

# Development of Molecular Markers in Medicinal *Salvia* Species

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## Abstract

We used the Illumina HiSeq 2500 technology to sequence the chloroplast genomes of *Salvia bowleyana*, *S. splendens* and *S. officinalis* in order to thoroughly establish their evolutionary relationships and create molecular markers for species classification. *S. bowleyana*, *S. splendens* and *S. officinalis* all had chloroplast genomes that were 151,387, 150,604 and 151,163 base pairs long, respectively. The IR areas contained the six genes *ndhB*, *rpl2*, *rpl23*, *rps7*, *rps12* and *ycf2*. There are 29 tandem repeats, 35 simple sequence repeats, 24 simple sequence repeats and 47, 49, 40 interspersed repeats in the chloroplast genomes of *S. bowleyana*, *S. splendens* and *S. officinalis*, respectively. The 23 *Salvia* species could be distinguished by the three distinct intergenic sequences (IGS) of *rps16-trnQ-UUG*, *trnL-UAA-trnF-GAA* and *trnM-CAU-atpE*. Genetic distance analysis allowed the identification of 91 intergenic spacer sequences in total. The two specific IGS areas (*ycf3-trnS-GGA* and *trnG-GCC-trnM-CAU*) exhibit the highest K2p values among the three *Salvia* species under investigation. The phylogenetic tree also demonstrated that the 23 species of *Salvia* formed a monophyletic group. The discovery of two sets of genus-specific DNA barcode primers. The findings will give a strong foundation for understanding how the three *Salvia* species are classified phylogenetically. Additionally, the unique intergenic regions can offer the potential to distinguish *Salvia* species based on both phenotypic and the differentiation of gene segments.

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

## Introduction

The sixth-largest family of flowering plants is the Lamiaceae group. It has 3500 species, 220 genera and 10 subfamilies [1]. The majority of species are mostly found in Asia, Europe and Africa. The Verbenaceae and Violaceae families are most closely connected to the Lamiaceae family historically [2]. The Lamiaceae family contains more than 800 species and 99 genera in China, including over 1050 *Salvia* species. Among these, 32 variations and 78 different species primarily flourish in tropical or temperate regions [3]. In terms of the categorization evolution of the *Salvia* genus, Benth and Briquet previously split it into four subgenera and 12 groupings. The *Salvia* genus has also been the subject of other taxonomic research in various geographical areas. For instance, the subgenus *Calosphaea* was split into 91 groups by the American scientist Carl Epling and the genus grew to 102 over the following 20 years. The *Salvia* genus is split into five groups in the flora of Europe, compared to four subgenera and eight groups in the flora of the USSR, Africa and Europe. The Chinese *Salvia* genus was split into five subgenus groups and 18 subbranches by the botanist of academician Wu Zhengyi in East Asia. The genus *Salvia* is not monophyletic, according to the molecular systems and data of *rbcL* and *trnL-F*; it has a sister taxon relationship imbedded with the genera of *Rosmarinus*, *Perovskia*, *Dorystaechas*, *Meriandra* and *Zhumeria*. Meanwhile, 15 species from the 5 genera of *Rosmarinus*, *Perovskia*, *Spear*, *Meriandra* and *Zhumeria* were formally united into the generic *Salvia* genus with 10 identified distinct clades using genetic systematics and morphological evidence. Based on comparisons between the *rbcL*, *trnL-F* and ITS sequences, the 11 *Salvia* species found in Japan were grouped into one branch.

The *Salvia* genus was clustered into one clade from China and Japan, with

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the exception of the species *Salvia deserta* and the three subgenera defined in Chinese plants are not the monophyletic groups, according to the molecular systems of 38 *Salvia* species in China that were classified using the ITS, *rbcL*, *psbA-trnH* and *matK* sequences. The phylogenetic tree with 78 species and 10 variants confirmed that the *Salvia* of East Asia is a monophyletic group, officially naming the clade IV (*S. Glutinaria* Clade) as East Asian *Salvia* with eight groups. This was done using a study on the divergence of ITS, ETS, *psbA-trnH*, *ycf1-rps15*, *trnL-trnF*. More intriguingly and significantly, the 345 species that make up the 77 Lamiaceae genera have been categorised and clustered into phylogenetic groups based on the components of phytochemicals and how they treat different ailments through the examination of NRI and NTI metrics. The outcomes demonstrated that *Salvia bowleyana* had an impact on the management of hepatic and reproductive problems. As a result, there are certain variations among the *Salvia* species in terms of their morphological traits, chemical makeup, effects as a form of treatment for disorders and molecular markers. To clarify the *Salvia* genus's position within the family, we are eager to carry out the integration of taxonomic study from multiple perspectives.

The most important organelle in plants is the chloroplast. Numerous genes involved in photosynthesis, evolution and genetic engineering are found in the chloroplast genome. Over 120 genes are often encoded by the chloroplast genome. These genes can be grouped into three categories: photosynthesis, biosynthesis of fatty acids and amino acids and transcription and translation. The genes primarily connected to photosynthetic systems I (PSA) and systems II are dispersed in the large-single copy (LSC) and small-single copy (SSC) regions (PSB). The tRNA gene (*tRNA*), the ATP enzyme gene (*ATP*), the NADH plastid-masking oxidoreductase gene (*NADH*) and the RNA polymerase gene (*RPO*) are also among them. They are also major subunits of Rubisco (encoded by *rbcL*). The majority of the genes found in the IRs region are those that encode rRNA (*RPS*), including the 16S and 23S genes. The intermediate genes are distinguished by those that encode 4.5S rRNA, 5S rRNA and 23S rRNA, as well as a few genes with unidentified gene functions.

Chloroplast genomes contain genes that can be exploited for species identification, phylogenetic evolution, genetic modification and molecular breeding of medicinal plants, giving the foundational information for resource identification and conservation. The chloroplast genome sequences of medicinal plants, including *psbA-trnH*, *matK* and *rbcL*, have been extensively employed for DNA molecular identification and have now been developed for

the analysis of polymorphic loci containing multiple genes and gene spacers. The 14 *Salvia* species in the Lamiaceae family have reported chloroplast genomes to date. The thorough development of chloroplast genomes, in contrast to the diversification of nuclear and mitochondrial genomes, could offer a fundamental resource for further investigation into structural variation, traits, genetic evolution and chemistry. In order to identify divergence hotspots of phylogenetic genome regions and determine the applicability of phylogenomics for further resolving the evolutionary and systematic relationship in the *Salvia* genus of the Lamiaceae family, we sequenced and analysed the chloroplast genomes of three *Salvia* species for the first time.

## Procedure

The three species of *Salvia* share the characteristics of the Lamiaceae family, including a quadrangular stem, opposing leaves, a lip on the corolla and four nutlets. However, they stand out clearly from the phenotypic of flower hues (1), which range from red to pink and purple (*S. bowleyana* and *S. officinalis*) (*S. splendens*). The three *Salvia* species are perennial herbs with cymose inflorescences, oblong or oval leaves and nutlets. However, only the veins of *S. bowleyana* are slightly pilose and the top of the fruit is hairy. The leaves are also glabrous on both sides. The stems, leaves on both sides and petioles of *S. splendens* are not glabrous and have glandular patches below. The fruits have narrow wings at the margin and top folds that are asymmetrical. The stems, many branches, leaf surfaces and petioles of *Salvia officinalis* are coated in white, short villi. The fruits are hairless and silky.

## Discussion

The total number of protein-coding genes in the chloroplast genomes of *S. bowleyana*, *S. splendens* and *S. officinalis* was equal, with the exception of *S. splendens* having one fewer gene. Similar to other *Salvia* species, the total number of tRNA and rRNA genes was also the same. These findings suggested that the *Salvia* species' chloroplast genomes were largely conserved. The selected Lamiaceae species (41 in total) and the two outgroup species (*L. chuanxiong* and *P. notoginseng*) shared many pharmacological properties, including improved hypoxia resistance, increased coronary flow, improved microcirculation, protection of the heart and anti-hepatitis, anti-tumor and antiviral properties. Because they contain genes from both the nuclear and mitochondrial genomes, chloroplasts play a crucial role in the synthesis of chemicals and the development of phenotypes. The average genetic distance between all strains of CWR and cultivated rice revealed that the variability of the nuclear genome was higher than that of the chloroplast and mitochondrial genomes. Analysis of the genetic difference in the chloroplast genomes of *Salvia* species is therefore essential [4].

It makes sense that the ten *Salvia* species can be differentiated using the DNA sequences of the hypervariable sections and comparison of the

chloroplast genomes in three IGS regions of rps16-trnQ-UUG, trnL-UAA-trnF-GAA and trnM(cau)-atpE. (*S. officinalis*, *S. japonica*, *S. sclarea*, *S. meiliensis*, *S. hispanica*, *S. tilifolia*, *S. yangii*, *S. splendens*, *S. nilotica* and *S. chanryoenica*). The *Zingiber officinale* and *Coffea alliance* species contain the first IGS region. The second one frequently happens in angiosperms. Since they share a common ancestor with living gymnosperms and angiosperms, the last one has diversified and some of the oldest mtDNAs of trnV(uac)-trnM(cau)-atpE-atpB-rbcL were transferred from cpDNA to mtDNA. Nuclear ribosomal DNA sequences (nrETS, 5S-NTS) from 148 accessions were used to rebuild the evolutionary connections in the Eurystachys clade. These genera included commonly known and well-defined segregates like *Prasium* and *Sideritis* [5].

## Conclusion

Using Illumina sequencing technology, the whole chloroplast genomes of *S. bowleyana*, *S. splendens* and *S. officinalis* were obtained. The phenotypic makes it simple to distinguish between these three species. The three investigated species were found to be part of the different branches of the one branch that was formed by 23 *Salvia* species and one Rosmarinus genus, according to phylogenetic analysis. rps16-trnQ-UUG, trnL-UAA-trnF-GAA, trnM-CAU-atpE, trnL-UAG-ccsA, ccsA-ndhD, rps15-ycf1 and ndhE-ndhG are the seven IGS areas that the sequence divergence found. Notably, the three *Salvia* species under study contained the two IGS regions trnG-GCC-trnM-CAU and ycf3-trnS-GGA. The considerable diversity of the sequences' divergence suggests they can be developed as DNA markers for future genus *Salvia* identification and phytotaxonomy. Overall, the information gathered will help to advance the taxonomy, ecology, taxonomy, phylogenetic evolution and conservation of the *Salvia* genus in China.

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