Acta fytotechn zootechn, 26, 2023(3): 324–331
http://www.acta.fapz.uniag.sk

Original Paper

Utilization of plant profilins as DNA markers

Simona Čerteková, Adam Kováčik, Lucia Klongová, Jana Žiarovská*

Slovak University of Agriculture in Nitra, Faculty of Agrobiology and Food Resources, Institute of Plant and Environmental Sciences, Slovakia

Article Details: Received: 2023-03-20 | Accepted: 2023-05-30 | Available online: 2023-09-30

https://doi.org/10.15414/afz.2023.26.03.324-331

This study aims to explore the possibility of utilizing markers derived from profilin sequences for the genomic fingerprinting of plant organisms. Profilins are a category of small actin-binding proteins that are present in all eukaryotic cells. Despite profilins being ubiquitous, some forms are also clinically relevant because of their ability to induce allergic responses in sensitized individuals. We conducted a PCR analysis on DNA samples obtained from 11 vegetable species (*Brassica oleracea* L. in 4 varieties) using two types of primers: non-degenerate and degenerate. In the case of degenerate primers, a total of 51 amplification products of different lengths were recorded, while their average amount was in the range of 7–8 amplicons for one species. The most frequently occurring product was the product with a length of 249 bp. A lower degree of polymorphism was noted when non-degenerate primers were used. The total number of different products created by amplification using non-degenerate primers was 33 and there was an average of 5 amplicons in one sample. As indicated by the findings, implementation of degenerate primers was more suitable for genomic fingerprinting based on profilin sequences in vegetable species, as it led to a higher level of variability in the amplification profiles of distinct species. It can be stated that amplification based on profilin sequences proved to be sufficient in its versatility and efficiency in generating variable-length polymorphism of PCR products.

Keywords: profilins, PCR, length polymorphism, DNA marker

1 Introduction

Profilins, a large protein family characterized by their relatively small size with a molecular weight of 14–16 kDa, were first identified by Carlsson and colleagues in 1977 (Carlsson et al., 1977). They are essential for the function of eukaryotic cells, because of their ability to bind to monomeric G-actin (globular actin), thus regulating the polymerization of actin filaments (Schlüter, Jockusch and Rothkegel, 1997; Rodríguez del Río et al., 2018). Actin filaments are one of the three basic elements of the cytoskeleton of cells, together these elements are enabling cells to change and adapt their shape during their existence based on stimuli coming from the internal or external environment. Rapid reorganization of the cytoskeleton is essential for the cell, especially in processes such as cytokinesis, cell movement, cell elongation, etc. (Mills and Shewry, 2004). In plant organisms, profilins as regulators of actin filament remodelling play an important role in processes such as the growth of the pollen sac (Qu et al., 2015), the formation of the phragmoplast involved in the formation of the cell wall (Maeda et al., 2020), or the photorelocation of chloroplasts (Yamashita et al., 2011). In addition to actin monomers, profilins can also bind to poly-l-proline derived sequences and polyphosphoinositides (Mahoney et al., 1997; Mahoney et al., 1999; Davey and Moens, 2020). The existence of several profilin isoforms, verified in higher plants, may be attributed to the complexity of actin proteins in these organisms, as opposed to animals, where the number of isoforms is comparatively lower (Huang et al., 1996; Jimez-Lopez et al., 2012). Profilins can be described as relatively well conserved proteins both in terms of their phylogeny and structure. They possess a noteworthy conservation of a critical actin-binding motif, while the C-terminal region displays a considerable degree of variation (Valenta et al., 1993; Sohn and Goldschmidt-Clermont, 1994). A sequence homology search indicated a high level of similarity among profilins. Alignment of the amino acid sequences of Cuc m 2, the major allergen of melon, with profilin sequences from

***Corresponding Author:** Jana Žiarovská, Slovak University of Agriculture in Nitra, Institute of Plant and Enviromental Sciences, Tr. Andreja Hlinku 2, 949 76 Nitra, Slovakia jana.ziarovska@uniag.sk

watermelon, tomato, Bermuda grass, banana, peach, and latex resulted in similarities of 89%, 84%, 80%, 77%, 87%, and 84%, respectively (Sankian et al., 2005). Amino acid sequences of profilins present in pollen of timothy grass and birch had identity of 79%. However, when these sequences were compared to profilin sequences from organisms belonging to different phyla, a similarity of only around 30% was recorded (Valenta et al., 1993). These results suggest that profilin sequences are well conserved among higher plants, both monocot and dicot species. Huang et al. (1996) compared sequences of 9 plant profilins and found that approximately 70 to 90% of the amino acids were identical. Nucleotide sequences coding for these profilins had an identity ranging from 68 to 90%. Taken together, these studies imply a relatively high level of conservation among plant profilin sequences, indicating their potential for use as molecular markers.

The first identified profilin capable of inducing an allergic response was Bet v 2, which was discovered in birch pollen in 1991 (Valenta et al., 1991) and since then many profilins capable of triggering allergenic reactions not only in pollen but also in plant foods and latex have been identified (Rodríguez del Río et al., 2018). Despite the fact that profilins were not considered relevant allergens in the past, nowadays, the presence of immunogenic profilins has been confirmed in almost all types of allergenic plant sources. Profilins are therefore one of the protein families, which, due to their widespread presence in nature, are also referred to as panallergenic (Wallner et al., 2017). High degree of similarity in some structural motifs, can also cause cross-allergic reactions between pollen, latex, and foods of plant origin. Primary sensitization usually occurs against profilins from pollen and subsequently formed IgE antibodies then cross-react with profilins present in food and latex. Potential cross-reactivity was demonstrated in a study conducted by Tordesillas et al. (2009) who analysed IgE antibodies derived from the sera of profilin-allergic patients. They found out that these antibodies are able to bind to various profilin homologues found in a large number of botanically distant plants. Clinical data, however, showed that these patients react only to a small number of possible profilin-containing allergen sources or do not show symptoms of food allergy at all (Tordesillas et al., 2009). Profilin-specific IgE has been found in around 20–30% of individuals with pollen allergy, with the prevalence varying depending on the geographical location (Ruiz-Hornillos et al., 2020). This suggests that despite being considered minor allergens, profilins have considerable clinical relevance.

Allergology has witnessed remarkable advancements in the past few decades, with new data on various allergens being constantly reported. However, most publications tend to focus on proteomic and clinical aspects of allergens. In this work, emphasis was placed mainly on the genetic characterization of variability of profilins originating from several types of commonly consumed vegetables. Several of the analysed vegetables contained profilins, which are officially recognized as allergenic by the WHO/IUIS subcommittee for allergen nomenclature. The primary objective of this study was to establish the applicability of plant profilins as DNA markers. This was achieved by investigating whether PCR amplification, using non-degenerate or degenerate primers derived from conserved domains of profilin sequences, could generate characteristic PCR amplification profiles for particular plant species. Compared to non-degenerate primers with specific sequences, degenerate primers incorporate one of multiple possible bases at specific positions, resulting in a collection of individual primers with slightly varying sequences. By employing this kind of primers, closely related sequences with non-identical nucleotides can be amplified (Kwok et al., 1994). The degeneracy of a primer refers to the count of distinct sequence combinations it encompasses (Linhart and Ahamir, 2005).

2 Material and methods

2.1 Biological material

In this work, we analysed 14 samples of commonly consumed vegetable species in Central Europe that were obtained from local supermarkets. These 14 samples originated from 11 different plant species, listed in Table 1.

2.2 DNA extraction

Genomic DNA was extracted from 100 mg of previously processed and frozen plant material. The frozen tissue of individual samples was ground to a fine powder and genomic DNA was subsequently isolated using GeneJET Plant Genomic DNA Purification Kit (ThermoFisher®). Nanophotometer P360 (Implen) was used to assess the quantity and quality of isolated DNA, which was subsequently diluted to a concentration of 1–5 ng.µl^{-1}, depending on the type of tissue and its contaminants. The quality and functionality of DNA were further verified by PCR with commonly used primers ITS5 and ITS2 (White et al., 1990). Extracted DNA was diluted 1 : 9 prior to PCR amplification.

2.3 PCR analysis

Two types of primers were used in PCR analysis: non-degenerate, with unique sequences designed for conserved regions of the profilin genes and degenerate which are specially designed to be able to simultaneously

Table 1 Vegetable species included in PCR analysis.

Family	Species	Vegetable
Alliaceae	*Allium cepa* L.	onion
Amaranthaceae	*Beta vulgaris* L.	beetroot
Apiaceae	*Apium graveolens* L.	celery
	Daucus carota L.	carrot
	Petroselinum crispum (Mill.) A. W. Hill	parsley
Asteraceae	*Lactuca sativa*	lettuce
Brassicaceae	*Brassica oleracea* L. var. *capitata* (L.) Alef. F. *alba* DC.	cabbage
	Brassica oleracea L. var. *capitata* (L.) Alef. F. *rubra* (L.) Thell	cabbage
	Brassica oleracea L. var. *gongylodes* (L.) Markgr.	kohlrabi/cabbage turnip
	Brassica oleracea L. var. *italica* (Plenck) Markgr.	broccoli
Cucurbitaceae	*Cucurbita maxima* Duch. ssp. *maxima* convar. *maxima*	Hokkaido
Convolvulaceae	*Ipomoea batatas* (L.) Poir	sweet potato
Solanaceae	*Solanum tuberosum* L.	potato
	Capsicum annuum L.	pepper bell

hybridize with multiple divergent regions of profilin homologues. The sequences of non-degenerate primers used were as follows: F: 5' ACC GGC GAT CTG GTT TT 3' and R: 5' AGG TAG TCT CCC AAC CTC TC 3' (Klongová et al., 2021). Forward and reverse degenerate primer with sequences F: 5'-AGA GAA TTC CAT ATG TCG TGG CAR RCG TAC GT 3' (degeneracy = 4), and R: 5' AGA AAG CTT YTA CAK GCC YTG TTC ABV AGG TA 3' (degeneracy = 72) were used. Amplification was carried out using 5 µl of MasterMix Robust HS Elizyme (Elizabeth Pharmacon). When using non-degenerate primers, 0.3 µl of each primer (forward and reverse) and 2 µl of DNA were used. In the case of degenerate primers, it was necessary to use a larger amount of primers (0.4 µl) as well as template DNA (4.2 µl) due to specifity of degenerative PCR approach. When utilizing degenerate primers, the success of PCR amplification can be compromised due to insufficient concentration of individual primers. To improve the likelihood of primer hybridization, it is necessary to increase the primer concentration compared to specific primers. HotStart PCR was performed according to the following program: initiation at 95 °C (5 min); 40 cycles of denaturation at 95 °C (45 s), annealing at 55 °C for non-degenerate primers or 49 °C for degenerate primers (45 °C), polymerization at 72 °C (35 s); and last cycle of final extension at 72 °C (10 min). The amplified products were subjected to electrophoresis in 1.5% agarose gel. The resulting bands were stained using the GelRed™ (Biotium) and visualized by transilluminator.

2.4 Data processing

Amplification profiles obtained by PCR were analyzed by GelAnalyzer software (Lazar Jr. and Lazar Sr.). Binary matrices, based on the presence or absence of amplicons with certain length were constructed and transformed to distance matrices using Jaccard coefficient (Jaccard, 1912). Pair-wise distances were subsequently visualized and represented in form of heatmaps. Distance matrices were also used for cluster analysis by the UPGMA method (Sokal and Michener, 1958) followed by dendrogram construction. The polymorphic information content of presented markers was calculated according to Roldán-Ruiz et al. (2000), whereas the discriminating power of the primers was estimated using the approach described by Tessier et al. (1999). Statistical analyses and heatmap visualizations were carried out using Pandas (McKinney, 2010), NumPy (Harris et al., 2020), SciPy (Virtanen et al., 2020) and Seaborn (Waskom, 2021) libraries in Python (Python Software Foundation, https://www.python.org/). Dendrogram was constructed using hclust function from the stats package in R (R Core Team, 2020) with the clustering method argument set to "average", which is equivalent to the UPGMA clustering method. Due to software limitations, italics cannot be used in figures.

3 Results and discussion

The PCR profiles of each sample were compared to one another, as well as compared to amplification profiles using different primers. Using a set of degenerate primers 51 length-variable products were created, while the total number of products was 111. On average, 8 amplicons were detected in each sample, except for *Allium sativum* which did not show any amplification despite functional DNA verified by ITS PCR and repeating of amplification reactions. Up to date, no genomic information exist for

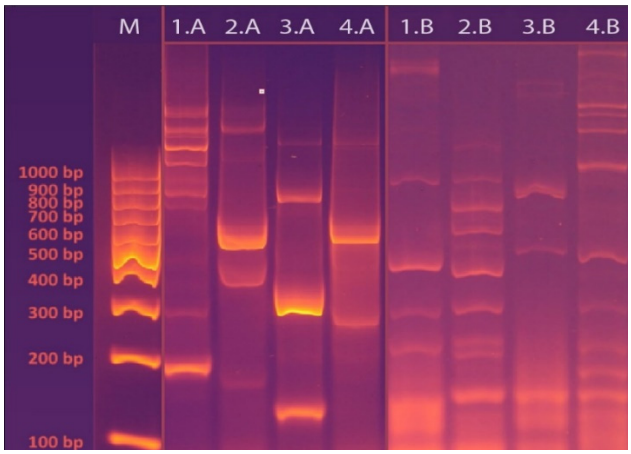

Figure 1 Comparison of PCR amplification profiles of selected analysed samples
M – ladder; A – degenerate primers, B – non-degenerate primers; 1 – *Brassica oleracea* var. *capitata* f. *rubra*, 2 – *Cucurbita maxima* ssp. *maxima*, 3 – *Brassica oleracea* var. *gongylodes*, 4 – *Ipomoea batatas*

profiling homologs in databases for *Allium sativum*. The highest number of products (17) of various lengths was observed in *Apium graveolens*. The most frequently generated amplicon (249 bp) was found in six samples (Figure 1). Cluster analysis grouped the samples into three branches, with one branch containing all analysed vegetables from the Brassicaceae family, including *Brassica oleracea* var. *gongylodes* (Kale), *Brassica oleracea* var. *capitata* f. *alba* (white cabbage), *Brassica oleracea* var. *capitata* f. *rubra* (blue cabbage), and *Brassica oleracea* var. *italica* (broccoli). The greatest similarity was observed between *Brassica oleracea* var. *capitata* f. *rubra* and *Brassica oleracea* var. *italica*, with a distance value of 0.64 (Figs 2, 3, 4). The greatest similarity was observed between *Brassica oleracea* var. *capitata* f. *rubra* and *Brassica oleracea* var. *capitata* f. *alba*, with a distance value of 0.64 (Figure 3). When using non-degenerate primers, all samples, except for *Petroselinum crispum* and *Brassica oleracea* var. *capitata* f. *rubra*, exhibited variability in length polymorphism generated by non-degenerate primers. The sample from *Brassica oleracea* var. *italica* (broccoli) produced the largest variety of products. Amplification using non-degenerate primers produced a total of 33 length-polymorphic products, while the total number of products formed was 71 (Figure 1). The strongest positive relationship was observed between *Beta vulgaris* and *Cucurbita maxima* samples (Figure 3).

Cluster analysis of matrices obtained from amplification with degenerate primers grouped the samples into four branches, with one branch containing all analysed vegetables from the Brassicaceae family, including *Brassica oleracea* var. *gongylodes* (Kale), *Brassica oleracea* var. *capitata* f. *alba* (white cabbage), *Brassica oleracea* var. *capitata* f. *rubra* (blue cabbage), and *Brassica oleracea* var. *italica* (broccoli). A high cophenetic correlation coefficient indicates that the clustering in the dendrogram represents pairwise distances between the original data with high accuracy and provides an accurate and reliable method for understanding the relationships between the data points (Figure 5).

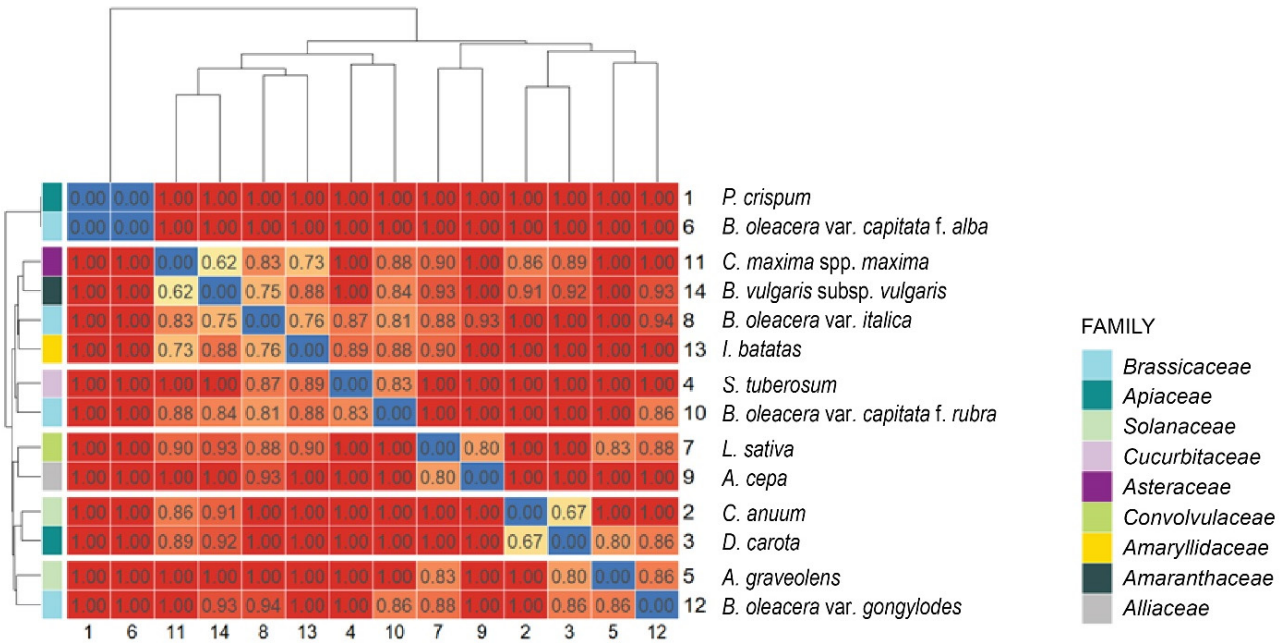

Figure 2 Heatmap showing the relationships between the resulting PCR profiles of individual species using specific primers
blue colour represents less distant pairs (lower values), while the colour red represents more distant pairs (higher values); the annotation bar on the right side of the heatmap shows the family affiliation of species

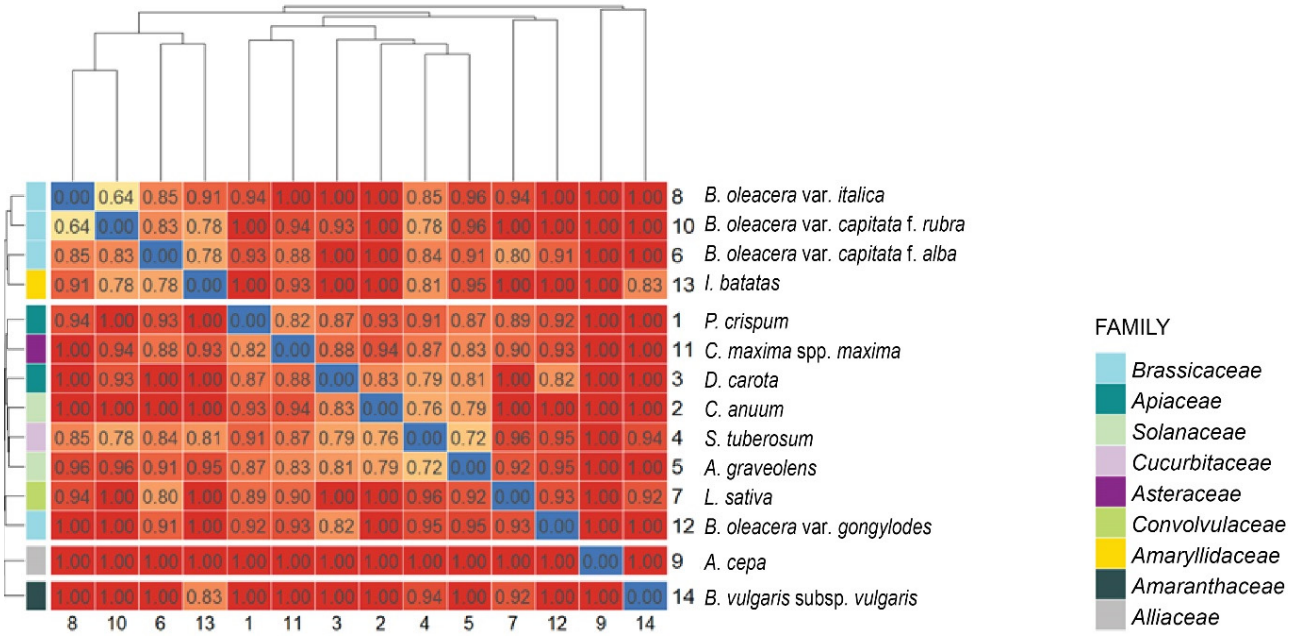

Figure 3 Heatmap showing the relationships between the resulting PCR profiles of individual species using degenerate primers
blue colour represents less distant pairs (lower values), while the colour red represents more distant pairs (higher values); the annotation bar on the right side of the heatmap shows the family affiliation of species

The degree of polymorphic information content for degenerate and non-degenerate primers was found to be 0.226 and 0.228, respectively. These values indicate that both primer sets offer a similar level of informativeness. An estimated high discriminating power level of around 0.97 was observed for both sets of primers. This parameter assesses the probability of differentiating between two randomly selected primers.

Molecular markers aid in identifying cultivars, determining genetic similarities, and are also used for

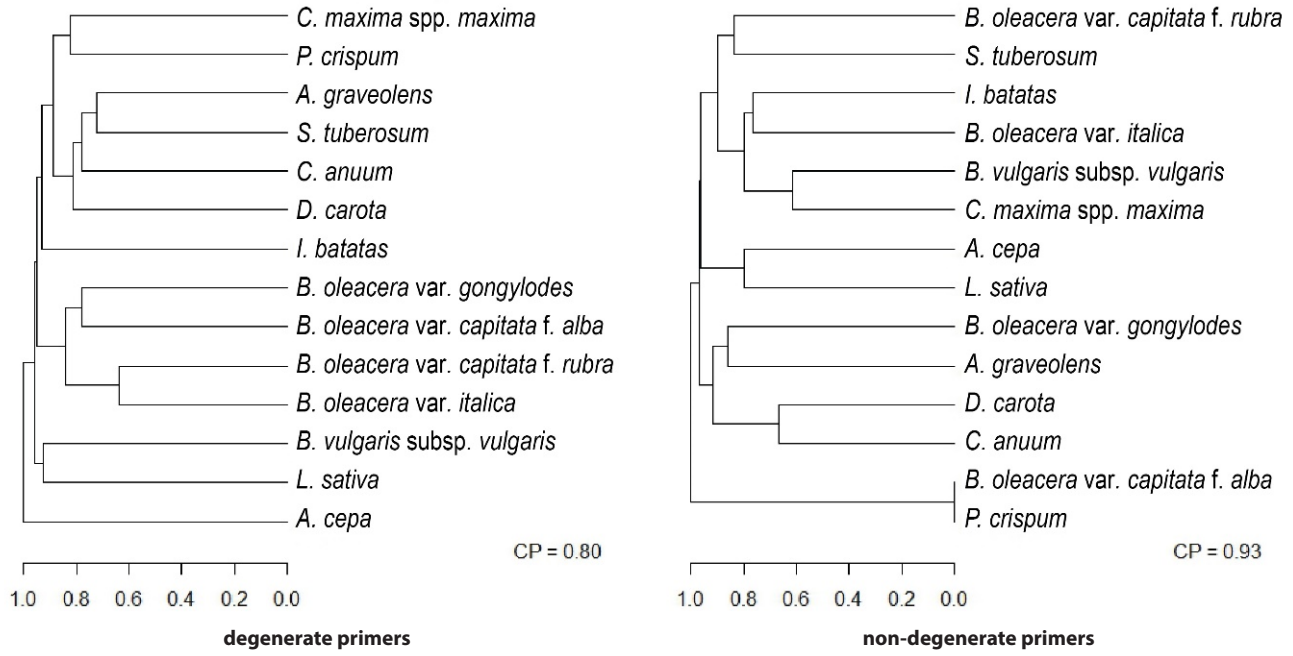

Figure 4 Dendrograms based on profilin derived amplification polymorphism obtained via degenerate and non-degenerate primer sequences
cophenetic correlation coefficient (CP) is displayed in the lower-right corner of each dendrogram

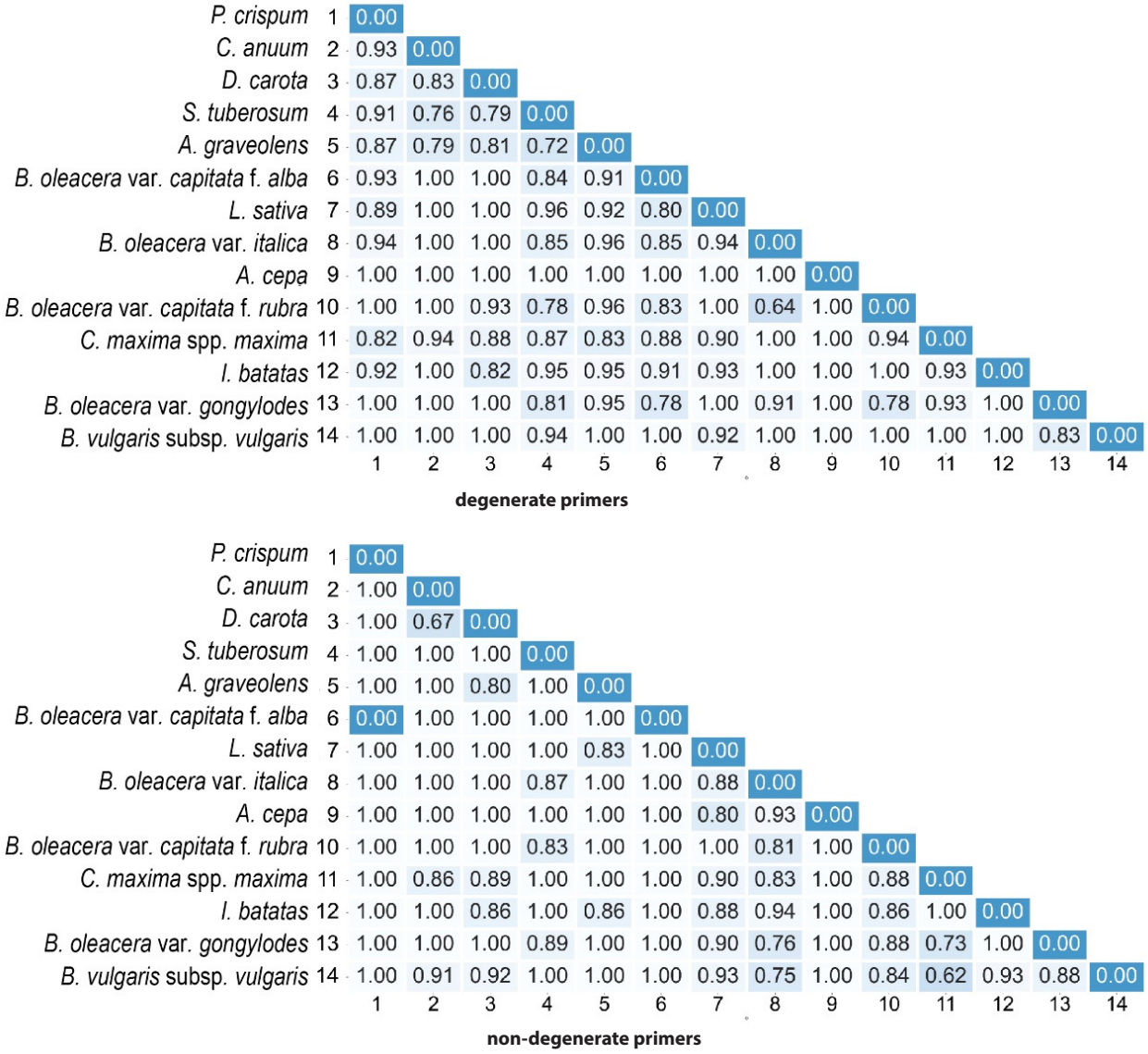

Figure 5 Heatmap showing the relationships between the resulting PCR profiles of individual species using non-degenerate and degenerate primers
blue colour represents less distant pairs (lower values), while the colour white represents more distant pairs (higher values)

detecting genetic polymorphism in related individuals. Molecular markers that reveal genetic polymorphism represent simple and cost-effective method that can be used for genetic characterization and evaluation of genetic relatedness. Probably the most significant benefit of molecular markers is their potential to be used in marker-assisted selection (Masojć, 2002). The use of molecular markers in selection has significantly reduced the time required to develop new crop varieties. Plant breeders have over the past few decades relied on various techniques to gain insights into molecular genetics and explore the potential of DNA markers (Hasan et al., 2021). Recent advances in plant molecular biology have led to a shift towards more informative and efficient gene-targeted markers, which are derived from sequences stored in public genomic databases and represent the coding sequences of genes. This allowed for more precise identification and utilization of molecular markers in higher plants (Gupta and Rustgi, 2004). To date, several approaches using gene targeted markers have been developed, including Conserved DNA-Derived Polymorphism (CDDP) (Collard and Mackill, 2009), P450-Based Analogue (PBA) markers (Yamanaka et al., 2003), Target Region Amplification Polymorphism (TRAP) (Hu et al., 2003) and Start Codon Targeted (SCoT) Polymorphism (Collard and Mackill, 2009).

The knowledge of plant food allergens has been greatly advanced by molecular biology and biochemical

techniques. Interestingly, the majority of the identified plant food allergens share homology with pathogenesis-related proteins (PRs), which are proteins induced by pathogens, wounding, or certain environmental stresses (Breiteneder and Ebner, 2000). The method based on the creation of a characteristic profile of PCR amplicons obtained on the basis of genes homologous to the PR-10 allergen, also called BBAP (from the English Bet v 1 homologues-based amplified profile), was used in the past for DNA fingerprinting of individual allergenic types of vegetables (Zamieškova and Žiarovská, 2021) and fruit (Žiarovská and Urbanová, 2022). Appropriately designed primers enabled the use of PR-10 allergens as a DNA marker, while the variability of individual profiles proved to be sufficient for the use of this technique for DNA fingerprinting of plant species. Despite not being considered relevant allergens in the past, recent publications have highlighted the significance of profilin in allergenic reactions. Profilins are cytosolic proteins with functional relationships in the root elongation, leaf morphology, epidermal expansion, flowering time phenotypes and seed germination in various plant species (Ramachandran et al., 2000; Pandey and Chaudhary, 2020). The functional diversity of their isovariants was studied for spatio-temporal regulation during vegetative development, pollen maturation, and pollen tube growth (Kandasamy et al., 2002). The role of profilin in inducing allergy is still evaluated because of their cross-reactivity. IgE cross-reactivity among profilins is associated with multiple pollen sensitization and with various pollen-food syndromes (Santos and Van Ree, 2011). A total of nine plant profilins were compared by Huang et al. (1996) and a range of 70 to 90% of the amino acids similarities were found as well as an identity ranging from 68 to 90%, wha imply a relatively high level of conservation among plant profilin sequences, indicating their potential for use as molecular markers. In this study, the possibility of using profilin sequences as molecular markers in the analysis of plant species was investigated. Using primers derived from profilin sequences proved to be an effective and cost-efficient technique for identifying genetic variability within the coding regions of analysed species.

4 Conclusions

Despite not being considered relevant allergens in the past, recent publications have highlighted the significance of profilin in allergenic reactions. In this study, the possibility of using profilin sequences as molecular markers in the genetic analysis of plant species was investigated. Two different sets of primers were used. Based on the data it can be inferred that greater variation in the length polymorphism of amplicons was achieved through the implementation of degenerate primers. Overall, using primers derived from profilin sequences proved to be an effective and cost-efficient technique for identifying genetic variability within the coding regions of analysed species.

Acknowledgments

This publication was supported by the Operational programme 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%) and KEGA 020SPU-4/2021 Innovation of the methodological background and content of profile food and gastronomic subjects with a focus on increasing the competitiveness of graduates (50%).

References

Breiteneder, H., & Ebner, C. (2000). Molecular and biochemical classification of plant-derived food allergens. *Journal of Allergy and Clinical Immunology*, 106(1), 27–36. https://doi.org/10.1067/mai.2000.106929

Carlsson, L. et al. (1977). Actin polymerizability is influenced by profilin, a low molecular weight protein in non-muscle cells. *Journal of Molecular Biology*, 115(3), 465–483.

Collard, B. C. Y., & Mackill, D. J. (2009). Conserved DNA-Derived Polymorphism (CDDP): A Simple and Novel Method for Generating DNA Markers in Plants. *Plant Molecular Biology Reporter*, 27(4), 558–562. https://doi.org/10.1007/s11105-009-0118-z

Davey, R. J., & Moens, P. D. (2020). Profilin: Many facets of a small protein. *Biophysical Reviews*, 12(4), 827–849. https://doi.org/10.1007/s12551-020-00723-3

Harris, C. R. et al. (2020). Array programming with NumPy. *Nature*, 585(7825). https://doi.org/10.1038/s41586-020-2649-2

Hasan, N. et al. (2021). Recent advancements in molecular marker-assisted selection and applications in plant breeding programmes. *Journal of Genetic Engineering and Biotechnology*, 19(1), 128. https://doi.org/10.1186/s43141-021-00231-1

Huang, S. et al. (1996). The *Arabidopsis* profilin gene family. Evidence for an ancient split between constitutive and pollen-specific profilin genes. *Plant Physiology*, 111(1), 115–126.

Kandasamy, M.K. et al. (2002). Plant profilin isovariants are distinctly regulated in vegetative and reproductive tissues. *Cell motility*, 52 (1). https://doi.org/10.1002/cm.10029

Klongová, L. et al. (2021). Utilization of specific primers in legume allergens based polymorphism screening. *Science, Technology and Innovation*, 13(2). https://doi.org/10.5604/01.3001.0015.5431

Kwok, S. et al. (1994). A guide to the design and use of mismatched and degenerate primers. *Genome Research*, 3(4), S39–S47. https://genome.cshlp.org/content/3/4/S39.long

Linhart, C., & Shamir, R. (2005). The degenerate primer design problem: Theory and applications. *A Journal of Computational Molecular Cell Biology*, 12(4), 431–456. https://doi.org/10.1089/cmb.2005.12.431

Maeda, K. et al. (2020). Actin Filament Disruption Alters Phragmoplast Microtubule Dynamics during the Initial Phase of Plant Cytokinesis. *Plant and Cell Physiology*, 61(3), 445–456. https://doi.org/10.1093/pcp/pcaa003

Mahoney, N. M. et al. (1997). Structure of the profilin-poly-L-proline complex involved in morphogenesis and cytoskeletal regulation. *Nature Structural & Molecular Biology*, 4(11), 953–960. https://doi.org/10.1038/nsb1197-953

Mahoney, N. M. et al. (1999). Profilin binds proline-rich ligands in two distinct amide backbone orientations. *Nature Structural Biology*, 6(7). https://doi.org/10.1038/10722

Masojć, P. (2002). The application of molecular markers in the process of selection. *Cellular & Molecular Biology Letters*, 7(2A), 499–509.

McKinney, W. (2010). *Data Structures for Statistical Computing in Python*. https://doi.org/10.25080/Majora-92bf1922-00a

Mills, E. N. C., & Shewry, P. R. (Ed.). (2004). *Plant food allergens*. Blackwell Science.

Qu, X. et al. (2015). Organization and regulation of the actin cytoskeleton in the pollen tube. *Frontiers in Plant Science*, 5. https://doi.org/10.3389/fpls.2014.00786

Pandey, D.K., & Chaudhary, B. (2020). Evolution of Functional Diversity Among Actin-Binding Profilin Genes in Land Plants. *Frontiers in Cell and Developmental Biology*. https://doi.org/10.3389/fcell.2020.588689

Ramachandran, S. et al. (2000). Profilin plays a role in cell elongation, cell shape maintenance, and flowering in *Arabidopsis*. *Plant Physiology*, 124, 1637–1647. https://doi.org/10.1104/pp.124.4.1637

Rodríguez del Río, P. et al. (2018). Profilin, a Change in the Paradigm. *Journal of Investigational Allergology and Clinical Immunology*, 28(1), 1–12. https://doi.org/10.18176/jiaci.0193

Roldán-Ruiz, I. et al. (2000). AFLP markers reveal high polymorphic rates in ryegrasses (*Lolium* spp.). *Molecular Breeding*, 6(2), 125–134. https://doi.org/10.1023/A:1009680614564

Ruiz-Hornillos, J. et al. (2020). Profilin is a marker of severity in allergic respiratory diseases. *Allergy*, 75(4), 853–861. https://doi.org/10.1111/all.14140

Sankian, M. et al. (2005). Sequence homology: A poor predictive value for profilins cross-reactivity. *Clinical and molecular allergy : CMA*, 3, 13. https://doi.org/10.1186/1476-7961-3-13

Schlüter, K., Jockusch, B. M., & Rothkegel, M. (1997). Profilins as regulators of actin dynamics. *Biochimica et Biophysica Acta (BBA) – Molecular Cell Research*, 1359(2), 97–109. https://doi.org/10.1016/S0167-4889(97)00100-6

Sokal, R., & Michener, C. (1902). A statistical method for evaluating systematic relationships. **http://archive.org/details/**cbarchive_33927_astatisticalmethodforevaluatin1902

Tessier, C. et al. (1999). Optimization of the choice of molecular markers for varietal identification in *Vitis vinifera* L. *Theoretical and Applied Genetics*, 98, 171–177. https://doi.org/10.1007/s001220051054

Tordesillas, L. et al. (2009). Characterization of IgE epitopes of Cuc m 2, the major melon allergen, and their role in cross-reactivity with pollen profilins: B epitopes of melon profiling. *Clinical & Experimental Allergy*, 40(1), 174–181. https://doi.org/10.1111/j.1365-2222.2009.03401.x

Valenta, R. et al. (1991). Identification of Profilin as a Novel Pollen Allergen; IgE Autoreactivity in Sensitized Individuals. *Science*, 253(5019), 557–560. https://doi.org/10.1126/science.1857985

Valenta, R. et al. (1993). Identification of profilin as an actin-binding protein in higher plants. *Journal of Biological Chemistry*, 268(30), 22777–22781. https://doi.org/10.1016/S0021-9258(18)41594-3

Virtanen, P. et al. (2020). SciPy 1.0: Fundamental algorithms for scientific computing in Python. *Nature Methods*, 17(3), 261–272. https://doi.org/10.1038/s41592-019-0686-2

Wallner, M. et al. (2017). The Concept of Pollen Panallergens: Profilins and Polcalcins. In J. Kleine-Tebbe & T. Jakob (Ed.), *Molecular Allergy Diagnostics: Innovation for a Better Patient Management* (pp. 43–56). Springer International Publishing. https://doi.org/10.1007/978-3-319-42499-6_3

Waskom, M. L. (2021). seaborn: Statistical data visualization. *Journal of Open Source Software*, 6(60), 3021. https://doi.org/10.21105/joss.03021

Yamashita, H. et al. (2011). Chloroplast actin filaments organize meshwork on the photorelocated chloroplasts in the moss *Physcomitrella patens*. *Planta*, 233(2), 357–368. https://doi.org/10.1007/s00425-010-1299-2

Zamiesková, 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. https://doi.org/10.15414/afz.2021.24.mi-apa.1-6

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