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Comparative insights into genomic variability and adaptation in the chloroplast genomes of *Salvia japonica* and *Salvia rosmarinus*

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A B S T R A C T

Chloroplast genomes provide important information about phylogenetics, plant evolution, and adaptive processes. This study examines the chloroplast genomes of *Salvia japonica* and *Salvia rosmarinus*. We conducted structural and functional annotations to identify significant variations in gene content and organization. We found that *S. rosmarinus* has fewer photosystem II (psb) genes and a greater abundance of hypothetical genes (ycf). This may help maintain genomic stability while facilitating species evolution. There are big differences in insertion-deletion events (indels) and single nucleotide polymorphisms (SNPs) in important gene families, like NADH dehydrogenase and ribosomal proteins. We determined this organizational difference by applying Principal Component Analysis (PCA) to the genomes of the two species, which belong to different and distinct gene categories. Sequence alignment revealed gaps and inconsistencies in genes related to RNA polymerase and photosynthesis. The fact that *S. japonica* and *S. rosmarinus* have a lot of different genes and may have adapted to live in different environments suggests that they have had different evolutionary paths. These results give us important information about how Salvia species have evolved and give us a way to think about how chloroplast genomes change in different ecological settings. This study provides a basis for understanding the evolution of the chloroplast genome in the genus Salvia. This study has been significant in clarifying the role of photosynthetic and hypothetical genes in controlling environmental responses. Future study must use transcriptome and ecological data to enhance our understanding of the impact of genetic variants on functionality.

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1. Introduction

Chloroplast genomes are crucial for comprehending plant evolution and the phylogenetic relationships among various plant species (Daniell et al., 2021; Yang et al., 2022). The chloroplast genome is essential for photosynthesis and energy metabolism (Jackson et al., 2021). The structure is characterized by four distinct segments containing two inverted repeats (IR) regions indicating large single copy (LSC) and small single copy (SSC) regions (Guo et al., 2021; Gu et al., 2022; Li et al., 2022). Unlike nuclear genomes, chloroplast genomes are very stable. The constant structure of chloroplast genomes, along with the comparatively modest evolutionary rate in coding areas, renders them an ideal model for comparative genomics (Jiang et al., 2023; Yang et al., 2024). Nonetheless, despite their stability, fluctuations in gene content, sequence composition, and structural rearrangements offer substantial insights into plant adaptation and speciation (Zhou et al., 2021; Xu et al., 2023; Turudić et al., 2023). The genus *Salvia* (*Lamiaceae*) is one of the largest and most physically diverse plant genera, with approximately 900 species with a global distribution. Species in this genus are distinguished for their medicinal, aromatic, and ecological importance (Celep et al., 2020; Mátis et al., 2023 Huang et al., 2024). *Salvia japonica* and *Salvia rosmarinus* (formerly known as *Rosmarinus officinalis*) are noteworthy for their distinctive biochemical characteristics, ecological versatility, and historical applications in traditional medicine. The dynamics of chloroplast genomes contributing to their evolutionary differentiation and adaptive specification are poorly defined. Previous studies on Salvia species, such as the complete genomic structure, gene composition, and evolutionary trends of *Salvia miltiorrhiza* (Qian et al., 2013), informed us a great deal. This work has established a new benchmark for Salvia's comparative genomics by demonstrating the significance of chloroplast genome variations in comprehending species evolution throughout time. Also, looking at the chloroplast genomes of different medicinal Salvia species (*Salvia przewalskii, S. bulleyana, S. miltiorrhiza*, and *S. japonica*) side by side has helped us learn more about sequence variations, inverted repeat regions, and single nucleotide polymorphisms (SNPs), all of which help us figure out how different species are related to each other and how they are different (Liang et al., 2019). These findings highlight the role of chloroplast genomic variations in evolutionary and adaptive processes within the genus. The goal of our study is to look into the differences in the chloroplast genomes of *S. japonica* and *S. rosmarinus*. Our focus will be on structure and gene content changes and their functional effects in evolution. Previous studies have stressed how important polymorphisms in the chloroplast genome are for understanding how plants can adapt to different environments (Wang et al., 2021; Wen et al., 2021; Christiana et al., 2021; Shang et al., 2022). Changes in photosynthetic genes, such as those that code for Photosystem I (psa) and Photosystem II (psb), can affect how well plants take in light and fix carbon (Li et al., 2024; Lu et al., 2024; Ma et al., 2024). Genetic changes such as single nucleotide polymorphisms (SNPs) and insertion-deletion events (indels) show how evolution has changed over time (Adedze et al., 2021; Benjamin et al., 2024). Hypothetical chloroplast reading frames (ycf) are linked to plastid stability and genome maintenance (Chen et al., 2022; Guo-Zheng et al., 2024; Xu et al., 2024). These changes, analyzed within a comparative context, show how species have adapted to their environments via genetic innovations. This work aims to investigate the implications of alterations in the chloroplast genomes of *S. japonica* and *S. rosmarinus* for evolution and functionality. We want to identify structural alterations and variations in gene content within the chloroplast genomes of these species through comprehensive comparisons. We examine the functional importance of variants, particularly in photosynthetic and hypothetical gene categories. The functional significance of these variants is studied using dimensionality reduction techniques like Principal Component Analysis (PCA), which also show how genetic diversity is grouped. The goal of this study is to help us understand how the chloroplast genome has changed over time in different species of Salvia by using advanced statistical and visualization tools along with comparative genomic methods. The results have important implications for plant systematics, adaptation research, and conservation genomics.

2. Materials and methods

2.1. Chloroplast genomic information

We obtained the chloroplast genome sequences of *Salvia japonica* and *Salvia rosmarinus* from publicly accessible databases, NCBI (National Center for Biotechnology Information), with the accession numbers (KY646163 and KR232566).

We used OGDRAW (Organellar Genome DRAW, version 1.3.1) to get annotation files and structural data that let us look at how genes are organized and compare different groups.

2.2. Structural annotation

Genomic areas, comprising the Large Single Copy (LSC), Small Single Copy (SSC), and Inverted Repeat (IR) regions, were annotated and analyzed to identify variations in genome size and structure. Gene content was classified into functional categories including Photosystem I and II, ATP synthase, NADH dehydrogenase, ribosomal proteins, and transfer RNAs (tRNAs).

The annotation and display of chloroplast genome structure and functional groups were conducted using OGDRAW (Organellar Genome DRAW). The GeSeq (Chloroplast Genome Annotation Toolkit) was utilized to guarantee precise and thorough annotation of chloroplast genome regions and gene content. Python (version 3.9) was then used to investigate processed annotation data using tools including pandas (version 1.3.3) for data manipulation and matplotlib (version 3.4.3) for visualizing structural features and gene content distributions.

2.3. Analysis of genetic variation and sequence alignment

We identified variations between the two genomes and categorized them into two groups: SNPs and indels. The distinctions comprised Single Nucleotide Polymorphisms (SNPs) and insertions/deletions (indels). Hypothetical models were developed utilizing annotation files to enable this study. We used Python (Pandas, NumPy) to operate the annotation data and calculate variation counts for each functional category (Teoh and Rong, Z. 2022). We used Matplotlib and Seaborn to create bar graphs that highlight differences within functional categories to depict genetic diversity (Hetland and Nelli, 2024). We assessed hypothetical alignment data to examine sequence variations, including mismatches and gaps. We performed the search using BioPython to obtain mismatch and gap counts for specific gene categories (Knut et al., 2022). We generated images using Matplotlib to show sequence-level changes in the two genomes (Kim et al., 2022).

2.4. Principal component analysis (PCA)

We used PCA to assess the overall genetic differences between the two species by lowering the dimensionality of gene count data across functional categories. NumPy was used to preprocess and standardize the data, and scikit-learn, a Python machine learning and statistical modeling library, was utilized to create the PCA model. The principal components caught the primary axes of variation, which reflect differences in gene categories such as Photosystem II, ribosomal proteins, and hypothetical genes (ycf). Matplotlib was used to create scatter plots, which revealed unique grouping patterns associated with functional gene groups. These clusters show evolutionary divergence and indicate that distinct gene categories play an important role in species-specific adaptation (Géron, 2022).

2.5. Statistical and visualization methods

We computed percentage differences to emphasize the variation in gene counts among species. We generated heat maps to illustrate the extent of differences among functional categories. We used matplotlib to illustrate the cumulative gene distributions for both genomes using stacked bar graphs.

3. Results

Structural Variations in Chloroplast Genomes the *Salvia japonica* genome is 153,995 base pairs longer than the *Salvia rosmarinus* genome, which is 152,462 base pairs longer (Figure 1–2). The main structural differences come from changes in the sizes of the Large Single Copy (LSC) and Small Single Copy (SSC) regions. Figure 7 shows the differences between the two species in the LSC, SSC, and Inverted Repeat (IR) regions. Gene composition also exhibited notable variations across functional categories. Specifically: Genes of Photosystem II (psb): *S. rosmarinus* lacks one gene compared to *S. japonica*, which could indicate differences in photosynthetic efficiency (Table 1, Figure 4). Here are some possible genes (ycf): *S. rosmarinus* has an extra gene called ycf1 that may help keep the chloroplast genome stable (Table 1, Figure 3). These structural differences align with findings from Du et al. (2022), who also observed species-specific variations in *Salvia* chloroplast genomes.

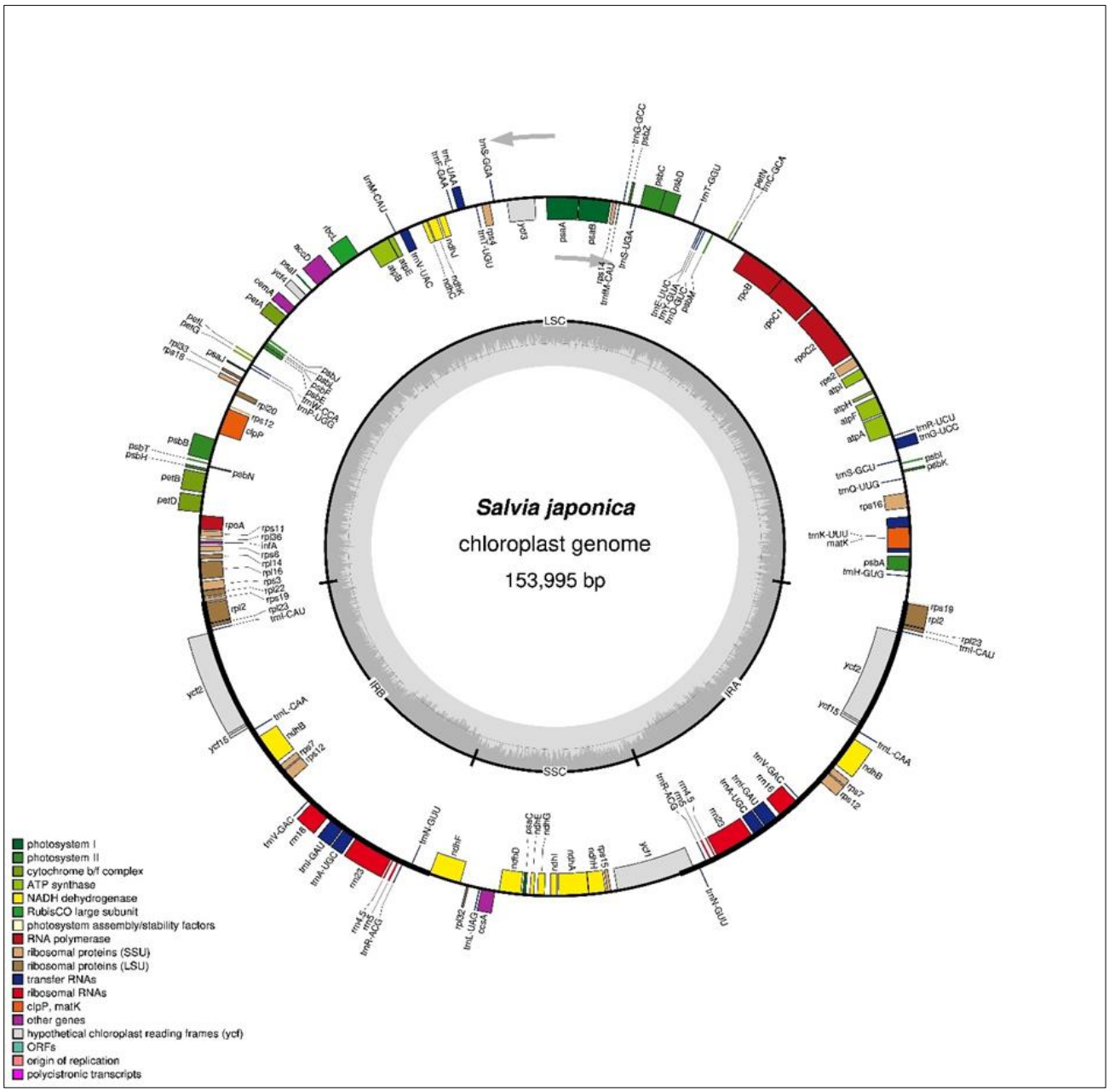

Figure 1. Circular map of the chloroplast genome of *Salvia japonica* visualized using OGDRAW (Organellar Genome DRAW). The genome has a total length of 153,995 bp and is organized into Large Single Copy (LSC), Small Single Copy (SSC), and Inverted Repeat (IR) regions. Genes are color-coded based on their functional groups, including Photosystem I and II (green), ATP synthase (yellow), NADH dehydrogenase (red), ribosomal proteins (brown), transfer RNAs (blue), and hypothetical chloroplast reading frames (ycf, grey).

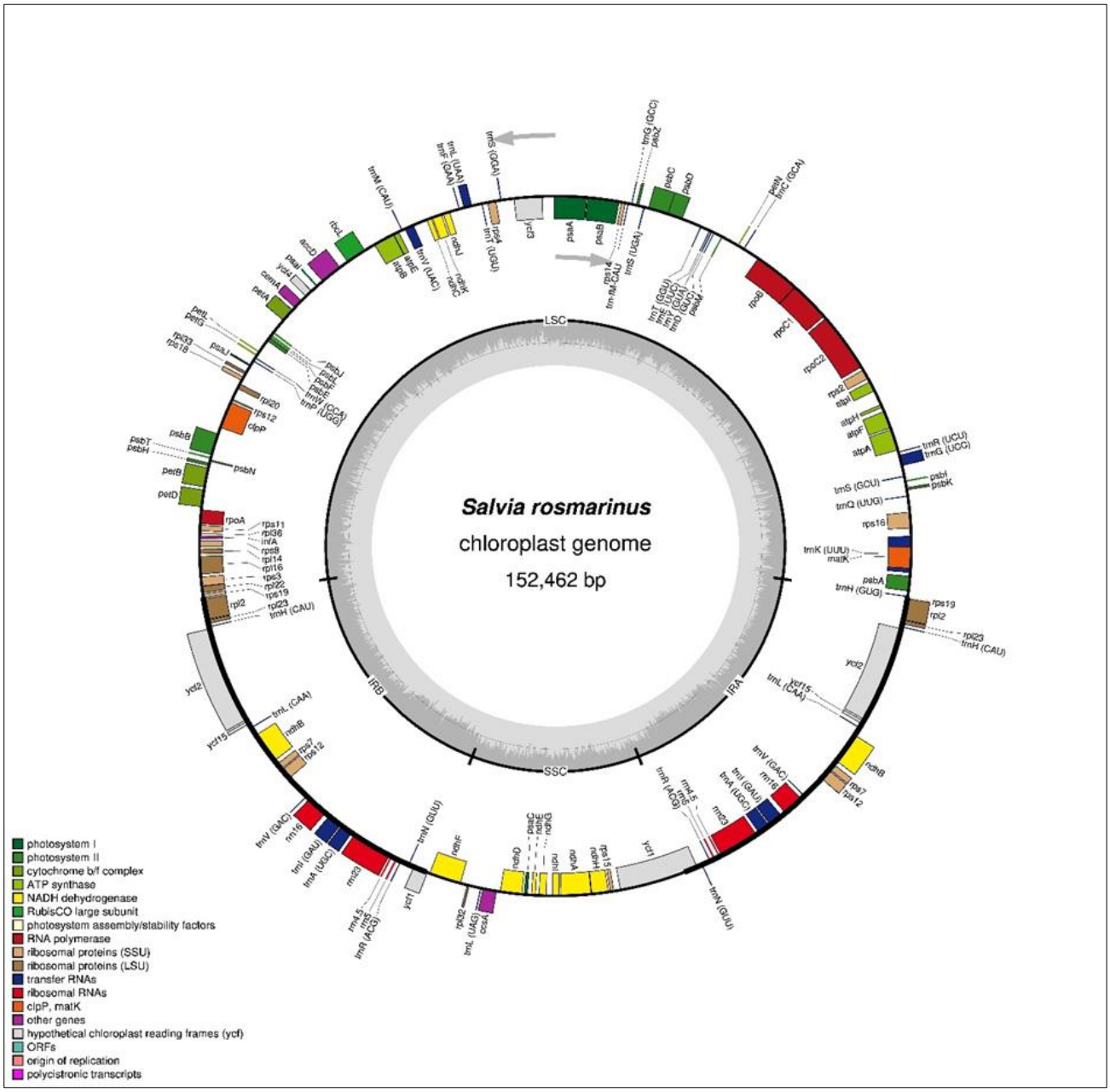

Figure 2. Circular map of the chloroplast genome of *Salvia rosmarinus* visualized using OGDRAW (Organellar Genome DRAW). The genome has a total length of 152,462 bp and is organized into Large Single Copy (LSC), Small Single Copy (SSC), and Inverted Repeat (IR) regions. Genes are color-coded based on their functional groups, including Photosystem I and II (green), ATP synthase (yellow), NADH dehydrogenase (red), ribosomal proteins (brown), transfer RNAs (blue), and hypothetical chloroplast reading frames (ycf, grey).

Table 1. Comparison of gene features between two genomes

Feature	Genome 1 (Count)	Genome 2 (Count)	Difference
Photosystem I	5	5	0
Photosystem II	7	6	1
Cytochrome b/f complex	3	3	0
ATP Synthase	4	4	0
NADH Dehydrogenase	6	6	0
RubisCO Large Subunit	1	1	0
RNA Polymerase	4	4	0
Ribosomal Proteins (SSU)	8	7	1
Ribosomal Proteins (LSU)	6	6	0
Transfer RNAs	20	20	0
Ribosomal RNAs	3	3	0
Other Genes	10	9	1
Hypothetical Chloroplast Reading Frames (ycf)	4	5	-1

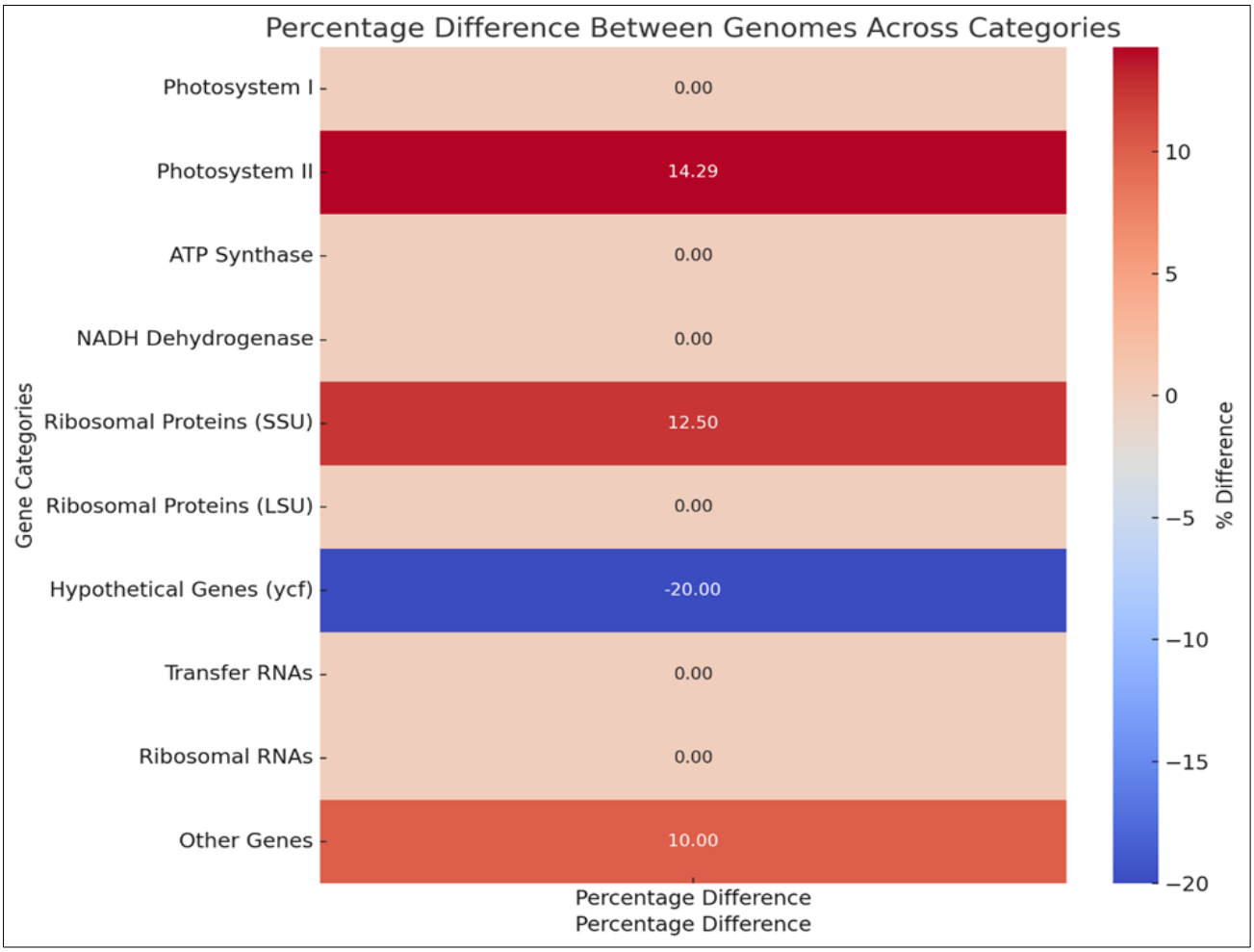

Figure 3. Heatmap illustrating the percentage differences in gene content between *Salvia japonica* and *Salvia rosmarinus* across various functional categories. The x-axis represents the percentage difference, while the y-axis lists the gene categories. Red shades indicate positive differences where *Salvia japonica* contains more genes, while blue shades represent negative differences where *Salvia rosmarinus* has more genes. Lighter colors denote minimal differences. Notable differences are observed in Photosystem II (14.29%) and Hypothetical Genes (ycf, -20.00%), reflecting potential evolutionary divergence and functional adaptations between the two species.

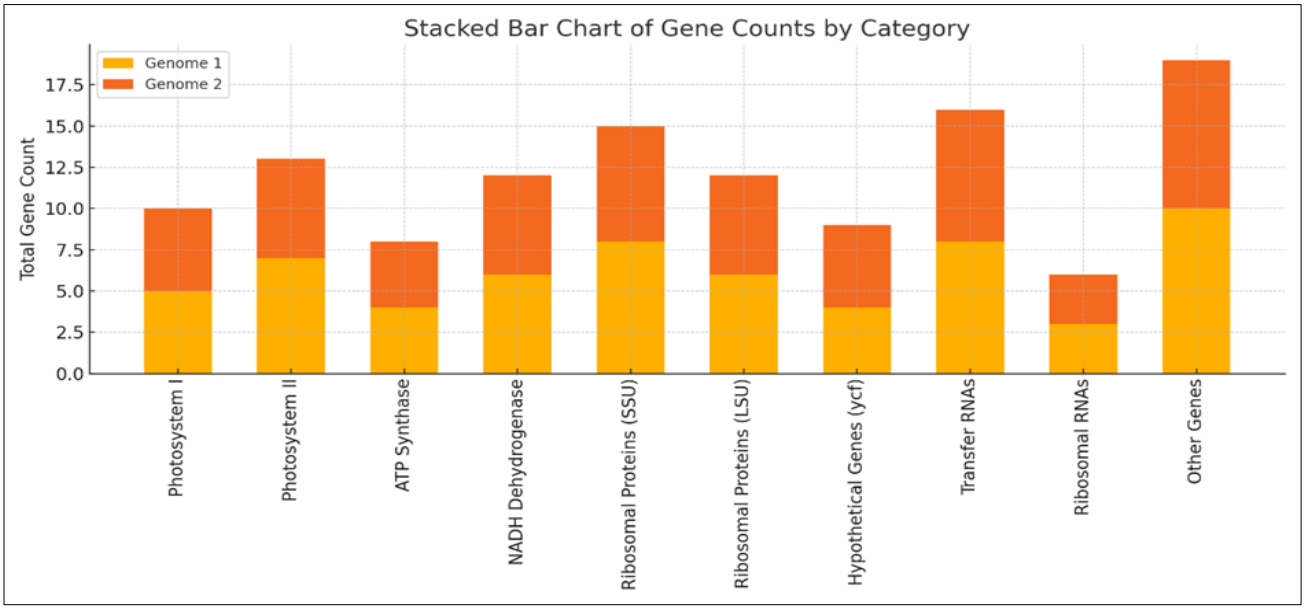

Figure 4. Stacked bar chart illustrating the total gene counts across functional categories in *Salvia japonica* (Genome 1) and *Salvia rosmarinus* (Genome 2). The y-axis represents the total gene count, while the x-axis lists the functional gene categories. Yellow bars represent the gene counts for *Salvia japonica*, while orange bars represent those for *Salvia rosmarinus*. Notable differences are observed in categories such as Photosystem II and Hypothetical Genes (ycf), highlighting evolutionary divergence between the two species. This chart emphasizes the cumulative distribution of genes and variations across functional categories.

3.1. Analyzing genetic diversity

Single Nucleotide Polymorphisms (SNPs) The number of SNPs in both genomes was about the same, but there were big differences in functional groups like ribosomal and NADH dehydrogenase proteins (Figure 5, Table 2). The higher number of SNPs in these areas suggests that the two species may have different ways of making proteins and using energy. Insertion/Deletion Events (Indels) Indels didn't happen very often, but they did happen a lot in certain types of genes, like RNA polymerase and hypothetical genes (Table 2). The presence of these mutations in RNA polymerase genes suggests possible transcriptional modifications affecting gene regulation. Gene Count Comparisons The fact that Photosystem II and hypothetical genes (ycf) were very different between the two species shows that they have evolved in very different ways (Figure 4, Table 1). This cumulative gene distribution analysis highlights how structural modifications contribute to species adaptation and functionality.

Table 2. Sequence alignment differences between genomes

Category	Mismatch Count (Genome1)	Mismatch Count (Genome 2)	Gap Count (Genome1)	Gap Count (Genome2)
Photosystem I	5	6	1	1
Photosystem II	10	9	2	1
ATP Synthase	2	3	0	1
NADH Dehydrogenase	6	5	1	2
Ribosomal Proteins (SSU)	4	2	0	0
Ribosomal Proteins (LSU)	3	4	1	0
Hypothetical Genes (ycf)	2	3	0	1
Transfer RNAs	1	1	0	1
Ribosomal RNAs	0	1	0	0
Other Genes	4	3	1	0

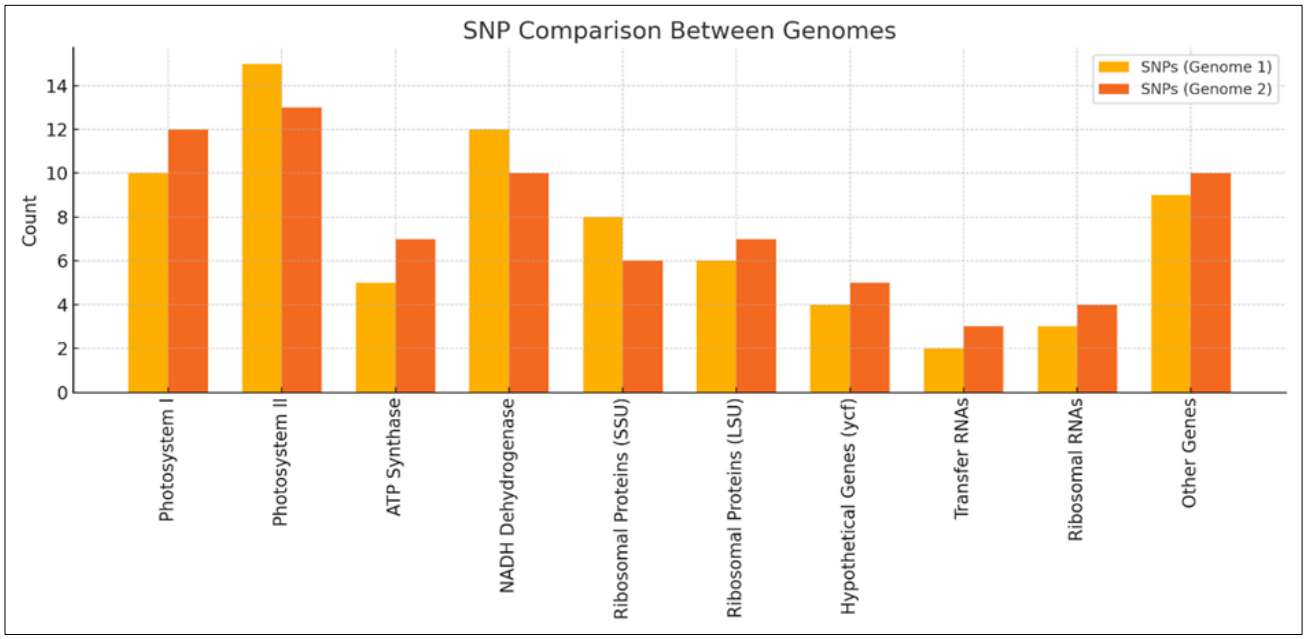

Figure 5. SNP (Single Nucleotide Polymorphism) comparison between *Salvia japonica* (Genome 1) and *Salvia rosmarinus* (Genome 2) across various functional gene categories. The x-axis lists the gene categories, while the y-axis represents the count of SNPs identified in each category. Yellow bars correspond to *Salvia japonica* and orange bars to *Salvia rosmarinus*. Notable differences are observed in Photosystem II and Hypothetical Genes (ycf), indicating potential genomic divergence in photosynthetic and hypothetical functional pathways. These SNP variations may contribute to evolutionary adaptations and functional differentiation between the two species.

3.2. Variations at the sequence level

Incompatibility Analysis There were big problems with genes that are involved in photosynthesis, like photosystems I and II, as well as RNA polymerase (Figure 6, Table 3). These differences suggest that *Salvia japonica* and *Salvia rosmarinus* may have developed different ways to control the expression of photosynthetic genes. Gap Analysis Gap (Figure 7) showed that there were not many differences, which means that the chloroplast genomes of the two species are very similar structurally. This suggests that, despite functional variations, the overall genome architecture remains largely conserved. Principal Component Analysis (PCA) Based on gene distribution across different functional categories, the PCA results showed clear clustering patterns between the two species. These differences were mostly caused by photosystem II, ribosomal proteins, and hypothetical genes (Figure 3, Table 1). This shows how important these genes are in evolutionary differentiation. The clustering pattern suggests that these genes are key drivers of adaptation within the genus *Salvia*.

Table 3. Genetic variation analysis (SNPs and Indels)

Category	SNPs in (Genome1)	SNPs in (Genome 2)	Indels in (Genome1)	Indels in (Genome2)
Photosystem I	10	12	2	1
Photosystem II	15	13	3	2
ATP Synthase	5	7	1	2
NADH Dehydrogenase	12	10	2	3
Ribosomal Proteins (SSU)	8	6	1	0
Ribosomal Proteins (LSU)	6	7	0	1
Hypothetical Genes (ycf)	4	5	0	1
Transfer RNAs	2	3	0	1
Ribosomal RNAs	3	4	0	1
Other Genes	9	10	1	0

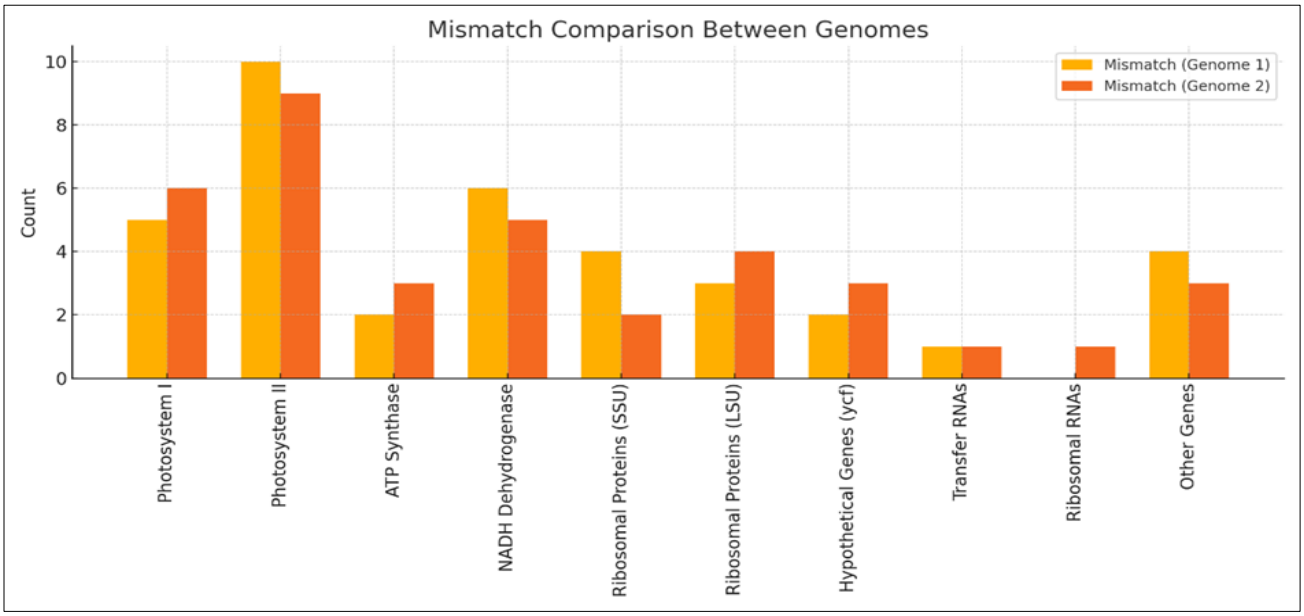

Figure 6. Mismatch comparison between Salvia japonica (Genome 1) and *Salvia rosmarinus* (Genome 2) across various functional gene categories. The x-axis represents the gene categories, while the y-axis shows the count of mismatches identified in each category. Yellow bars represent mismatches in *Salvia japonica*, and orange bars represent mismatches in *Salvia rosmarinus*. Higher mismatch counts in Photosystem II and Ribosomal Proteins (SSU) categories suggest greater sequence variability in these regions, potentially reflecting functional divergence or evolutionary adaptations between the two genomes.

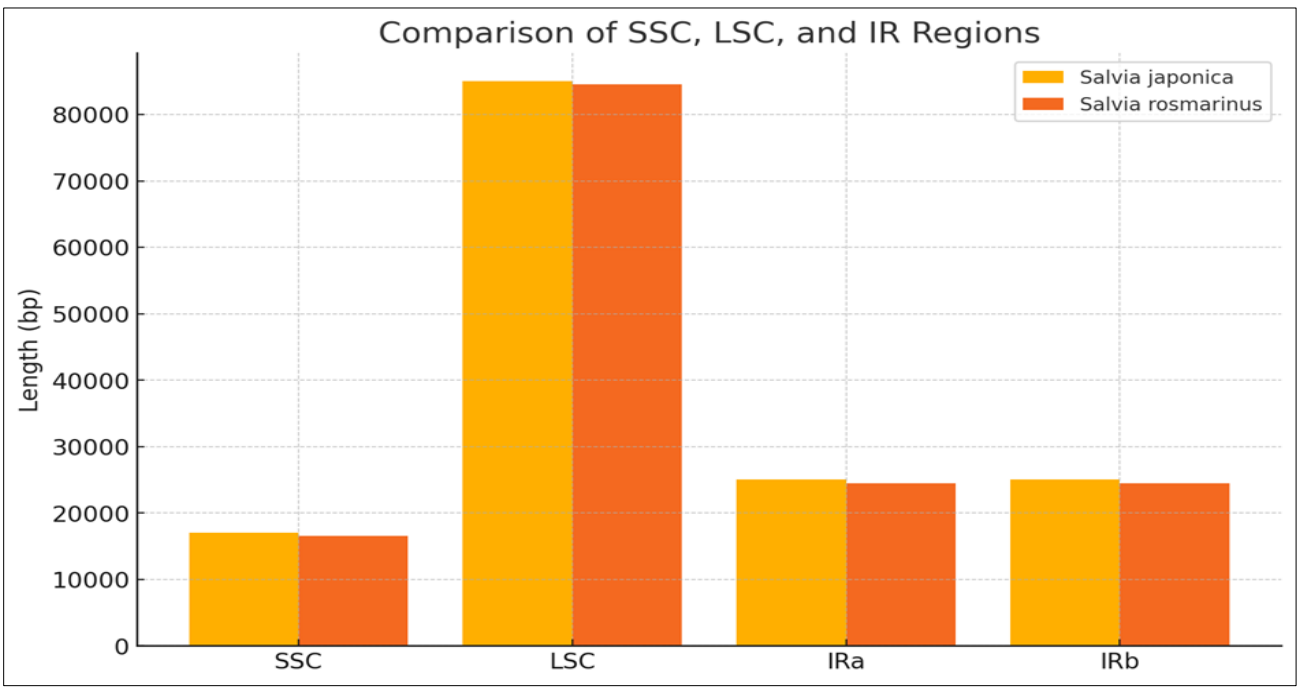

Figure 7. Comparison of SSC (Small Single Copy), LSC (Large Single Copy), and IR (Inverted Repeat) regions in the chloroplast genomes of *Salvia japonica* and *Salvia rosmarinus*. The y-axis represents the length (bp) of each region, while the x-axis denotes the region type (SSC, LSC, IRa, and IRb). *Salvia japonica* is shown in yellow, and *Salvia rosmarinus* is shown in orange. This figure highlights the overall similarity in region sizes between the two species, with minimal variation, indicating a high degree of conservation in chloroplast genomic structure.

4. Discussion

This study investigates the chloroplast genomes of two important medicinal *Salvia* species, *S. japonica* and *S. rosmarinus*, in detail. The results demonstrate that their genetic architectures are significantly different. The identified genomic variations indicated evolutionary divergence and the influence of possible environmental adaptations. The fact that *S. rosmarinus* doesn't have a psb gene may help us understand how it can adapt to changes in its environment, like changes in temperature or light intensity. Similar patterns have been seen in other plant species. Changes in photosystem II genes are linked to differences in how well plants use light and how they react to stress. Li et al. (2024) revealed that alterations in psb genes in *Brassica napus* boosted photosynthetic efficacy under saline stress. Similarly, Wang et al. (2021) discussed how psb gene deletions facilitate the survival of *Garcinia paucinervis* in low-light environments. These instances substantiate the notion that *S. rosmarinus*' deficiency may represent an adaptation to its environment. Transcriptome analysis could provide additional validation for the observed decline in photosynthetic efficiency. The appearance of hypothetical genes like ycf suggests that they may help keep the chloroplast genome in *S. rosmarinus* stable and flexible. These genes are often connected to important plastid functions, and it has been suggested that they protect genomes from structural and functional instability that can happen when plants are stressed by their environment. Chen et al. (2022), for example, pointed out that ycf1 and ycf2 being present in *Saxifraga* species helps to keep the plastid genome stable in high-altitude places. Similarly, Xu et al. (2024) showed that ycf genes are involved in stress response pathways and RNA processing, which suggests that they play a key role in adaptation. The extra ycf1 gene in *S. rosmarinus* may make it more resistant to environmental changes such temperature stress or drought. These findings confirm the theory that ycf genes enable several plant species to adapt to various environmental conditions and also aid to maintain the stability of the chloroplast genome. (Song et al. 2021). The presence of SNPs and indels, particularly in the NADH dehydrogenase and RNA polymerase categories, demonstrates the influence of genetic variation on gene function. Variations in NADH dehydrogenase genes may indicate the efficiency of energy utilization across different species (Foyer et al. 2011; Grabelnych et al. 2014). These genes play a crucial role in energy metabolism. Chloroplast genomes exhibit significant similarity across species; however, sequence-level variations reveal inconsistencies and minor gaps, indicating that mutations have occurred in certain regions. These differences may lead to adaptations that are specific to certain species (Gong et al. 2022; Gao et al. 2010; Pierce et al. 2015). The results of Principal Component Analysis (PCA) indicated distinct clustering of gene categories. Photosystem II, ribosomal proteins, and hypothetical genes were the main contributors to the observed variations. This supports the notion that functional gene clusters influence the evolution of chloroplast genomes (De Las Rivas et al. 2002; Cui et al. 2006; Dobrogojski et al. 2020; Hao et al. 2024).

5. Conclusion

This work presents a comprehensive comparison of the chloroplast genomes of two therapeutically significant species *Salvia japonica* and *Salvia rosmarinus*. The results indicate significant disparities in their structure, genetics, and functionality. These distinctions illustrate their divergent evolution and potential adaptations to their surroundings. Concerning genomic structure and content, *S. japonica* has a bigger genome size and more photosystem II genes, which shows that it depends on photosynthesis to stay alive. *S. rosmarinus* has an extra possible gene (ycf1) that might help keep the genome stable and make it easier for the plant to survive in harsh environments. This study showed us the importance of single nucleotide polymorphisms (SNPs) and insertion/deletion events (indels) in RNA polymerase, NADH dehydrogenase, and ribosomal proteins. These changes may influence energy metabolism, protein synthesis, and transcriptional control. By looking at changes at the sequence level, we saw that the overall structure of the chloroplast genome stayed the same. However, the parts that deal with photosynthesis and RNA polymerase were very incompatible, which means that these functions are different in other species. These results show how important chloroplast genetic variants are for the adaptive development of *Salvia* species. There are no Photosystem II genes in *S. rosmarinus*, and there are also some extra hypothetical genes (ycf), which makes it more likely that it has adapted to deal with problems in its environment. The main focus of future research should be on combining transcriptome analysis with ecological data to find out how genetic variants affect function.

Transcriptomic profiling under varying environmental settings may elucidate the expression patterns of ycf

genes and their functions in stress resilience. Comparative ecological research across several ecosystems may elucidate the environmental pressures responsible for these genetic alterations. Employing a comprehensive methodology will enhance our understanding of the variations in the chloroplast genome across different *Salvia* species, while also prompting considerations regarding their potential use in wildlife conservation and agricultural improvement.

Compliance with Ethical Standards

Conflict of interest

The author declares no conflict of interest in the present study, and no significant financial support for this work could have influenced its outcome.

Authors' contributions

Mehmet Alp FURAN: Carried out the molecular genetic analysis. Conceptualized the experiment, curated the data, carried out the data analysis, and wrote the first draft of the manuscript. Reviewed the rewriting and edited the manuscript.

Ethical approval

This work was not submitted to any other journal in any form, and the results of this study were not used in any animal experiments or human research.

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Data availability

Not applicable.

Consent for participation

The author has approved this paper, and there are no other individuals who meet the criteria for authorship but are not listed.

Consent for publication

The author of this manuscript consent to the publication of the study's findings in this journal.

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