

Original Research

Assessment of Genetic Diversity and Polymorphism in *Corylus avellana* L. Pollen by Inter-Primer Binding Site Marker Analysis

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Abstract

Genetically conditioned variation in pollen has been demonstrated to have a substantial influence on reproductive mechanisms and population dynamics. The present study evaluated the genetic variability of 28 pollen samples of *Corylus avellana* L. using iPBS (Inter Primer Binding Sites Polymorphism) markers 1882 and 2152. The collection of biological material was undertaken at various locations across five European countries, with the objective of capturing a broad spectrum of environmental conditions. The genetic relationships among the samples were evaluated by constructing a dendrogram using the UPGMA (Unweighted Pair Group Method with Arithmetic mean) with genetic similarity estimated based on the Jaccard coefficient. Clustering analysis of the 1882 iPBS marker identified four major clusters. Similarly, applying this analysis to the 2152 iPBS marker identified six major clusters. The average Jaccard genetic similarity coefficient was 0.415 and 0.608 for iPBS markers 1882 and 2152, respectively. With regard to the iPBS marker 1882, the highest degree of genetic similarity, indicated by a Jaccard coefficient of 0.73, was observed between the Ukrainian sample from Kyiv park and the sample from Germany, Darmstadt. The iPBS marker 2152 revealed that the



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Slovak sample originating from the field near Ivanka pri Nitre and the Ukrainian sample from Kyiv park shared the highest genetic resemblance, with a Jaccard coefficient of 0.815. The results obtained demonstrate a high degree of genetic diversity among retrotransposon insertions in hazelnut pollen. Furthermore, the iPBS technique has been shown to be reliable for assessing genomic variability in *Corylus avellana* L.

Keywords

Cluster analysis; genetic variability; hazelnut; iPBS; polymorphism

1. Introduction

Understanding genetic variability within and between plant species is essential for conservation biology, plant breeding, and germplasm management. DNA markers are widely used, and many have been developed and applied in various studies investigating plant genome characteristics. These specific DNA sequences serve as reliable indicators of gene presence, such as those associated with agronomically important plant traits [1]. The application of PCR-based molecular markers allows the investigation of population diversity and distinguishes individuals and groups based on their unique genetic characteristics [2]. They were used in assessing genetic diversity across a wide range of plant species [3-5].

Retrotransposon sequence-based markers are considered among the most effective for describing intraspecific genetic variability. This is due to their wide genomic distribution and high polymorphism rates. One such system is the inter-primer binding site (iPBS) retrotransposon marker [6], a universal DNA fingerprinting technique that targets conserved primer-binding sites associated with retrotransposon elements across genomes, regardless of prior sequence information [7, 8].

iPBS markers reduce the need for species-specific primer design, making iPBS a versatile tool for use with a variety of species. In plant genetic research, iPBS markers have been widely used to evaluate genetic diversity and population structure in crop species and their wild relatives [7-11].

Analysing the genetic variability of *Corylus avellana* L. (European hazelnut) populations is essential for the effective conservation and breeding of this economically important nut crop. Over the past decades, various marker systems have been applied to assess genetic variation in *C. avellana*. Simple sequence repeats (SSR) markers have been used to characterise hazelnut germplasm collections, evaluate population structure, and distinguish genetic relationships among cultivars of diverse geographical origins. Investigations involving 16 SSR loci in 78 hazelnut cultivars demonstrated considerable polymorphism and enabled the resolution of genetic relationships according to pedigree and geographic origin [12]. SSRs have also been used to fingerprint Sicilian hazelnut accessions, revealing clear genetic differentiation between local and commercial varieties [13]. Comparative analyses of natural German hazel populations using Amplified Fragment Length Polymorphism (AFLP) alongside SSR and isozyme markers showed that AFLP markers revealed significant inter-population differentiation and provided high-resolution genetic structure [14]. AFLPs have also been applied with SSRs in Slovenian germplasm to reveal high levels of genetic diversity and to support association mapping for nut and kernel traits [15]. Randomly Amplified Polymorphic DNA (RAPD) markers were used to construct early genetic linkage maps of *C. avellana*

when combined with SSR loci, enabling the placement of agronomically important loci such as those linked to resistance to eastern filbert blight and incompatibility systems [16]. RAPDs have also been used to differentiate Turkish hazelnut cultivars alongside Inter Simple Sequence Repeats (ISSR) and AFLP markers, demonstrating their utility in cultivar identification [17].

Hazelnut pollen is released early in spring and is one of the most common pollen allergens in Central Europe [18]. Direct analysis of pollen polymorphism in plant species is rare, mainly because of the amount of tissue required for DNA extraction. SSR markers were used to analyze pollen dispersal [19], but to our knowledge, iPBS markers have not yet been applied. Here, pure pollen spread from catkins was chosen as a tissue for which no specific surface decontamination is needed, and the possible contamination with environmental DNA is minimal. The aim of this study was to characterise the natural iPBS polymorphism in the pollen DNA of common hazelnut genotypes from different localities in Central and Eastern Europe, and to prove the effectiveness of iPBS markers for this purpose.

2. Materials and Methods

2.1 Biological Material and DNA Extraction

This study analysed 28 pollen samples of *Corylus avellana* L. (Table 1), which were collected from 5 European countries: 4 samples were collected in Ukraine (accessions UA1-5), 12 samples in Slovakia (accessions SK6-10, 13-19), 2 samples in Germany (accessions DE11-12), 5 samples in Czechia (accessions CZ20-24) and 4 samples in Russia (accessions RU25-28). Every sample is a pool of pollen collected from catkins from a single tree.

Table 1 List of *Corylus avellana* L. accessions.

Sample	Country	Locality	Description of locality
UA1	Ukraine	Kyiv	botanical garden
UA2	Ukraine	Kyiv	city
UA3	Ukraine	Kyiv	park
UA4	Ukraine	Kamenets-Podolskyi	botanical garden
UA5	Ukraine	Kamenets-Podelskyi	city
SK6	Slovakia	Jelenec	forest
SK7	Slovakia	Jelenec	village
SK8	Slovakia	Ivanka pri Nitre	field
SK9	Slovakia	Ivanka pri Nitre	village
SK10	Slovakia	Ivanka pri Nitre	road
DE11	Germany	Darmstadt	road
DE12	Germany	Darmstadt	park
SK13	Slovakia	Nitra Zobor	forest
SK15	Slovakia	Banka	village
SK16	Slovakia	Modrovka	village
SK17	Slovakia	Zemianske podhradie	village
SK18	Slovakia	Moravské lieskové	village
SK19	Slovakia	Nitra	botanical garden

CZ20	Czechia	Prague - Petřín - Strahov Monastery	park
CZ21	Czechia	Prague - Petřín - Pod Rozhlednou	park
CZ22	Czechia	Prague - Lobkowitz Garden	park
CZ23	Czechia	Chocerady	village
CZ24	Czechia	Chocerady	road
RU25	Russia	Orel	park
RU26	Russia	Moscow	park
RU27	Russia	Orel	road
RU28	Russia	Orel	botanical garden

Sampling was conducted across a range of environments, including urban areas, parks, botanical gardens, forests, fields, and roads, in order to capture variability in both natural and human-modified landscapes. The samples were stored at -70°C in a deep freezer until their analysis.

Genomic DNA was extracted using the commercial GeneJET Plant Genomic DNA Purification Kit (Thermo Fisher Scientific, USA) following the supplier's instructions. Pollen disruption was performed directly in the lysis buffer using a mortar and pestle. The quantity and quality of the obtained DNA were determined spectrophotometrically using a NanoPhotometer P360 (IMPLEN, Slovak Republic). The yields range from 50 to 150 ng/μL and were unified to 50 ng/μL for further analysis.

A control PCR reaction using ITS1 and ITS4 primers [20] was performed to validate the functionality of the DNA.

2.2 PCR and Gel Electrophoresis

Two iPBS markers were selected for analysis based on previous tests (data not shown), specifically 1882 (5'TCGACTTCTCATGCATGGCAGCACC3') and 2152 (5'AGTGAGCATGGGAGCGGACAAGC3') [6]. The PCR reaction was carried out in triplicate using the DreamTaq PCR Master Mix (2×) (Thermo Fisher Scientific, USA) and 1200 nmol of primer, with a reaction volume of 20 μl on the SureCycler 8800 (Agilent, USA). The following temperature-time profile was applied: initial denaturation step at 95°C for 3 minutes, followed by 40 cycles of denaturation at 95°C for 30 seconds, annealing at 55°C for 40 seconds, and elongation at 72°C for 1 minute, concluding with a final elongation step at 72°C for 10 minutes.

The amplified fragments were analysed by electrophoretic separation on a 15% polyacrylamide gel (PAGE) and by visualisation under default conditions, using UV light in a transilluminator BDA digital System 30 (Biometra, Germany).

2.3 Data Analysis

The analysis of DNA fingerprint gel images was performed using open-source, freely available software tools - GelJ [21] and GelAnalyzer (<http://www.gelanalyzer.com>). The presence (1) or absence (0) of bands at specific sizes was used to compile a binary matrix. Subsequently, the dendrogram of genetic similarity was generated. The Jaccard coefficient of genetic similarity [22] was also used to assess the similarity between the iPBS profiles of the studied accessions. In accordance with the binary data, various indices (Polymorphism information content - PIC, Marker index - MI, Heterozygosity index - HI, Discrimination power - D, Resolving power - R) reflecting the

degree of polymorphism were computed using the online Marker Efficiency Calculator (iMEC) according to the formulas given in the study [23]. Fingerprint profiles of the most similar and most distinctive hazelnut genotypes were generated using the software tool GelAnalyzer.

3. Results

3.1 *iPBS* Marker 1882

A total of 548 polymorphic fragments were obtained with 86 distinct size levels, ranging from 1550 bp to 140 bp, and were identified across all the analysed samples (Figure 1). The highest number of fragments (38) was observed in the pollen sample from Orel, Russia (RU27), whereas the lowest (6) was recorded in the accession from Zobor, Nitra (SK14). A 205 bp amplicon was identified in all obtained results. Several unique fragments were detected.

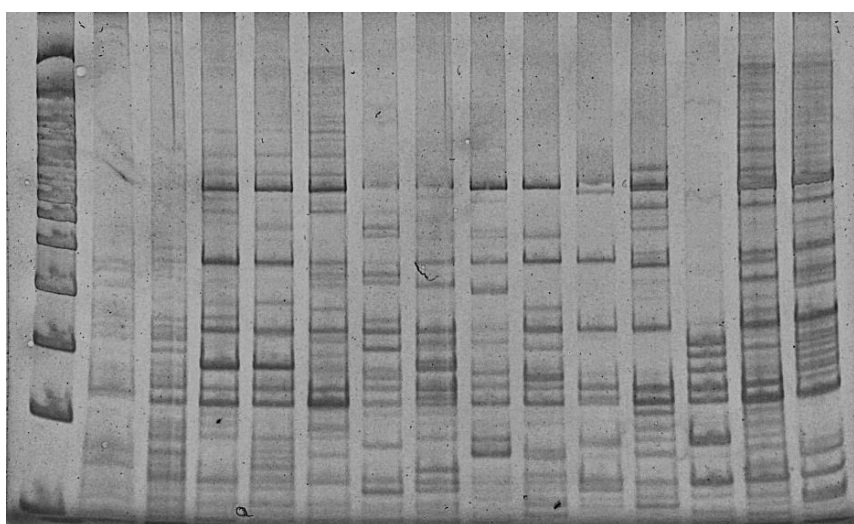


Figure 1 Obtained *iPBS* profiles for 1882 marker. First line - ladder; following lines genotypes SK15 - RU28.

The constructed dendrogram divided the analysed genotypes into several clusters, with varying Jaccard coefficients of genetic similarity. Clear separation of the fingerprint profiles of all of the analysed hazel genotypes was observed in four major clusters (Figure 2) with a cophenetic coefficient of 0.97.

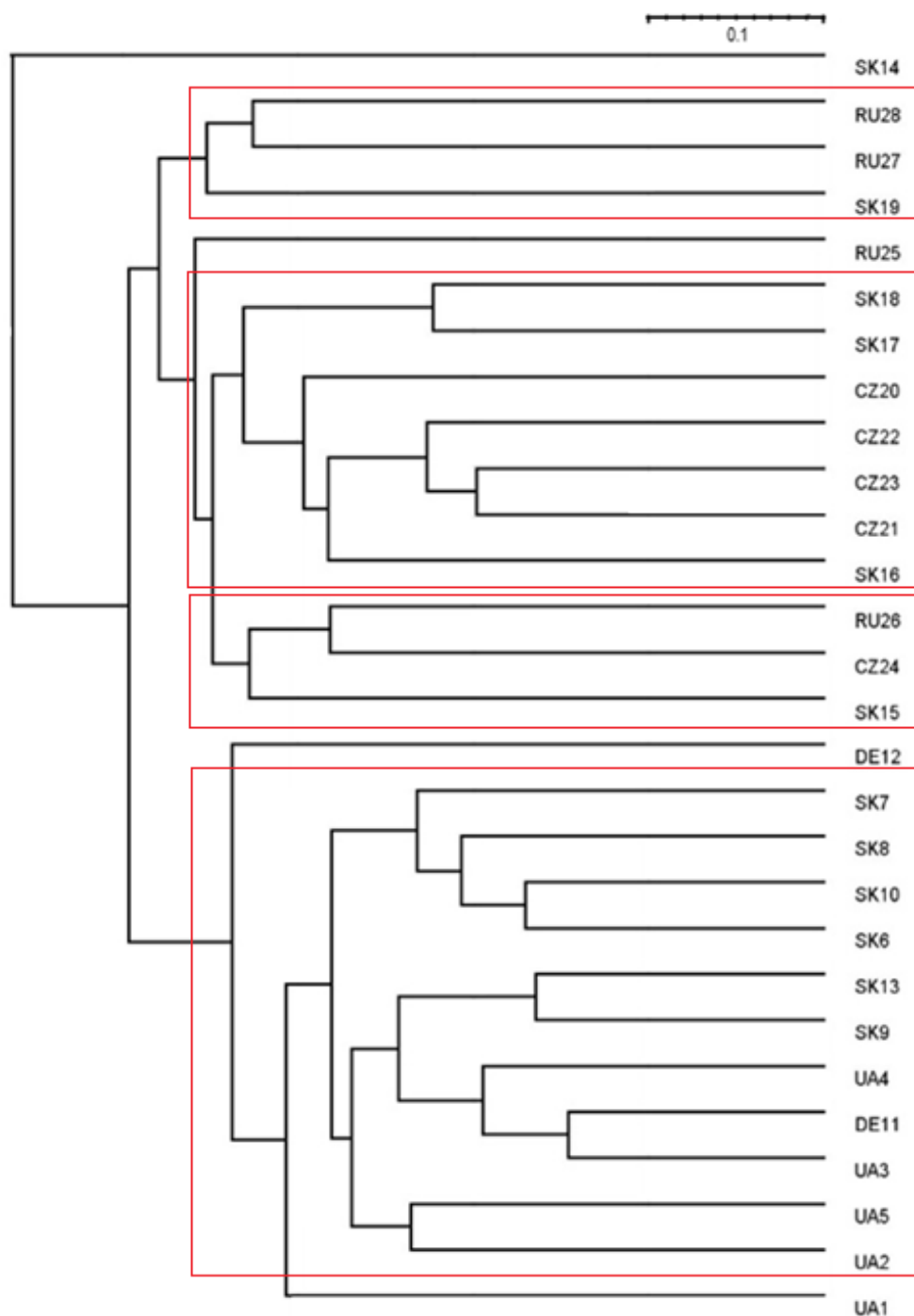


Figure 2 UPGMA dendrogram based on Jaccard coefficients of similarity illustrating the clustering of analysed hazel genotypes based on iPBS fingerprint data of the marker 1882.

All the analysed genotypes were separated based on their iPBS fingerprints. The most similar profiles were observed between the UA3 and DE11 genotypes (Figure 3), while the most distinctive profiles were observed between the SK14 and RU28 genotypes (Figure 4). This corresponds to their location in the dendrogram branches.

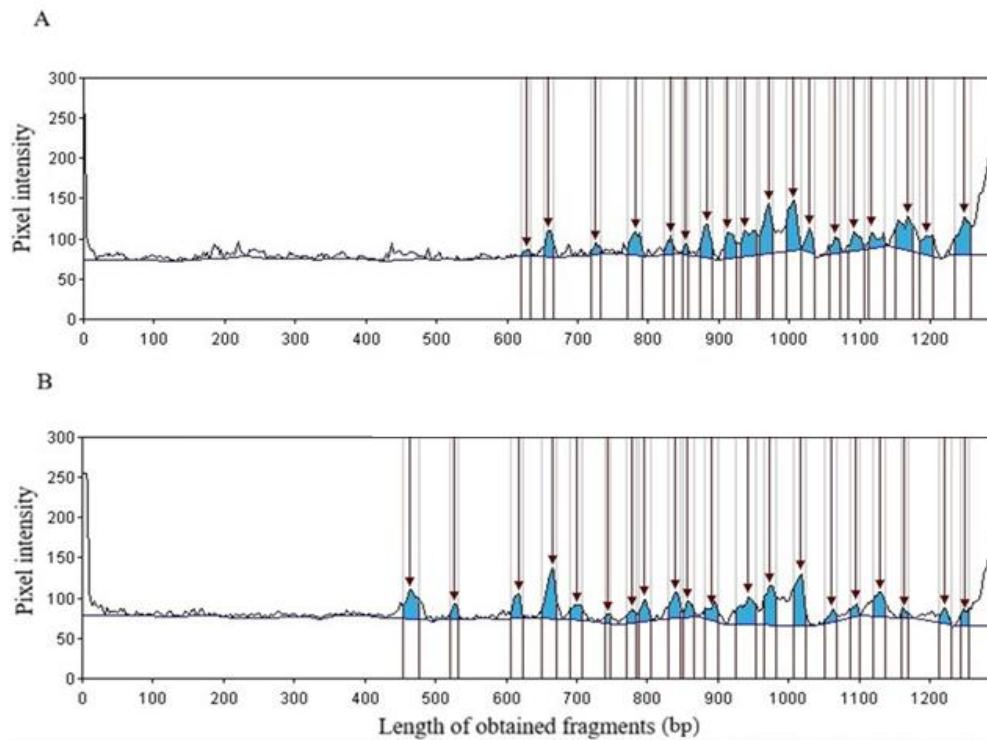


Figure 3 Fingerprints profiles of the most similar hazelnut genotypes (UA3 - A, DE11 - B) based on 1882 marker amplification results.

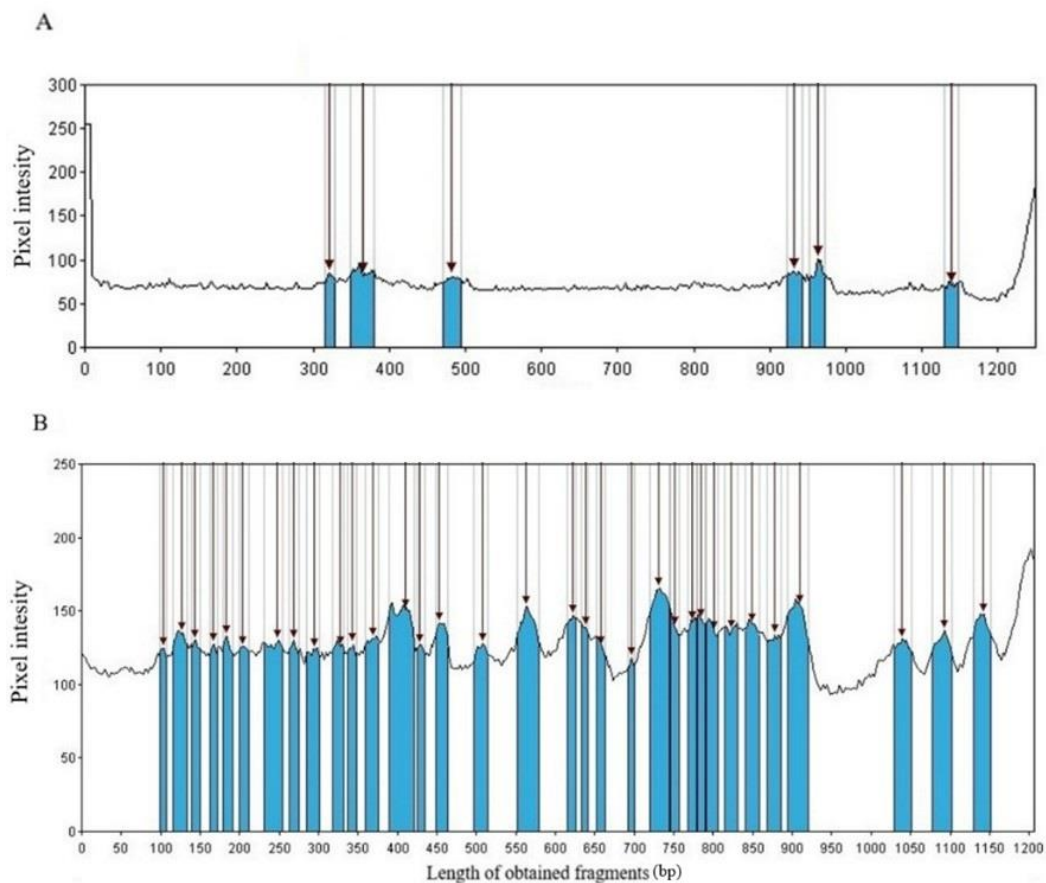


Figure 4 Fingerprints profiles of the most distinctive hazelnut genotypes (SK14 - A, RU28 - B) based on 1882 marker amplification results.

The obtained Jaccard coefficients of similarity ranged from 0.08 to 0.73 across the analysed genotypes, with an average of 0.415 and a median of 0.405. As demonstrated by the coefficient, the highest level of genetic similarity was observed between the Ukrainian samples from the Kyiv park (UA3) and the German sample from Darmstadt (DE11), with a coefficient value of 0.73. The lowest genetic similarity, indicated by a Jaccard coefficient of 0.08, was observed among the Russian accession from the botanical garden in Orel (RU28) and the Slovak pollen sample from Nitra, Zobor (SK14), the botanical garden in Nitra (SK19), and the village Banka (SK15) (Figure 5).

	UA1	UA2	UA3	UA4	UA5	SK6	SK7	SK8	SK9	SK10	DE11	DE12	SK13	SK14	SK15	SK16	SK17	SK18	SK19	CZ20	CZ21	CZ22	CZ23	CZ24	RU25	RU26	RU27	RU28
UA1	1	0.447	0.486	0.488	0.405	0.553	0.606	0.444	0.525	0.524	0.447	0.462	0.528	0.5	0.543	0.432	0.575	0.5	0.488	0.39	0.611	0.512	0.488	0.489	0.5	0.558	0.55	0.45
UA2	0.447	1	0.529	0.381	0.531	0.514	0.471	0.4	0.487	0.488	0.486	0.543	0.576	0.417	0.5	0.563	0.538	0.465	0.605	0.35	0.486	0.475	0.45	0.455	0.538	0.455	0.475	0.571
UA3	0.486	0.529	1	0.611	0.581	0.4	0.724	0.815	0.568	0.694	0.576	0.636	0.486	0.5	0.545	0.563	0.579	0.5	0.564	0.421	0.719	0.639	0.568	0.455	0.714	0.488	0.595	0.528
UA4	0.488	0.381	0.611	1	0.571	0.55	0.556	0.667	0.524	0.634	0.568	0.538	0.487	0.425	0.5	0.514	0.571	0.605	0.523	0.463	0.649	0.625	0.561	0.489	0.61	0.458	0.548	0.605
UA5	0.405	0.531	0.581	0.571	1	0.472	0.567	0.643	0.571	0.526	0.581	0.5	0.581	0.5	0.455	0.567	0.5	0.5	0.526	0.457	0.625	0.647	0.528	0.525	0.5	0.452	0.6	0.625
SK6	0.553	0.514	0.4	0.55	0.472	1	0.5	0.472	0.512	0.477	0.556	0.526	0.436	0.571	0.41	0.459	0.488	0.595	0.548	0.289	0.475	0.465	0.512	0.511	0.524	0.511	0.615	0.475
SK7	0.606	0.471	0.724	0.556	0.567	0.5	1	0.621	0.514	0.639	0.563	0.576	0.563	0.633	0.485	0.455	0.526	0.564	0.595	0.405	0.559	0.541	0.556	0.409	0.568	0.409	0.583	0.514
SK8	0.444	0.4	0.815	0.667	0.643	0.472	0.621	1	0.571	0.568	0.531	0.594	0.441	0.455	0.455	0.469	0.462	0.579	0.526	0.417	0.529	0.514	0.571	0.488	0.541	0.419	0.514	0.625
SK9	0.525	0.487	0.568	0.524	0.571	0.512	0.514	0.571	1	0.634	0.657	0.579	0.611	0.462	0.462	0.514	0.571	0.468	0.489	0.395	0.605	0.585	0.488	0.591	0.61	0.489	0.667	0.564
SK10	0.524	0.488	0.694	0.634	0.526	0.477	0.639	0.568	0.634	1	0.564	0.537	0.488	0.579	0.5	0.475	0.533	0.6	0.667	0.4	0.684	0.581	0.595	0.622	0.683	0.49	0.619	0.684
DE11	0.447	0.486	0.576	0.568	0.581	0.556	0.563	0.531	0.657	0.564	1	0.636	0.625	0.5	0.545	0.613	0.538	0.658	0.525	0.421	0.667	0.686	0.611	0.6	0.622	0.455	0.595	0.667
DE12	0.462	0.543	0.636	0.538	0.5	0.526	0.576	0.594	0.579	0.537	0.636	1	0.588	0.559	0.514	0.486	0.55	0.711	0.537	0.333	0.629	0.525	0.579	0.571	0.676	0.61	0.564	0.629
SK13	0.528	0.576	0.486	0.487	0.581	0.436	0.563	0.441	0.611	0.488	0.625	0.588	1	0.5	0.545	0.563	0.5	0.465	0.419	0.421	0.571	0.595	0.526	0.488	0.667	0.561	0.553	0.528
SK14	0.5	0.417	0.5	0.425	0.5	0.571	0.633	0.455	0.462	0.579	0.5	0.559	0.5	1	0.471	0.441	0.439	0.512	0.5	0.359	0.5	0.487	0.5	0.5	0.639	0.465	0.568	0.421
SK15	0.543	0.5	0.545	0.5	0.455	0.41	0.485	0.455	0.462	0.5	0.545	0.514	0.545	0.471	1	0.531	0.439	0.476	0.429	0.432	0.588	0.487	0.5	0.465	0.513	0.37	0.526	0.543
SK16	0.432	0.563	0.563	0.514	0.567	0.459	0.455	0.469	0.514	0.475	0.613	0.486	0.563	0.441	0.531	1	0.526	0.488	0.475	0.444	0.606	0.541	0.6	0.476	0.487	0.442	0.583	0.559
SK17	0.575	0.538	0.579	0.571	0.5	0.488	0.526	0.462	0.571	0.533	0.538	0.55	0.5	0.439	0.439	0.526	1	0.543	0.605	0.476	0.575	0.634	0.65	0.565	0.545	0.565	0.718	0.5
SK18	0.5	0.465	0.5	0.605	0.5	0.595	0.564	0.579	0.468	0.6	0.658	0.711	0.465	0.512	0.476	0.488	0.543	1	0.674	0.383	0.535	0.628	0.568	0.667	0.543	0.63	0.75	0.535
SK19	0.488	0.605	0.564	0.523	0.526	0.548	0.595	0.526	0.489	0.667	0.525	0.537	0.419	0.5	0.429	0.475	0.605	0.674	1	0.4	0.6	0.619	0.558	0.46	0.683	0.553	0.619	0.561
CZ20	0.39	0.35	0.421	0.463	0.457	0.289	0.405	0.417	0.395	0.4	0.421	0.333	0.421	0.359	0.432	0.444	0.476	0.383	0.4	1	0.462	0.419	0.463	0.435	0.409	0.375	0.419	0.357
CZ21	0.611	0.486	0.719	0.649	0.625	0.475	0.559	0.529	0.605	0.684	0.667	0.629	0.571	0.5	0.588	0.606	0.575	0.535	0.6	0.462	1	0.632	0.649	0.523	0.575	0.558	0.55	0.611
CZ22	0.512	0.475	0.639	0.625	0.647	0.465	0.541	0.514	0.585	0.581	0.686	0.525	0.595	0.487	0.487	0.541	0.634	0.628	0.619	0.419	0.632	1	0.667	0.543	0.634	0.543	0.65	0.512
CZ23	0.488	0.45	0.568	0.561	0.528	0.512	0.556	0.571	0.488	0.595	0.611	0.579	0.526	0.5	0.5	0.6	0.65	0.568	0.558	0.463	0.649	0.667	1	0.628	0.692	0.591	0.711	0.488
CZ24	0.489	0.455	0.455	0.489	0.525	0.511	0.409	0.488	0.591	0.622	0.6	0.571	0.488	0.5	0.465	0.476	0.565	0.667	0.46	0.435	0.523	0.543	0.628	1	0.565	0.617	0.651	0.634
RU25	0.5	0.538	0.714	0.61	0.5	0.524	0.568	0.541	0.61	0.683	0.622	0.676	0.667	0.639	0.513	0.487	0.545	0.543	0.683	0.409	0.575	0.634	0.692	0.565	1	0.5	0.595	0.537
RU26	0.558	0.455	0.488	0.458	0.452	0.511	0.409	0.419	0.489	0.49	0.455	0.61	0.561	0.465	0.37	0.442	0.565	0.63	0.553	0.375	0.558	0.543	0.591	0.617	0.5	1	0.578	0.489
RU27	0.55	0.475	0.595	0.548	0.6	0.615	0.583	0.514	0.667	0.619	0.595	0.564	0.553	0.568	0.526	0.583	0.718	0.75	0.619	0.419	0.55	0.65	0.711	0.651	0.595	0.578	1	0.59
RU28	0.45	0.571	0.528	0.605	0.625	0.475	0.514	0.625	0.564	0.684	0.667	0.629	0.528	0.421	0.543	0.559	0.5	0.535	0.561	0.357	0.611	0.512	0.488	0.634	0.537	0.489	0.59	1

Figure 5 Heatmap visualizing values of Jaccard coefficients of similarity across hazelnut genotypes analysed by iPBS marker 1882.

3.2 iPBS Marker 2152

For this marker, a total of 814 polymorphic fragments were obtained with 63 distinct size levels ranging from 1535 bp to 135 bp within hazel genotypes that were analysed (Figure 6). The genotype from the Chocerady (CZ24) exhibited the highest fragment number (37), while the lowest number (20) was observed in the genotype from Jelenec village (SK7). In the analysed genotypes, several uniform and unique amplicons were consistently detected. The fingerprint profiles of the analysed hazel genotypes were clearly separated in the constructed dendrogram, which is divided into six major clusters (Figure 7) with the cophenetic coefficient of 0.91.

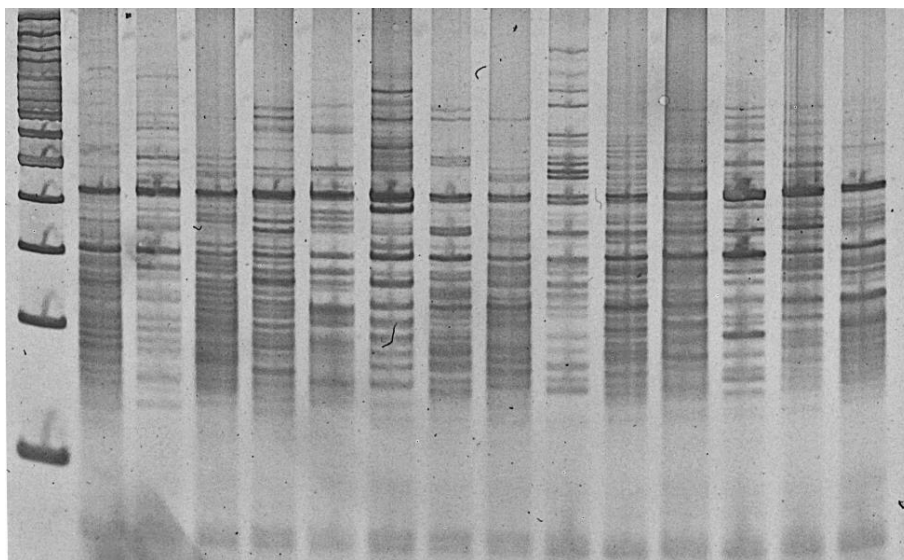


Figure 6 Obtained iPBS profiles for the 1882 marker. First line - ladder; following lines genotypes UA1 - SK15.

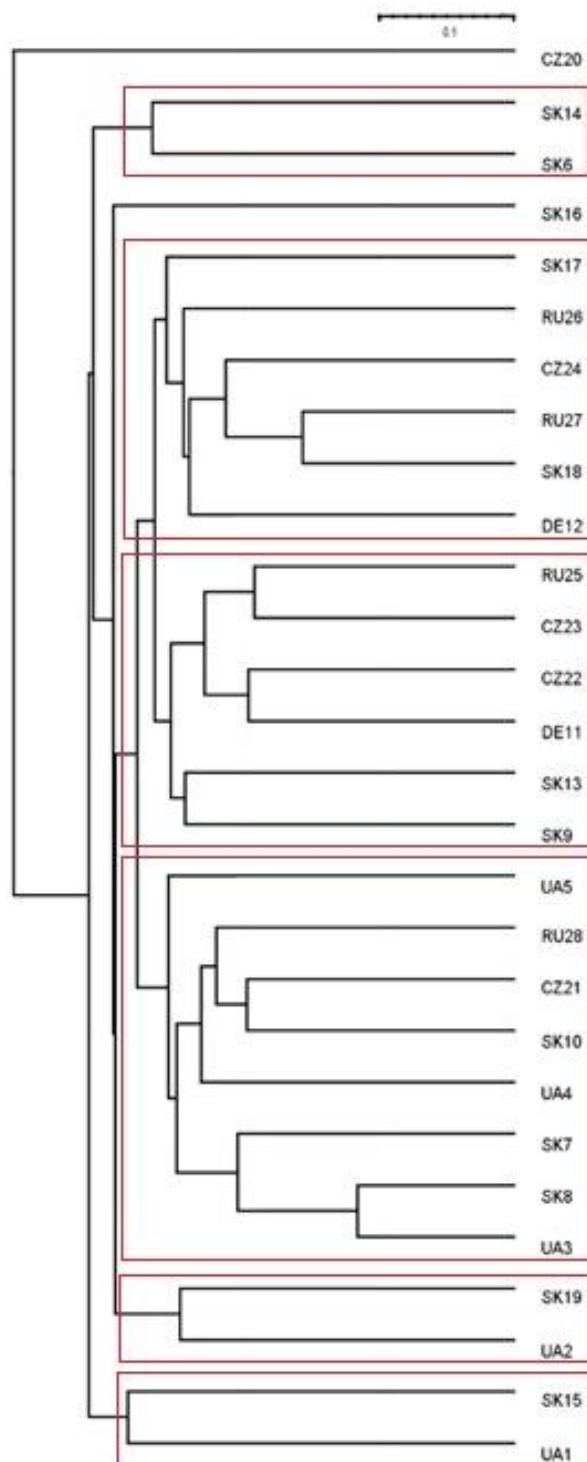


Figure 7 UPGMA dendrogram based on Jaccard coefficients of similarity illustrating the clustering of analysed hazel genotypes based on iPBS fingerprint data of the marker 2152.

For 2152, all the analysed genotypes were also separated based on their obtained iPBS fingerprints. The most similar profiles were observed between the UA3 and SK8 genotypes (Figure 8), while the most distinctive profiles were observed between the SK6 and CZ20 genotypes (Figure 9). This corresponds to their location in the dendrogram branches.

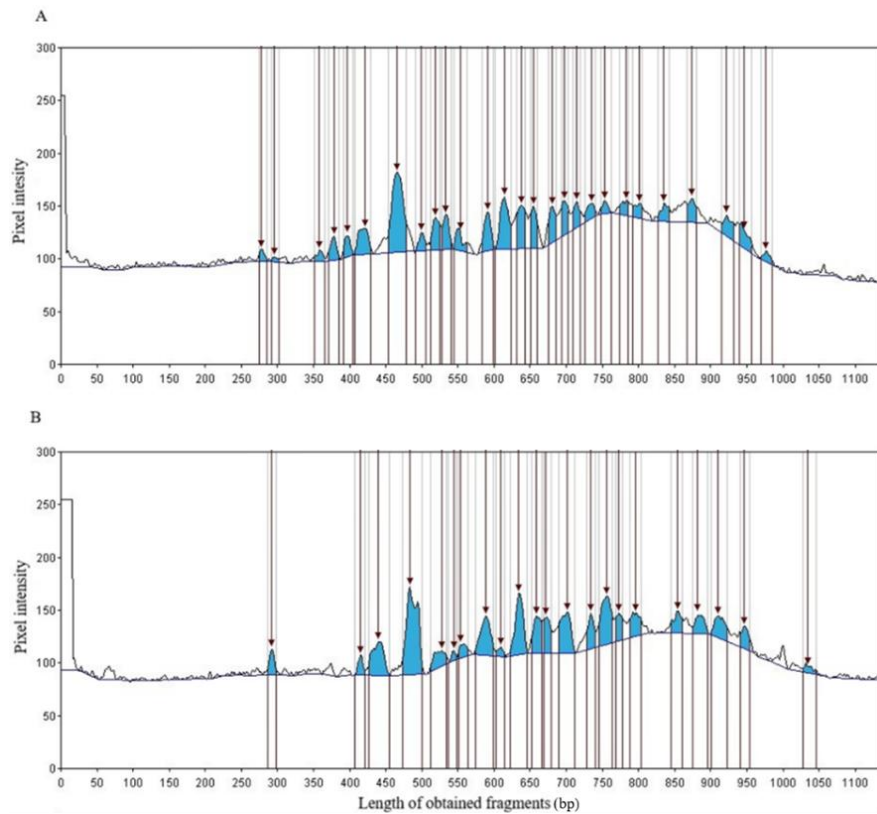


Figure 8 Fingerprints profiles of the most similar hazelnut genotypes (UA3 - A, SK8 - B) based on 2152 marker amplification results.

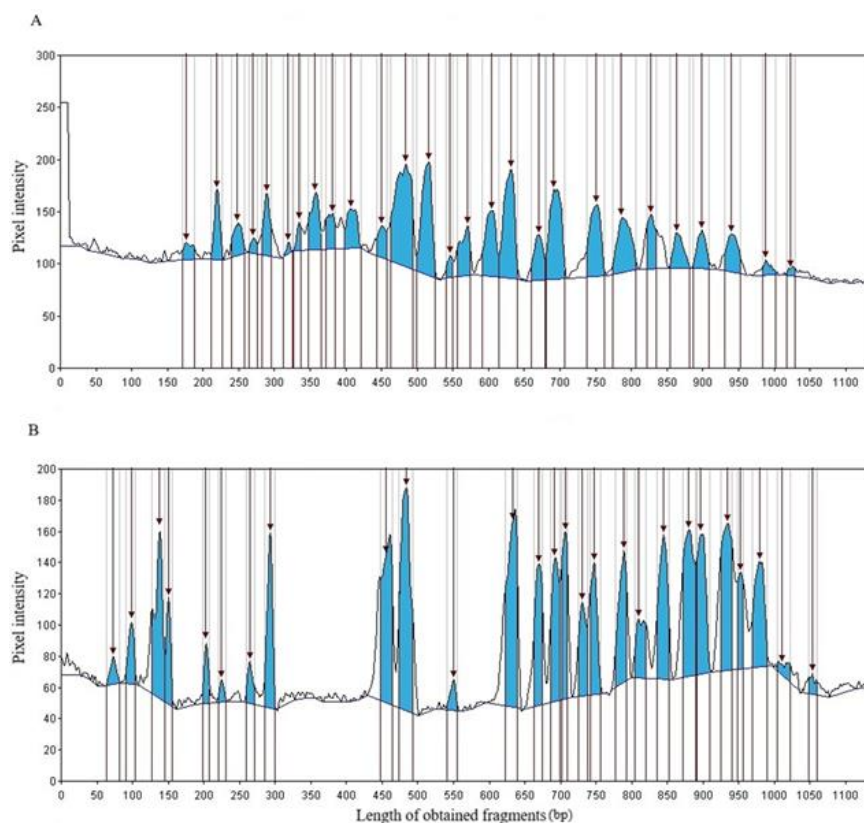


Figure 9 Fingerprints profiles of the most distinctive hazelnut genotypes (SK6 - A, CZ20 - B) based on 2152 marker amplification results.

The Jaccard similarity coefficient ranged from 0.289 to 0.815, averaging 0.608 and median 0.552. The Slovak genotype SK8 and the Ukrainian genotype UA3 exhibited the highest genetic similarity, with a Jaccard coefficient of 0.815. The minimum value of 0.289 was observed between the Slovak forest genotype Jelenec (SK6) and the Czech monastery genotype Prague - Strahov Monastery (CZ20) (Figure 10).

	UA1	UA2	UA3	UA4	UA5	SK6	SK7	SK8	SK9	SK10	DE11	DE12	SK13	SK14	SK15	SK16	SK17	SK18	SK19	CZ20	CZ21	CZ22	CZ23	CZ24	RU25	RU26	RU27	RU28
UA1	1	0.447	0.486	0.488	0.405	0.553	0.606	0.444	0.525	0.524	0.447	0.462	0.528	0.5	0.543	0.432	0.575	0.5	0.488	0.39	0.611	0.512	0.488	0.489	0.5	0.558	0.55	0.45
UA2	0.447	1	0.529	0.381	0.531	0.514	0.471	0.4	0.487	0.488	0.486	0.543	0.576	0.417	0.5	0.563	0.538	0.465	0.605	0.35	0.486	0.475	0.45	0.455	0.538	0.455	0.475	0.571
UA3	0.486	0.529	1	0.611	0.581	0.4	0.724	0.815	0.568	0.694	0.576	0.636	0.486	0.5	0.545	0.563	0.579	0.5	0.564	0.421	0.719	0.639	0.568	0.455	0.714	0.488	0.595	0.528
UA4	0.488	0.381	0.611	1	0.571	0.55	0.556	0.667	0.524	0.634	0.568	0.538	0.487	0.425	0.5	0.514	0.571	0.605	0.523	0.463	0.649	0.625	0.561	0.489	0.61	0.458	0.548	0.605
UA5	0.405	0.531	0.581	0.571	1	0.472	0.567	0.643	0.571	0.526	0.581	0.5	0.581	0.5	0.455	0.567	0.5	0.5	0.526	0.457	0.625	0.647	0.528	0.525	0.5	0.452	0.6	0.625
SK6	0.553	0.514	0.4	0.55	0.472	1	0.5	0.472	0.512	0.477	0.556	0.526	0.436	0.571	0.41	0.459	0.488	0.595	0.548	0.289	0.475	0.465	0.512	0.511	0.524	0.511	0.615	0.475
SK7	0.606	0.471	0.724	0.556	0.567	0.5	1	0.621	0.514	0.639	0.563	0.576	0.563	0.633	0.485	0.455	0.526	0.564	0.595	0.405	0.559	0.541	0.556	0.409	0.568	0.409	0.583	0.514
SK8	0.444	0.4	0.815	0.667	0.643	0.472	0.621	1	0.571	0.568	0.531	0.594	0.441	0.455	0.455	0.469	0.462	0.579	0.526	0.417	0.529	0.514	0.571	0.488	0.541	0.419	0.514	0.625
SK9	0.525	0.487	0.568	0.524	0.571	0.512	0.514	0.571	1	0.634	0.657	0.579	0.611	0.462	0.462	0.514	0.571	0.468	0.489	0.395	0.605	0.585	0.488	0.591	0.61	0.489	0.667	0.564
SK10	0.524	0.488	0.694	0.634	0.526	0.477	0.639	0.568	0.634	1	0.564	0.537	0.488	0.579	0.5	0.475	0.533	0.6	0.667	0.4	0.684	0.581	0.595	0.622	0.683	0.49	0.619	0.684
DE11	0.447	0.486	0.576	0.568	0.581	0.556	0.563	0.531	0.657	0.564	1	0.636	0.625	0.5	0.545	0.613	0.538	0.658	0.525	0.421	0.667	0.686	0.611	0.6	0.622	0.455	0.595	0.667
DE12	0.462	0.543	0.636	0.538	0.5	0.526	0.576	0.594	0.579	0.537	0.636	1	0.588	0.559	0.514	0.486	0.55	0.711	0.537	0.333	0.629	0.525	0.579	0.571	0.676	0.61	0.564	0.629
SK13	0.528	0.576	0.486	0.487	0.581	0.436	0.563	0.441	0.611	0.488	0.625	0.588	1	0.5	0.545	0.563	0.5	0.465	0.419	0.421	0.571	0.595	0.526	0.488	0.667	0.561	0.553	0.528
SK14	0.5	0.417	0.5	0.425	0.5	0.571	0.633	0.455	0.462	0.579	0.5	0.559	0.5	1	0.471	0.441	0.439	0.512	0.5	0.359	0.5	0.487	0.5	0.5	0.639	0.465	0.568	0.421
SK15	0.543	0.5	0.545	0.5	0.455	0.41	0.485	0.455	0.462	0.5	0.545	0.514	0.545	0.471	1	0.531	0.439	0.476	0.429	0.432	0.588	0.487	0.5	0.465	0.513	0.37	0.526	0.543
SK16	0.432	0.563	0.563	0.514	0.567	0.459	0.455	0.469	0.514	0.475	0.613	0.486	0.563	0.441	0.531	1	0.526	0.488	0.475	0.444	0.606	0.541	0.6	0.476	0.487	0.442	0.583	0.559
SK17	0.575	0.538	0.579	0.571	0.5	0.488	0.526	0.462	0.571	0.533	0.538	0.55	0.5	0.439	0.439	0.526	1	0.543	0.605	0.476	0.575	0.634	0.65	0.565	0.545	0.565	0.718	0.5
SK18	0.5	0.465	0.5	0.605	0.5	0.595	0.564	0.579	0.468	0.6	0.658	0.711	0.465	0.512	0.476	0.488	0.543	1	0.674	0.383	0.535	0.628	0.568	0.667	0.543	0.63	0.75	0.535
SK19	0.488	0.605	0.564	0.523	0.526	0.548	0.595	0.526	0.489	0.667	0.525	0.537	0.419	0.5	0.429	0.475	0.605	0.674	1	0.4	0.6	0.619	0.558	0.46	0.683	0.553	0.619	0.561
CZ20	0.39	0.35	0.421	0.463	0.457	0.289	0.405	0.417	0.395	0.4	0.421	0.333	0.421	0.359	0.432	0.444	0.476	0.383	0.4	1	0.462	0.419	0.463	0.435	0.409	0.375	0.419	0.357
CZ21	0.611	0.486	0.719	0.649	0.625	0.475	0.559	0.529	0.605	0.684	0.667	0.629	0.571	0.5	0.588	0.606	0.575	0.535	0.6	0.462	1	0.632	0.649	0.523	0.575	0.558	0.55	0.611
CZ22	0.512	0.475	0.639	0.625	0.647	0.465	0.541	0.514	0.585	0.581	0.686	0.525	0.595	0.487	0.487	0.541	0.634	0.628	0.619	0.419	0.632	1	0.667	0.543	0.634	0.543	0.65	0.512
CZ23	0.488	0.45	0.568	0.561	0.528	0.512	0.556	0.571	0.488	0.595	0.611	0.579	0.526	0.5	0.5	0.6	0.65	0.568	0.558	0.463	0.649	0.667	1	0.628	0.692	0.591	0.711	0.488
CZ24	0.489	0.455	0.455	0.489	0.525	0.511	0.409	0.488	0.591	0.622	0.6	0.571	0.488	0.5	0.465	0.476	0.565	0.667	0.46	0.435	0.523	0.543	0.628	1	0.565	0.617	0.651	0.634
RU25	0.5	0.538	0.714	0.61	0.5	0.524	0.568	0.541	0.61	0.683	0.622	0.676	0.667	0.639	0.513	0.487	0.545	0.543	0.683	0.409	0.575	0.634	0.692	0.565	1	0.5	0.595	0.537
RU26	0.558	0.455	0.488	0.458	0.452	0.511	0.409	0.419	0.489	0.49	0.455	0.61	0.561	0.465	0.37	0.442	0.565	0.63	0.553	0.375	0.558	0.543	0.591	0.617	0.5	1	0.578	0.489
RU27	0.55	0.475	0.595	0.548	0.6	0.615	0.583	0.514	0.667	0.619	0.595	0.564	0.553	0.568	0.526	0.583	0.718	0.75	0.619	0.419	0.55	0.65	0.711	0.651	0.595	0.578	1	0.59
RU28	0.45	0.571	0.528	0.605	0.625	0.475	0.514	0.625	0.564	0.684	0.667	0.629	0.528	0.421	0.543	0.559	0.5	0.535	0.561	0.357	0.611	0.512	0.488	0.634	0.537	0.489	0.59	1

Figure 10 Heatmap visualizing values of Jaccard coefficients of similarity across hazelnut genotypes analysed by iPBS marker 2152.

Comparing the iPBS markers used in the study, 1882 was more effective at distinguishing the analysed hazelnut genotypes (Table 2). The ability to detect polymorphisms was comparable, with an average PIC value of 0.33. The 2152 iPBS marker was more effective in analysing hazelnut variability, as the marker index value was higher. The marker 1882 had a higher discrimination power index, corresponding to the more distinct fingerprints for individual genotypes analysed by this marker.

Table 2 Characteristics of fingerprints obtained by the iPBS marker used in this study.

Marker	PIC	AN	MI	H	D	R
1882	0.289769	19.6	0.002857	0.351569	0.948283	27.14286
2152	0.37351	29.1	0.008191	0.000282	0.787204	26.42857

PIC - polymorphism information content; AN - average number of amplified alleles per accession; MI - marker index; H - heterozygosity index ; D - discrimination power; R - resolving power.

PCoA analysis was performed on the polymorphic profiles obtained for both markers used in the study (Figure 11). A clear separation of all of them and the visualisation of intra-accession polymorphisms within individual accessions were obtained.

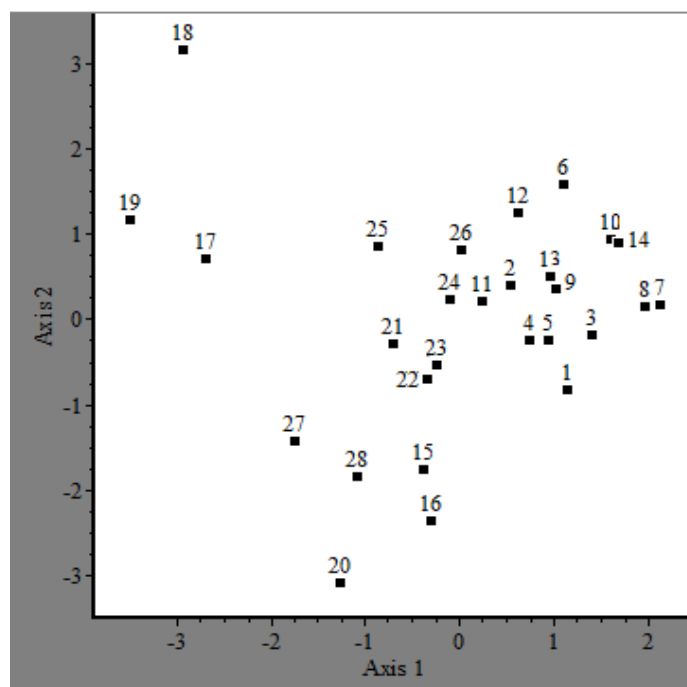


Figure 11 PCoA analysis visualizing the joined fingerprints of both iPBS markers used in this study.

In PCoA, a group of Slovak genotypes (SK16, SK18, SK19) was separated into distinct iPBS profiles.

4. Discussion

Corylus avellana L. is a species that has exhibited a high degree of polymorphism. Several studies utilised molecular marker approaches to examine the genetic diversity of common hazel [17], thereby providing evidence that RAPD and AFLP markers are valuable tools for hazelnut cultivar

identification. Significant genetic variability in common hazel has been reported [24, 25] using SSR (Simple Sequence Repeats) markers. The ISSR technique was also employed [17, 26]. In Corylaceae, DNA markers have been essential for resolving phylogeny and genetic diversity across these taxa, where morphology alone is insufficient [27]. Despite decades of research, phylogenetic resolution within *Corylus* and related genera remains incomplete, motivating continued marker development across RAPD, SSR, ISSR, AFLP, and sequence-based markers [28].

In the genomes of various *Corylus* spp., diverse retrotransposon sequences have been identified. In *Corylus heterophylla* L., repetitive elements account for 57.99% of the assembled genome [29]. In *Corylus avellana* L., 92.7% of the repetitive DNA comprises retroelements with long terminal repeats, and over half of which are degenerated, with internal deletions and divergent sequences. However, Copia-like elements are almost twice as abundant as Gypsy-like elements [30]. This data provides a solid foundation for the application of iPBS markers, which are expected to deliver highly polymorphic results capable of distinguishing hazelnut genotypes at the interspecific level. The strengths of iPBS markers include high polymorphism and reproducibility, the absence of a requirement for prior sequence data, and broad applicability across plant taxa. This makes them valuable in studies of genetic variability and diversity conservation, as well as in the design of breeding programs. Their effectiveness has been confirmed in both self and cross-pollinating species, and in annual, perennial, and wild plant populations [8]. As retrotransposons are dynamic components of genomes, iPBS markers also provide indirect insights into genome evolution and structural variation. This expands their utility beyond simple diversity assessment [31]. In legumes such as the common bean (*Phaseolus vulgaris* L.), iPBS markers generated a large number of polymorphic bands and enabled the identification of subpopulations, indicating their strong potential for use in germplasm identification and genetic improvement strategies [32]. Other applications include assessing genetic relationships in wild wheat and forage pea, where iPBS markers have provided insights into population structure and differentiation among accessions. This information is useful for implementing conservation and adaptive breeding strategies [33]. To date, only one study has been reported on iPBS markers for *Corylus avellana* L., demonstrating the ability to generate polymorphic markers at levels ranging from 60% to 100% [34]. In this study, 24 iPBS markers were tested in total, of which only six of the most polymorphic were evaluated in more detail across a set of 30 hazelnut varieties. The discrimination power and heterozygosity index obtained are in concordance with our results for marker 1882. However, both of these values were lower in our study for the iPBS marker 2152. This confirms the previous findings about iPBS markers: their effectiveness in discriminating analysed accessions is based on the species-specificity of retrotransposon sequences, and they differ in this parameter [35-37]. The polymorphic information content obtained in this study for markers 1882 and 2152 was 0.29 and 0.37, respectively, within the expected range for dominant iPBS markers and consistent with previous studies of iPBS markers in different plant species [38, 39]. SSR markers are the most widely used markers in Corylaceae and Betulaceae due to co-dominance and reproducibility. SSRs developed in one genus (e.g., *Betula* or *Corylus*) often transfer across related genera. However, amplification success declines across subfamilies [27], and the PIC range here is 0.09-0.58, indicating moderate genetic diversity and locus-specific variability [40]. Here, similar PIC values were obtained for the used iPBS markers. Earlier phylogenetic work in *Corylus* relied heavily on RAPD and AFLP markers, with PIC values that were variable and often with lower reproducibility [28].

5. Conclusions

The iPBS markers 1882 and 2152 were employed to evaluate genetic relationships among pollen samples of *Corylus avellana* L. The genetic similarity patterns differed between the two chosen iPBS markers. Using the iPBS marker 1882, a total of 548 polymorphic fragments were detected. The strongest genetic similarity was observed in the generated iPBS fingerprint pattern recorded between the Ukrainian accession from the Kyiv park (UA3) and the German accession from Darmstadt (DE11). In comparison, the iPBS marker 2152 produced 814 polymorphic fragments, with the highest level of genetic similarity observed between the Ukrainian accession from the Kyiv park (UA3) and the Slovakian accessions from the field near Ivanka pri Nitre (SK8).

These results reveal marker-specific patterns of genetic relatedness among the studied samples. iPBS markers were proven to exhibit moderate polymorphism and high informativeness for common hazelnut genotypes. The analysis provides valuable insights into the extent of genetic diversity and the potential patterns of gene flow within hazelnut populations. This demonstrates that the iPBS technique is both reliable and effective for this purpose. These findings are of considerable significance for breeding programmes, cultivar identification, and the long-term conservation of hazelnut genetic resources, as they contribute to a deeper understanding of the species' genetic structure.

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Author Contributions

Conceptualization - J.Ž.; methodology - J.Ž.; software - A.J., L.K.; validation - A.J., J.Ž.; formal analysis - A.J., L.K.; investigation - J.Ž.; resources - J.Ž.; writing original draft preparation - A.J., J.Ž.; writing review and editing - L.K.; visualization - A.J., J.Ž., L.K.; supervision - J.Ž.; project administration - J.Ž.

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Competing Interests

The authors have declared that no competing interests exist.

AI-Assisted Technologies Statement

Artificial intelligence (AI) tools of OpenAI's ChatGPT were used solely for basic grammar correction and English syntax control of this manuscript. All scientific content, data interpretation, and conclusions are original and were developed independently by the author. The authors have thoroughly reviewed and edited the AI-assisted text to ensure its accuracy and accept full responsibility for the content of the manuscript.

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