chowdhury, M.M.U., Cooper, S.M., Czarnecka-Operacz, M., Dugonik, A., Gallo, R., Giménez-Arnau, A., Johansen, J.D., John, S.M., Kieć-Świerczyńska, M., Kmecl, T., Kręcisz, B., Larese Filon, T., Mahler, V., Pesonen, M., Rustemeyer, T., Sadowska-Przytocka, A., Sánchez-Pérez, J., Schliemann, S., Schuttelaar, M.L., Simon, D., Spiewak, R., Valiukevičienė, S., Weisshaar, E., White, I.R., & Wilkinson, S.M. 2017. European Surveillance System on Contact Allergies (ESSCA): results with the European baseline series, 2013/14. In *Wiley online library*. https://doi.org/10.1111/jdv.14423

Vereda, A., van Hage, M., Ahlstedt, S., Ahlstedt, S., Ibañez, M.D., Cuesta-Herranz, J., van Odijk, J., Wickman, M., & Sampson, H.A. 2011. Peanut allergy: Clinical and immunologic differences among patients from 3 different geographic regions. In *Journal of Allergy and Clinical Immunology*, 127(3), 603–607. https://doi.org/10.1016/j.jaci.2010.09.010

Yeats, T.H., & Rose, J.K. 2008. The biochemistry and biology of extracellular plant lipid-transfer proteins (LTPs). In *Protein Science*, 17(2), 191–198. https://doi.org/10.1110/ps.073300108

Žiarovská, J., & Urbanová, L. 2022. Utilization of Bet v 1 homologs based amplified profile (BBAP) variability in allergenic plants fingerprinting. In *Biologia*, 77, 517–523. https://doi.org/10.1007/s11756-021-00943-2