Original Paper

Regional Patterns of Genetic Variation in Lavender Revealed by RAPD Analysis

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Lavender is primarily cultivated for its essential oil, which is rich in terpenoids and widely used in cosmetics, aromatherapy, and medicine. In this study, we assessed the genomic variability of lavender grown in six regions of Slovakia across two species, five varieties, and two tissue types (leaves and flowers) using molecular markers. Molecular profiles generated with five RAPD primers were largely shaped by taxonomic affiliation, with individual species forming clearly distinguishable groups. Additionally, primer SM_03 demonstrated a strong ability to differentiate samples by tissue type. Although the primers were highly effective in species discrimination, they did not reveal variability associated with environmental conditions. Overall, the applied RAPD markers showed strong discriminatory power for taxonomic differentiation but were insufficient to distinguish samples by geographic origin.

Keywords: genetic diversity, *Lavandula angustifolia* Mill., *Lavandula* × *intermedia* Emeric ex Loisel., medicinal plants, RAPD markers, Slovakia

1 Introduction

Lavender (*Lavandula* spp.) is an extensively cultivated medicinal plant, grown to produce the essential oil as well as ornamental value, and medicinal properties. Originally native to the Mediterranean, it is now grown globally. The essential oil of this plant, produced and stored in glandular trichomes, contains bioactive compounds such as linalool and linalyl acetate and is recognised for its anti-inflammatory, antimicrobial, and calming effects. These properties have long been used traditionally to treat anxiety, insomnia, and skin conditions (Lis-Balchin, 2002). There are around 47 known lavender species, with *Lavandula angustifolia* Mill. and *Lavandula* × *intermedia* Emeric ex Loisel. being the most widely cultivated and commercially valuable lavender species. *L. angustifolia*, recognised for its medicinal properties, is frequently used as a complementary treatment for anxiety, stress relief, and sleep disorders due to its calming effects (Ghavami et al., 2022). The species is mainly cultivated for its essential oil, which has broad applications in perfumery, cosmetics, culinary products, and aromatherapy (Lis-Balchin, 2002). *L.* × *intermedia* is a sterile hybrid of *L. latifolia* Medik. and *L. angustifolia*. Known for its vigorous growth and strong aroma, it is often used in the production of perfumes, but is not suitable for medical use (Koulivand et al., 2013).

In this work, the genetic variability of lavender samples grown in Slovakia was analysed using five sets of RAPD markers to access their determinative ability in the context of species (*L. angustifolia*, *L.* × *intermedia*), tissue type (leaf, flower), or their ability to point to a geographical origin of the samples, including differences related to soil types.

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2 Material and Methods

2.1 Biological Material and Localities

Biological material was collected during the flowering period, from 14 June to 12 July 2023. The flowering period varied considerably depending on the location and species cultivated. These included two species (*L. angustifolia* and *L. × intermedia*) and five different varieties (*L. × intermedia*: 'Grappenhall', 'Grosso'; *L. angustifolia*: 'Krajova', 'Hidcote' and 'Hidcote Blue') (Table 1). Three plants were randomly selected from each genotype grown on individual farms. From these 10 stems, together with leaves and flowers, were taken. These were labelled immediately after collection and stored separately in a portable cooling box. The samples were documented, divided into individual parts (flowers, leaves and stems) and stored at -20 °C until further processing. The soil types at the collection sites were determined using the Geochemical Atlas of Slovakia (Čurlík & Šefčík, 1999). The geographical location of selected farms from which samples were taken is shown in Figure 1.

Table 1 Sample collection sites and sample specifications

Location	Soil type	Species	Genotype	Tissue	Sample
Zbehy	chernozem	*L. angustifolia*	'Hidcote'	Leaf	1.A.1
				Flower	1.A.2
		L. × intermedia	'Grappenhall'	Leaf	1.X.1
				Flower	1.X.2
Šaľa	fluvisol	*L. angustifolia*	'Krajova'	Leaf	2.A.1
				Flower	2.A.2
		L. × intermedia	–	Leaf	2.X.1
				Flower	2.X.2
Dolná Breznica	rendzina	*L. angustifolia*	'Hidcote blue'	Leaf	3.A.1
				Flower	3.A.2
		L. × intermedia	'Grosso'	Leaf	3.X.1
				Flower	3.X.2
Trenčín	fluvisol	*L. angustifolia*	'Hidcote'	Leaf	4.A.1
				Flower	4.A.2
		L. × intermedia	'Grosso'	Leaf	4.X.1
				Flower	4.X.2
Vištuk	fluvisol	*L. angustifolia*	'Hidcote'	Leaf	5.A.1
				Flower	5.A.2
		L. × intermedia	'Grosso'	Leaf	5.X.1
				Flower	5.X.2
Malé Leváre	regosol	*L. angustifolia*	'Hidcote blue'	Leaf	6.A.1
				Flower	6.A.2
		L. × intermedia	–	Leaf	6.X.1
				Flower	6.X.2

Figure 1 Locations for collecting lavender samples in Slovakia

2.2 DNA Extraction and Molecular Analyses

DNA extraction from the collected samples was performed using the ISOLATE II Plant DNA Kit (Meridian Bioscience®) according to the manufacturer's instructions. Quality and purity of the extracted DNA were assessed using a NanoPhotometer P360 spectrophotometer (IMPLEN).

To verify the DNA's functionality in the samples, PCR analysis was performed using primers specific to the ITS (Internal Transcribed Spacer) region. The ITS1 primer was used, the sequence of which is 5′ TCC GTA GGT GAA CCT GCG G, and the ITS4 primer with the sequence 5′ TCC TCC GCT TAT TGA TAT CC. The reaction mixture with a volume of 10 µl contained 0.4 µmol/dm^3 of the ITS1 primer, 0.4 µmol/dm^3 of the ITS4 primer, and 2x EliZyme HS Robust MIX Red (ELISABETH PHARMACON Ltd.). PCR was performed in a C1000TM Thermal Cycler (BIORAD) following the temperature protocol: an initial HotStart at 95 °C for 5 minutes, followed by 34 cycles of denaturation at 95 °C for 30 seconds, primer annealing at 55 °C for 30 seconds, and polymerization at 72 °C for 1 minute, concluded with a final polymerization step at 72 °C for 15 minutes.

The RAPD (Random Amplified Polymorphic DNA) technique was used to generate unique genetic profiles. The primers used in the study, along with their sequences (5′ to 3′), were as follows: OPB11 (GTAGACCCGT), OPB18 (CCACAGCAGT), SM_03 (GAGCCCTCCA), SM_06 (ACGCGAGAGG), and SM_10 (TCCCGCCTAC). The reaction mixture contained 30 ng DNA, 2× EliZyme HS Robust MIX Red, 0.6 µmol/dm^3 primer and H2O in a total volume of 10 µl. The PCR was performed in a C1000TM Thermal Cycler (BIORAD) and the protocol began with an initial denaturation at 94 °C for 5 minutes, followed by 45 cycles of denaturation at 94 °C for 60 seconds, primer annealing at 36 °C for 60 seconds, and polymerisation at 72 °C for 2 minutes. The reaction ended with a final polymerisation step at 72 °C for 7 minutes. The resulting products were then loaded onto a 1.5% agarose gel and visualised using GelRed™ Nucleic Acid Stain (Biotinum) and G-Box electrophoretic documentation system (SYNGENE). The obtained electropherograms were analysed using the GeneTools software (SYNGENE), which allows automatic search for prominent amplicons based on signal intensity.

2.3 Statistical Analysis

Based on the presence or absence of individual amplicons, a binary matrix was created from the obtained electropherograms and further analysed using the R programming language (R Core Team, 2020), the dplyr, stats, pheatmap, and RColorBrewer libraries in the RStudio environment (RStudio Team, 2021). The binary matrices were transformed into distance matrices by calculating the Jaccard index, which quantifies similarity based on the presence/absence of amplified fragments. These distance matrices were used to create a dendrogram and a heatmap using the UPGMA (unweighted pair-group method with arithmetic mean) clustering method. To calculate basic indices of polymorphism, such as polymorphic information content (PIC) and discriminatory power (DP), binary matrices were analysed using the online tool iMEC (Amiryousefi et al., 2018, https://irscope.shinyapps.io/iMEC/). To analyse the possible influence of location and soil type on polymorphism, binary profiles obtained with all RAPD primers were combined into a single matrix, from which pairwise distances were subsequently calculated using the Jaccard coefficient, as in the previous case. The data thus prepared were analysed using t-SNE (t-Distributed Stochastic Neighbour Embedding) with the following parameters: perplexity = 13, learning_rate = 200, random_state = 42. The calculations and subsequent visualisation were performed in Python 3.12 using the scikit-learn and matplotlib libraries.

3 Results and Discussion

3.1 Results

Genomic screening of lavender variability using RAPD primer SM_03 demonstrated an exceptionally good ability to distinguish samples by tissue type and subsequently by plant species. A total of 160 products were generated with primer SM_03, averaging 6.7 per sample. In general, more fragments were generated by amplification of DNA originating from *L.* × *intermedia* ($\bar{x}$ = 7.7) compared to *L. angustifolia* ($\bar{x}$ = 5.7) and also in flowers ($\bar{x}$ = 8.5) compared to leaves ($\bar{x}$ = 5). A prominent fragment of approximately 2,300 bp in length can be observed specifically in leaf tissues. Two flower-specific fragments of approximately 2,500 bp and 1800 bp in length can also be observed. From a species perspective, a unique amplicon of 700 bp was identified, specific to *L.* × *intermedia* . The polymorphism indices for the primer SM_03 reached the following values: PIC = 0.374 and DP = 0.717. The heatmap (Fig. 2) shows that the hierarchical clustering algorithm accurately divided the samples into two groups based on tissue type. Within

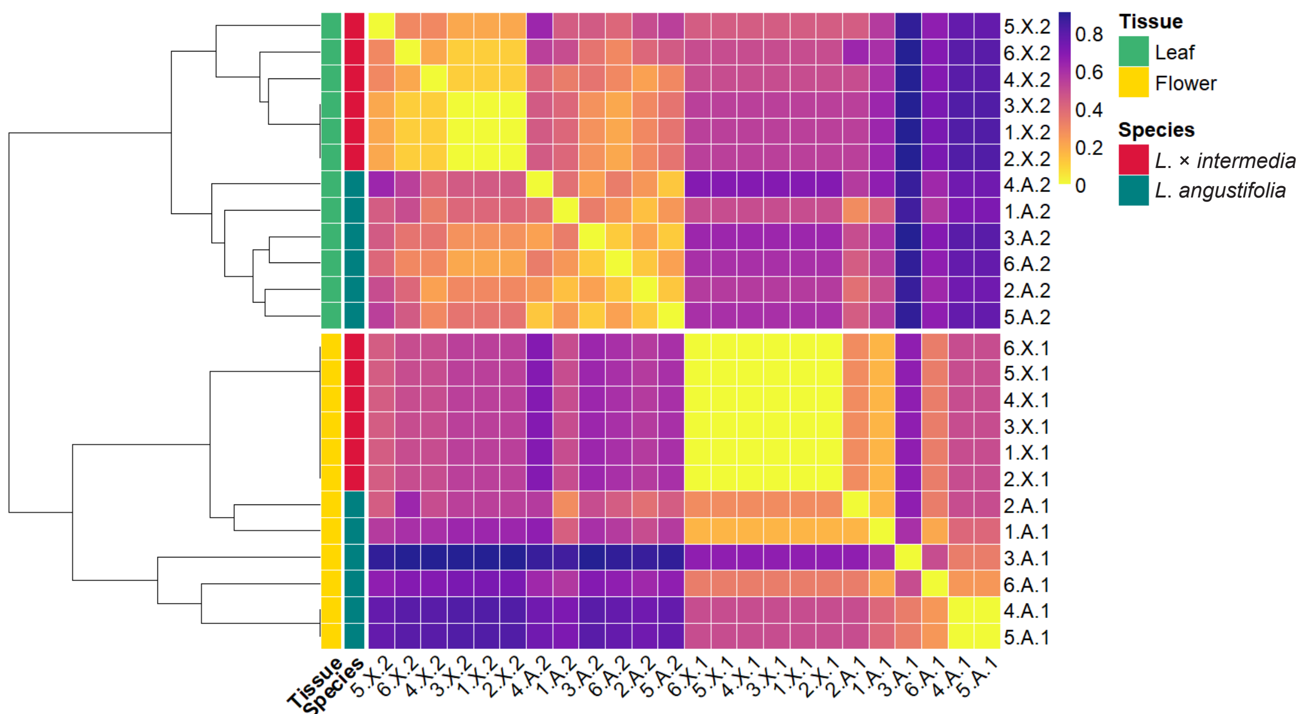

Figure 2 Heatmap showing similarities between profiles of individual lavender samples obtained using primer SM_03. The colour scale from yellow (highest similarity) to blue (lowest similarity) represents distance values based on the Jaccard coefficient. The annotation bar on the left edge indicates the species (*L. angustifolia*, *L.* × *intermedia*) or tissue (leaf, flower) to which each sample belongs. The dendrogram on the left side of the image shows the hierarchical clustering of samples based on their similarities. Created using the pheatmap library and R 4.3.3

these tissue-based groups, a secondary level of clustering is also clearly visible, with samples further grouped by species affiliation.

Primer SM_06 demonstrated high efficiency in distinguishing samples by plant species (Fig. 3). A total of 202 amplification products were generated with this primer, yielding an average of 8.4 products per sample. The results showed that DNA isolated from *L. angustifolia* produced more fragments ($\bar{x}$ = 9.4) compared to *L.* × *intermedia* ($\bar{x}$ = 7.7). Similarly, a higher number of fragments was observed in leaf samples ($\bar{x}$ = 8.5) compared to flowers ($\bar{x}$ = 8.3). The following values were determined for primer SM_06: PIC = 0.370 and DP = 0.678. Analysis of the presence or absence of individual amplicons based on a binary matrix revealed significant species-specific differences. In addition to the fragment of approximately 1200 bp, which was monomorphic across all analysed samples regardless of the species or tissue type, several species – specific fragments were also amplified. This is, e.g. a fragment with a length of approximately 1350 bp, unique for profiles generated by amplification of DNA from the species *L.* × *intermedia*. On the contrary, for the species *L. angustifolia*, one to two fragments in the range of 700 to 750 bp were typical, which did not occur in the profiles of the species *L.* × *intermedia*. No unique amplicons were observed for tissue specificity. The heat map indicates that the hierarchical clustering algorithm grouped the samples with relatively high accuracy by species (Fig. 2). An exception was observed, where one *L.* × *intermedia* sample clustered with *L. angustifolia* samples, despite its species origin.

Using the primer SM_10, 155 products were generated, with an average of 6.5 amplicons per sample. No significant difference in the average number of fragments was observed between species; however, the number of amplicons obtained from leaf DNA ($\bar{x}$ = 7.5) was significantly higher compared to flower samples ($\bar{x}$ = 5.4). Despite the relatively high inefficiency of this primer for distinguishing individual species and tissues, the polymorphism indices were similar to those in the previous cases: PIC = 0.370 and DP = 0.814. This fact may indicate possible clustering based on other factors, such as location and growing conditions. The primer SM_10 showed the lowest ability to cluster samples based on plant species or tissue type. A heatmap associated with a dendrogram visualisation indicates the presence of a significantly differentiated branch composed of several samples belonging to *L.* × *intermedia*, which exhibit almost identical profiles (Fig. 4).

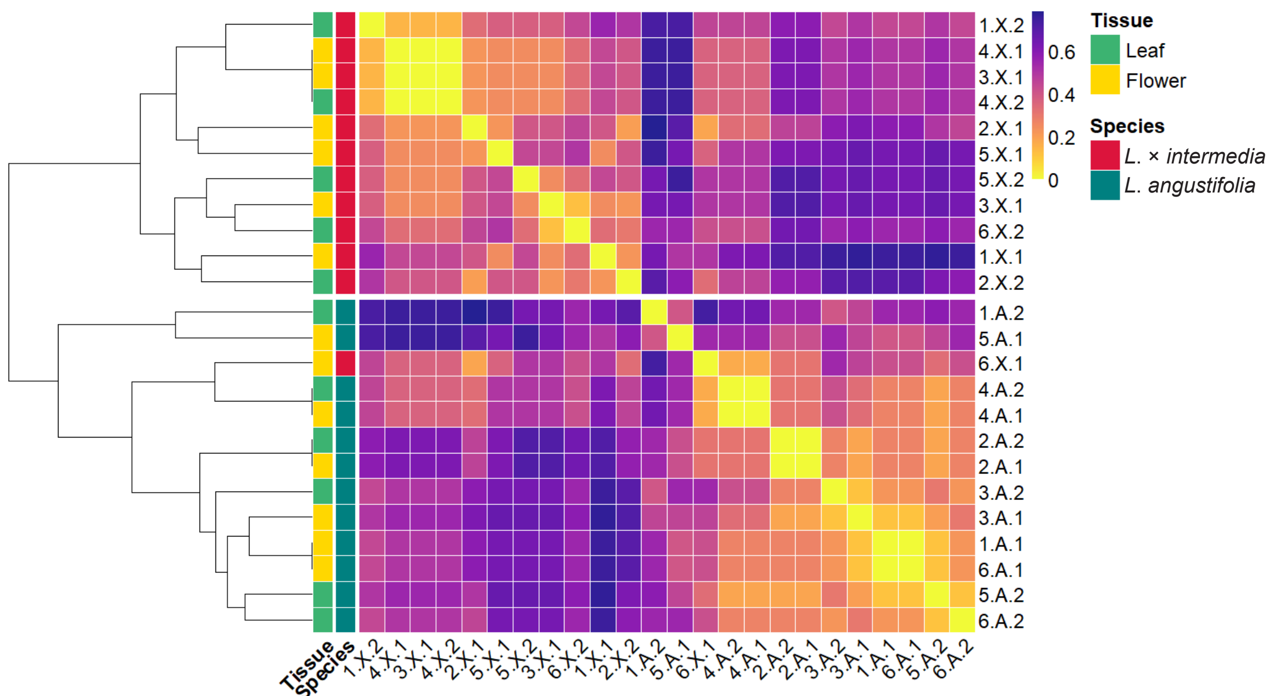

Figure 3 Heatmap showing similarities between profiles of individual lavender samples obtained using primer SM_06. The colour scale from yellow (highest similarity) to blue (lowest similarity) represents distance values based on the Jaccard coefficient. The annotation bar on the left edge indicates the species (*L. angustifolia*, *L.* × *intermedia*) or tissue (leaf, flower) to which each sample belongs. The dendrogram on the left side of the image shows the hierarchical clustering of samples based on their similarities. Created using the pheatmap library and R 4.3.3

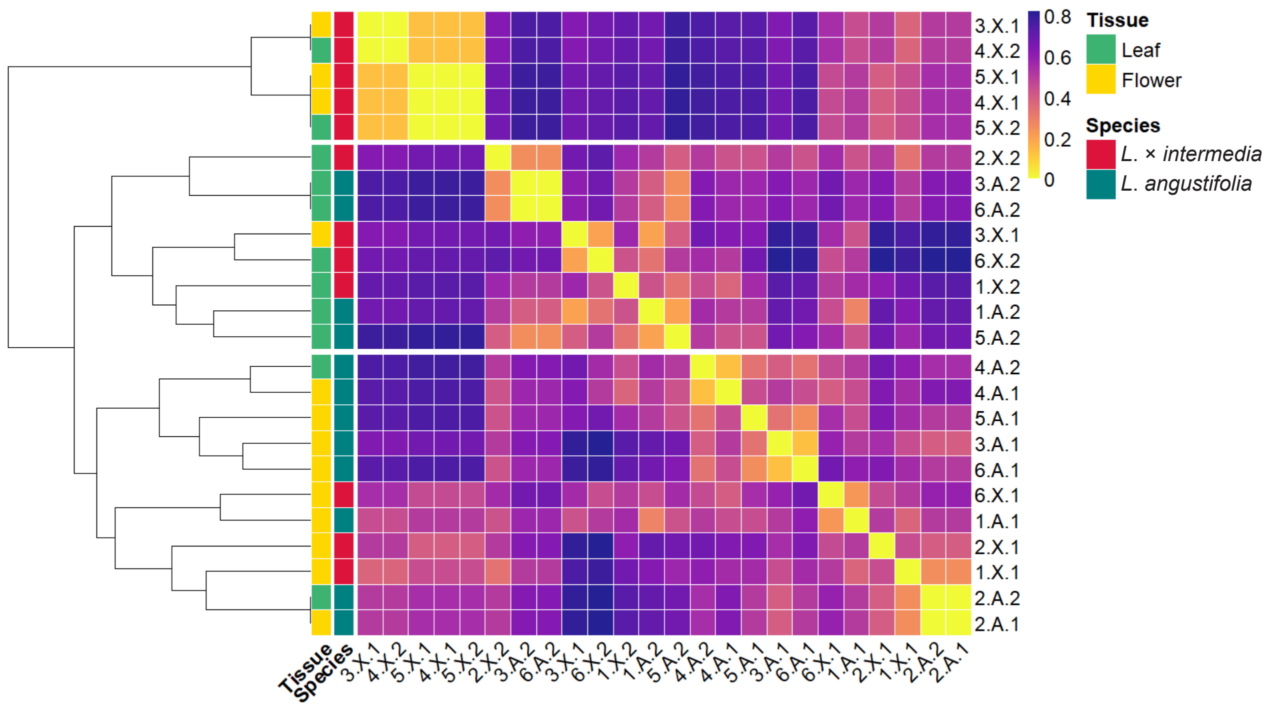

Figure 4 Heatmap showing similarities between profiles of individual lavender samples obtained using primer SM_10. The colour scale from yellow (highest similarity) to blue (lowest similarity) represents distance values based on the Jaccard coefficient. The annotation bar on the left edge indicates the species (*L. angustifolia*, *L.* × *intermedia*) or tissue (leaf, flower) to which each sample belongs. The dendrogram on the left side of the image shows the hierarchical clustering of samples based on their similarities. Created using the pheatmap library and R 4.3.3

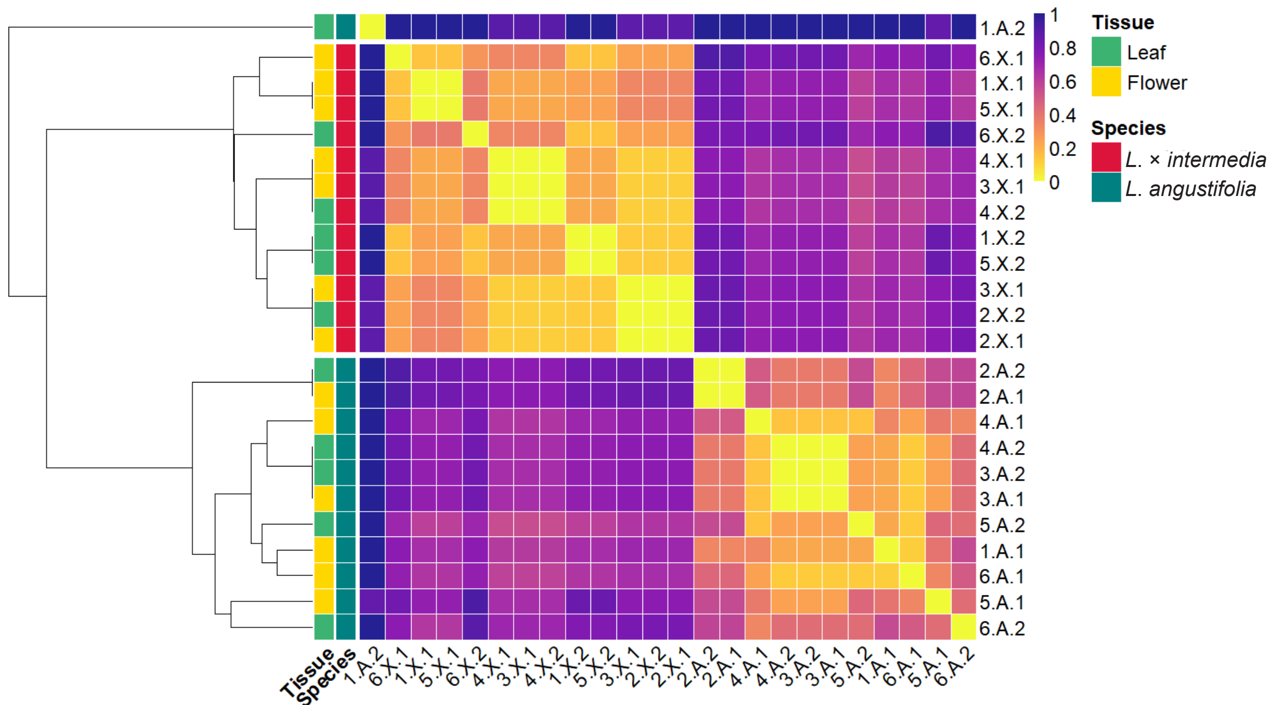

Figure 5 Heatmap showing similarities between profiles of individual lavender samples obtained using primer OPB_11. The colour scale from yellow (highest similarity) to blue (lowest similarity) represents distance values based on the Jaccard coefficient. The annotation bar on the left edge indicates the species (*L. angustifolia*, *L.* × *intermedia*) or tissue (leaf, flower) to which each sample belongs. The dendrogram on the left side of the image shows the hierarchical clustering of samples based on their similarities. Created using the pheatmap library and R 4.3.3

Primer OPB_11, similar to primers SM_03 and SM_06, showed high efficiency in distinguishing samples based on plant species (Fig. 5). The total number of generated amplicons in this case was equal to 166, and the average number of amplicons per sample was 6.91. In the case of *L.* × *intermedia*, a slightly increased number of generated amplicons was observed ($\bar{x}$ = 7.6) compared to *L. angustifolia* ($\bar{x}$ = 6.3). Similarly, a higher number of fragments was observed in profiles generated from leaf DNA ($\bar{x}$ = 7.4) than from flower DNA ($\bar{x}$ = 6.4). Only one weak fragment was present in sample 1.A.2, which also influenced the subsequent placement of this sample on a separate branch in the resulting dendrogram, while the other samples were classified into two clusters, mirroring the species composition of the samples. In the resulting electropherogram, several species-specific amplicons were observed (e.g., an amplicon approximately 600 bp in size specific for plants of *L.* × *intermedia*). However, the amplification profiles of samples 2.A.1 and 2.A.2 (*L. angustifolia*, genotype 'Krajova') are also interesting, as they differ from those of other samples of the same species belonging to the genotypes 'Hidcote ' and 'Hidcote blue'. In the case of both samples (2.A.1 and 2.A.2), a unique amplicon with a size of approximately 2400 bp was observed. This difference is also confirmed by visualisation using a heat map, which shows a significant difference between the genotype 'Krajova' and the other *L. angustifolia* genotypes represented in this analysis. PCR using primer OPB_11 generated profiles with PIC = 0.374 and DP = 0.718.

Although only 125 amplicons were obtained using primer OPB_18, the last of the analysed primers also showed effective discrimination between the two most popular lavender species (Fig. 6). However, with an average number of only 5.21 amplicons per sample, this primer was not effective in distinguishing samples beyond the species level. In general, more fragments were generated by amplifying DNA originating from *L. angustifolia* ($\bar{x}$ = 6) compared to *L.* × *intermedia* ($\bar{x}$ = 4.42). In Fig. 5, two distinct clusters are observed, with the only exception to the otherwise fairly accurate classification based on species being sample 1.A.2 (*L. angustifolia*), which was again grouped with *L.* × *intermedia* samples in the resulting heatmap. Also noteworthy is the similarity between the *L. angustifolia* samples collected in the Trenčín and Vištuk localities (4.A.2, 4.A.1, 5.A.2 and 5.A.1), based on which these samples were placed on the same branch similarly to the *L.* × *intermedia* samples from the same localities (4.X.2, 4.X.1, 5.X.2 and 5.X.1) in the case of using the SM_10 primer.

Figure 6 Heatmap showing similarities between profiles of individual lavender samples obtained using primer OPB_18. The colour scale from yellow (highest similarity) to blue (lowest similarity) represents distance values based on the Jaccard coefficient. The annotation bar on the left edge indicates the species (*L. angustifolia*, *L.* × *intermedia*) or tissue (leaf, flower) to which each sample belongs. The dendrogram on the left side of the image shows the hierarchical clustering of samples based on their similarities. Created using the pheatmap library and R 4.3.3

Despite the two localities being relatively distant from each other, the data in the Geochemical Atlas of Slovakia (Čurlík & Šefčík, 2012) indicate that they are connected by the same soil type – fluvial soil. These observations may indicate that the OPB_18 and SM_10 markers are sensitive to soil environmental conditions. Although this hypothesis is interesting, the discriminatory power of the analysed markers is insufficient for their practical use in this context. In the case of primer OPB_18, the following polymorphic index values were determined: PIC = 0.372 and DP = 0.802.

The different profiles of individual species and tissue types are shown in Fig. 7, which presents representative profiles obtained with individual RAPD primers. These profiles reflect genetic variability among the samples studied and indicate differences in the size and number of amplified DNA fragments specific to individual species or tissues.

Analysis using the t-SNE algorithm confirmed that the primers employed are not capable of unambiguously determining the origin of the samples based on geographical location or soil type (Fig. 8). Samples from different locations did not form clearly defined clusters in the two-dimensional space, suggesting that the genetic markers used lack sensitivity to these environmental factors. Nevertheless, the analysis revealed a strong tendency toward species-based clustering. These results indicate that the molecular profiles generated by the selected primers are primarily influenced by taxonomic affiliation, with distinct separation between species. Thus, while the primers demonstrate high specificity for detecting interspecific differences, they are not suitable for assessing intraspecific variation related to geography or environmental conditions.

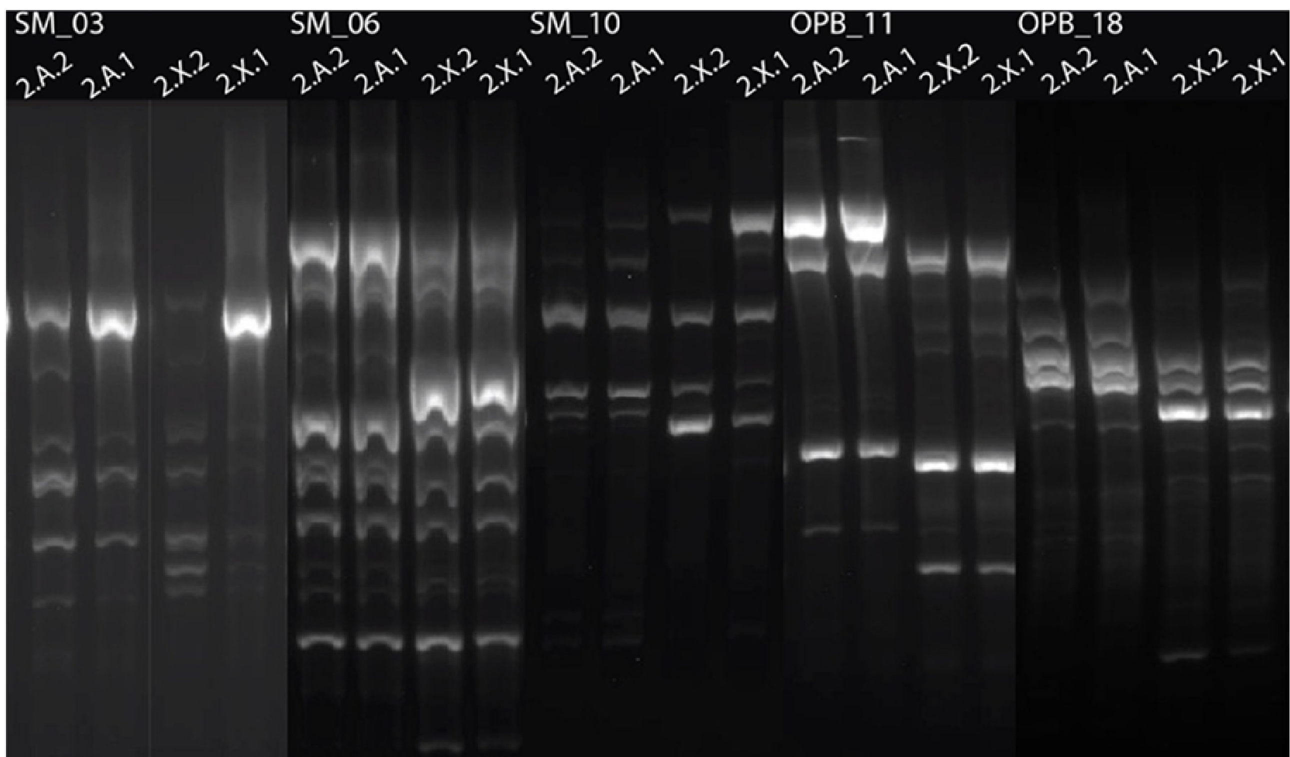

Figure 7 Representative profiles obtained using individual RAPD markers
2.A.2 – *L. angustifolia* flower, 2.A.1 – *L. angustifolia* leaf, 2.X.2 – *L.* × *intermedia* flower, 2.X.1 – *L.* × *intermedia* leaf

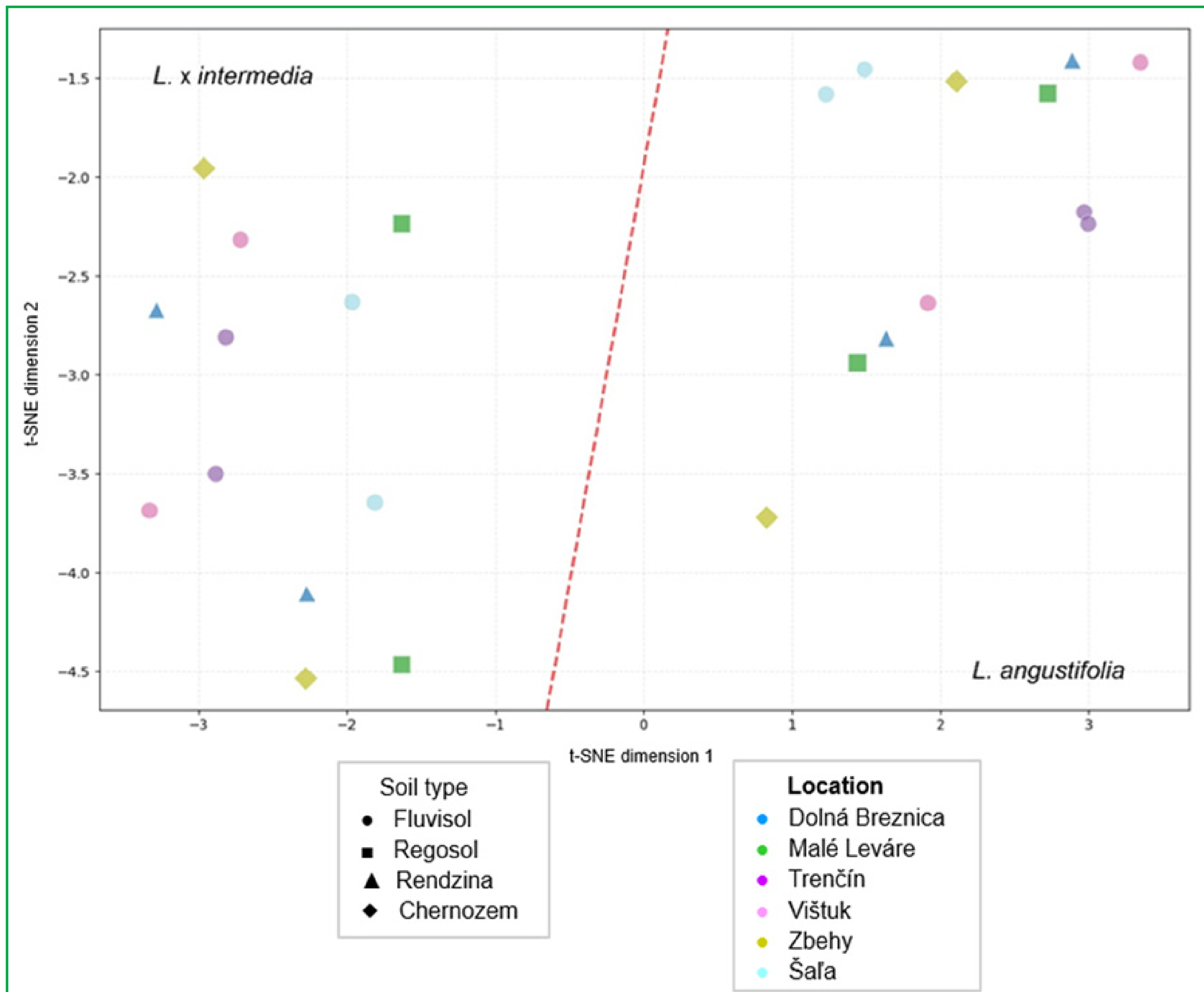

Figure 8 Displaying the output of t-SNE analysis based on Jaccard distances. The points are colour-coded by location, and their shapes represent different soil types. The x and y axes represent the two main dimensions obtained by t-SNE analysis. The red dashed line represents the boundary between *L. angustifolia* and *L.* × *intermedia*. Created using scikit-learn and matplotlib libraries in Python 3.12

3.2 Discussion

The use of RAPD (Random Amplified Polymorphic DNA) markers in the molecular characterisation of *Lavandula* spp. has proven to be a practical and efficient approach for assessing genetic diversity. The study on *Lavandula multifida* populations in Tunisia revealed substantial genetic diversity within populations using both allozymes and RAPD markers, with slightly higher polymorphism observed in populations from lower semi-arid regions. Despite this internal diversity, there was strong genetic differentiation among populations, particularly evident in RAPD results, likely driven by habitat fragmentation, small population sizes, and limited gene flow. Notably, the clustering of populations based on genetic data did not align with geographic or bioclimatic regions, suggesting unique local adaptations (Hnia et al., 2013). The genetic analysis of *Lavandula dentata* populations along the Algerian littoral revealed moderate levels of polymorphism, with 50.49% of bands polymorphic using RAPD-PCR with eight primers. Despite the geographic separation of the six sampled populations, cluster analysis grouped them into two main genetic clusters with over 68% similarity, indicating relatively close genetic relationships (Gadouche et al., 2019).

RAPD primers have also been widely used in various other medicinal plants to differentiate specific species or cultivars and to assess population genetic diversity. The study by Berindean et al. (2021) demonstrated the usefulness of RAPD markers in assessing genetic diversity among ten *Mentha* varieties. Despite testing 13 primers, 8 were polymorphic and yielded 270 bands, indicating a high level of genetic variability. The resulting dendrogram grouped the varieties into two main

clusters, reflecting both their genetic background and shared morphological traits.

In a comparative study of 11 *Mentha* accessions, Khanuja et al. (2000) reported 93.5% polymorphism from 630 amplified bands, demonstrating the high discriminatory capacity of RAPD markers in resolving close taxonomic relationships. These results validate the use of RAPD profiling not only for evaluating genetic diversity but also for developing reliable markers for varietal identification (Khanuja et al., 2000).

Saeidnia et al. (2009) successfully employed RAPD analysis to investigate the phylogenetic relationships among four *Salvia* species and one *Nepeta* species. Using 20 primers, a total of 548 amplification products were generated, allowing for clear differentiation of all species.

Bruna et al. (2006) also successfully demonstrated the utility of RAPD markers in distinguishing *Salvia* species and assessing their geographic and genetic diversity by evaluating genetic relationships among 17 *Salvia* genotypes originating from Central and South America, Ethiopia-Somalia, Morocco, and the Canary Islands. The study utilised 10 random primers, of which 7 produced 143 polymorphic fragments.

In conclusion, RAPD markers have proven effective for assessing genetic variation and population structure in *Lavandula* spp. Yet the method's inherent limitations call for a multi-marker approach to fully capture the genomic complexity. Integrating RAPD with higher-resolution markers and environmental metadata would facilitate more accurate conservation strategies, guiding the sustainable utilisation of aromatic and medicinal plant resources in changing climates.

Random Amplified Polymorphic DNA (RAPD) analysis has emerged as a powerful molecular technique for authenticating medicinal plants, providing a rapid, cost-effective method for detecting genetic polymorphisms without prior sequence data. Studies on *Glycyrrhiza glabra* Linn and *Senna angustifolia* have demonstrated the utility of RAPD profiling in generating species-specific markers that distinguish genuine samples from adulterants, thereby ensuring pharmacological integrity. Beyond these examples, RAPD has been successfully applied to *Embelia ribes*, a red-listed medicinal plant, where unique polymorphic bands were identified to differentiate it from closely related species and substitutes (Khan et al., 2009). Similarly, Shinde et al. employed RAPD to authenticate the components of the Ayurvedic formulation Rasayana Churna, identifying distinct markers for *Tinospora cordifolia*, *Emblica officinalis*, and *Tribulus terrestris*, thereby validating the presence of each constituent in the polyherbal blend (Khan et al., 2011). A broader review by Kumar et al. (2025) highlights the role of RAPD and other PCR-based techniques in overcoming challenges posed by morphological ambiguity and chemical variability in herbal preparations(Kumar et al.,2025). Collectively, these studies underscore the reliability of RAPD as a molecular fingerprinting tool for medicinal plant authentication, contributing to quality control, regulatory compliance, and consumer safety in the herbal medicine industry.

Recent studies underscore the effectiveness of Random Amplified Polymorphic DNA (RAPD) markers in assessing genetic diversity and authenticating medicinal plants. In an investigation of selected Asclepiadaceae species, RAPD analysis revealed significant polymorphism among *Ceropegia juncea*, *Gymnema sylvestre*, *Oxystelma esculentum*, *Pentatropis capensis*, and *Wattakaka volubilis*, with over 936 bands amplified and distinct clustering patterns observed in UPGMA analysis (Boomibalagan et al., 2021). Similarly, RAPD profiling of 36 anticancer drug plants – including *Podophyllum hexandrum* and *Curcuma longa* – demonstrated its utility in identifying species-specific markers and evaluating interspecies relationships, which is crucial for conservation and drug standardisation efforts (Shivashankar, 2014). Complementing these findings, a combined RAPD and ISSR study on *Ocimum sanctum*, *O. basilicum*, and *O. gratissimum* revealed high levels of polymorphism (over 96% for RAPD and 98% for ISSR), with species-specific alleles and clear genetic clustering that support their taxonomic distinction and potential for targeted breeding (Patel et al., 2015). Collectively, these studies affirm that RAPD, especially when paired with ISSR, offers a robust molecular toolkit for characterising genetic variability, guiding conservation strategies, and ensuring the authenticity of medicinal plant resources.

4 Conclusion

Genomic screening of lavender variability using RAPD primer SM_03 demonstrated a strong capacity to differentiate samples primarily by tissue type and, subsequently, by plant species. The primer SM_06 showed particularly high discriminatory power for separating samples according to species. Leaf-derived DNA consistently produced a higher number of amplicons than flower DNA for three of the five primers tested. Among them, aAlthough tSNE analysis confirmed that the applied primers cannot reliably resolve sample origin based on geographical location or soil characteristics, it revealed a clear tendency toward species-level clustering. Overall, these findings indicate that the molecular profiles generated by the selected RAPD primers are shaped predominantly by taxonomic relationships, resulting in distinct interspecific separation.

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Conflict of Interest

The authors declare that there is no conflict of interest.

AI and AI-Assisted Technologies use Declaration

No generative AI tools/AI-assisted technologies were used during the preparation of the manuscript.

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