

Advancements in Molecular Marker Development for Medicinal *Salvia*

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Abstract

We employed Illumina HiSeq 2500 technology to conduct chloroplast genome sequencing for *Salvia bowleyana*, *S. splendens*, and *S. officinalis*. This endeavor aimed to elucidate their evolutionary relationships comprehensively and establish molecular markers for species identification. The chloroplast genomes of these species exhibited lengths of 151,387, 150,604, and 151,163 base pairs, respectively. Within these genomes, the inverted repeat (IR) regions harbored six genes: *ndhB*, *rpl2*, *rpl23*, *rps7*, *rps12*, and *ycf2*. In terms of repeat structures, *S. bowleyana*, *S. splendens*, and *S. officinalis* showcased 29 tandem repeats, 35 simple sequence repeats, and 24 simple sequence repeats, along with 47, 49, and 40 interspersed repeats, respectively. Notably, three distinct intergenic sequences (IGS)-*rps16-trnQ-UUG*, *trnL-UAA-trnF-GAA*, and *trnM-CAU-atpE*-enabled the differentiation of the 23 *Salvia* species. Genetic distance analysis identified a total of 91 intergenic spacer sequences, with the *ycf3-trnS-GGA* and *trnG-GCC-trnM-CAU* regions demonstrating the highest K2p values among the three *Salvia* species under investigation. Furthermore, our phylogenetic analysis revealed that the 23 *Salvia* species formed a monophyletic group. This study also unveiled the development of two sets of genus-specific DNA barcode primers. These findings serve as a robust foundation for understanding the phylogenetic classification of the three *Salvia* species. Additionally, the distinctive intergenic regions hold the potential to distinguish *Salvia* species based on both phenotypic traits and gene segment differentiation.

Keywords: *Salvia bowleyana* • *Salvia splendens* • *Salvia officinalis*

Introduction

The Lamiaceae family, which ranks as the sixth-largest among flowering plant families, encompasses a rich diversity of life. Within this family, there are approximately 3,500 species distributed across 220 genera and further classified into 10 subfamilies [1]. The primary distribution of these species spans across Asia, Europe, and Africa. Historically, the Lamiaceae family shares its closest connections with the Verbenaceae and Violaceae families [2].

In the expansive realm of the Lamiaceae family, China alone hosts more than 800 species and 99 genera, with a remarkable 1,050 *Salvia* species. Among these, 32 variations and 78 distinct species thrive predominantly in tropical or temperate regions [3]. Taxonomically, the *Salvia* genus has undergone several evolutionary categorizations. Bentham and Briquet, for instance, divided it into four subgenera and 12 groupings. Furthermore, American scientist Carl Epling dissected the subgenus *Calospatha* into an astonishing 91 groups, expanding the genus to 102 groups within the following two decades. Geographically, the *Salvia* genus exhibits variation, with five groups in the European flora, contrasting with four subgenera and eight groups in the flora spanning the USSR, Africa, and Europe. In East Asia, the Chinese *Salvia* genus was meticulously categorized into five subgenus groups and 18 subbranches by the esteemed botanist, academician Wu Zhengyi.

However, it's worth noting that the *Salvia* genus is not monophyletic, as evidenced by molecular data from *rbcL* and *trnL-F* sequences. This genus shares a sister taxon relationship with other genera such as *Rosmarinus*,

Perovskia, *Dorystaechas*, *Meriandra*, and *Zhumeria*. Moreover, genetic systematics and morphological evidence have led to the formal inclusion of 15 species from the genera *Rosmarinus*, *Perovskia*, *Spear*, *Meriandra*, and *Zhumeria* within the broader *Salvia* genus, revealing ten distinct clades.

The diversity within the *Salvia* genus is not limited to geography and morphology but extends to genetic makeup. The divergence of *Salvia* species in Japan, for example, groups 11 species into a single branch based on *rbcL*, *trnL-F*, and ITS sequences. Meanwhile, the classification of 38 *Salvia* species in China using ITS, *rbcL*, *psbA-trnH*, and *matK* sequences challenges the monophyly of the three subgenera previously defined in Chinese plants.

Literature Review

East Asian *Salvia*, as confirmed by a phylogenetic tree featuring 78 species and 10 variants, forms a monophyletic group, officially designated as Clade IV and is further divided into eight groups. This classification was established through a comprehensive study of ITS, ETS, *psbA-trnH*, *ycf1-rps15*, and *trnL-trnF* sequences.

Beyond phylogeny, the *Salvia* genus's significance extends to its phytochemical components and their therapeutic properties, assessed through NRI and NTI metrics. *Salvia boweyara*, for instance, has demonstrated effectiveness in managing hepatic and reproductive issues. Consequently, variations within *Salvia* species encompass morphological traits, chemical compositions, therapeutic effects, and molecular markers. To elucidate the *Salvia* genus's position within the Lamiaceae family, a holistic approach to taxonomic research is warranted [4].

Switching gears to plant organelles, the chloroplast stands as the paramount entity in plants. It houses numerous genes critical for photosynthesis, evolutionary processes, and genetic engineering. The chloroplast genome encodes over 120 genes, classified into three categories: those associated with photosynthesis, biosynthesis of fatty acids and amino acids, and transcription and translation. Notably, genes linked to photosystems I (PSA) and II are dispersed within the large-single copy (LSC) and small-

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single copy (SSC) regions, such as PSB, alongside tRNA, ATP enzyme, NADH plastid-masking oxidoreductase, and RNA polymerase genes. Additionally, major subunits of Rubisco, encoded by *rbcL*, are present. The inverted repeat (IR) region mainly comprises genes for rRNA (RPS), including 16S and 23S genes. Intermediate genes encode 4.5S rRNA, 5S rRNA, 2 tRNA, and several genes with undefined functions.

Chloroplast genomes offer a treasure trove of genes suitable for applications ranging from species identification and phylogenetic analysis to genetic modification and molecular breeding of medicinal plants. These genomes, including *psbA-trnH*, *matK*, and *rbcL*, have been extensively utilized for DNA-based molecular identification, allowing the analysis of polymorphic loci containing multiple genes and gene spacers. While 14 *Salvia* species within the Lamiaceae family have had their chloroplast genomes documented, the comprehensive exploration of chloroplast genomes presents a foundational resource for investigating structural variations, traits, genetic evolution, and chemistry. In a concerted effort to pinpoint phylogenetic genome regions of divergence and assess the potential of phylogenomics in unraveling the evolutionary and systematic relationships within the *Salvia* genus of the Lamiaceae family, we have undertaken the pioneering sequencing and analysis of chloroplast genomes for three *Salvia* species.

Discussion

Distinguishing among the ten *Salvia* species becomes comprehensible when we delve into the DNA sequences found within the highly variable regions. A comparison of chloroplast genomes in three distinct intergenic spacer (IGS) regions, namely *rps16-trnQ-UUG*, *trnL-UAA-trnF-GAA*, and *trnM(cau)-atpE*, facilitates this differentiation. These *Salvia* species, namely *S. officinalis*, *S. japonica*, *S. sclarea*, *S. meiliensis*, *S. hispanica*, *S. tiliifolia*, *S. yangii*, *S. splendens*, *S. nilotica*, and *S. chanryoenica*, can be distinguished based on these genetic markers. Notably, the first IGS region is shared with species belonging to the Zingiber officinale and Coffeae alliance, while the second one is a common occurrence among angiosperms. The third region has evolved over time and has historical ties to both gymnosperms and angiosperms, with some of the oldest mitochondrial DNA (mtDNA) segments, including *trnV(uac)-trnM(cau)-atpE-atpB-rbcL*, being transferred from chloroplast DNA (cpDNA) to mtDNA. Additionally, to reconstruct the evolutionary relationships within the Eurystachys clade, nuclear ribosomal DNA sequences (nrETS, 5S-NTS) from a diverse pool of 148 accessions were employed. This investigation encompassed genera with well-defined distinctions, such as *Prasium* and *Sideritis* [5,6].

Conclusion

Utilizing advanced Illumina sequencing technology, we successfully obtained the complete chloroplast genomes of three distinct *Salvia* species: *S. bowleyana*, *S. splendens*, and *S. officinalis*. These species exhibit distinctive phenotypic traits, facilitating straightforward differentiation among them. Through phylogenetic analysis, we established that these three species

occupy separate branches within a broader grouping comprising 23 *Salvia* species and the *Rosmarinus* genus. The examination of seven intergenic spacer (IGS) regions, namely *rps16-trnQ-UUG*, *trnL-UAA-trnF-GAA*, *trnM-CAU-atpE*, *trnL-UAG-ccsA*, *ccsA-ndhD*, *rps15-ycf1*, and *ndhE-ndhG*, revealed significant sequence divergence among these species. Notably, the three *Salvia* species in focus shared commonalities in two specific IGS regions: *trnG-GCC-trnM-CAU* and *ycf3-trnS-GGA*. The remarkable diversity observed in these sequence divergences suggests their potential utility as valuable DNA markers for future identification and phytotaxonomy of the *Salvia* genus. Collectively, the information acquired through this study promises to advance our understanding of *Salvia* taxonomy, ecology, phylogenetic evolution, and conservation efforts within the Chinese context.

Acknowledgement

None.

Conflict of Interest

None.

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