Bioinformatics Study of the Important Medicinal Plant: *Salvia* Genus

ELMIRA ZIYA MOTALEBIPOUR [134]*, AKBAR PIRESTANI [234]

[1]*Department of Agronomy and Plant Breeding, Isfahan (Khorasgan) Branch, Islamic Azad University, Isfahan, Iran.*
[2]*Department of Animal Science, Isfahan (Khorasgan) Branch, Islamic Azad University, Isfahan, Iran.*
[3]*Medicinal plants research center, Isfahan (Khorasgan) Branch, Islamic Azad University, Isfahan, Iran.*
[4]*Transgenesis Center of Excellence, Isfahan (Khorasgan) Branch, Islamic Azad University, Isfahan, Iran.*

*Corresponding Author E-mail:*e.ziyamotalebipour@khuisf.ac.ir.

ABSTRACT

Wide variety of medicinal plants showed the high amount of secondary metabolites. Flavonoids are a class of important secondary metabolites with medicinal benefits. One of the well-known traditional medicinal herb is *Salvia* genus with a broad diversity of flavonoids. Flavonol synthase (FLS) is one of the flavonoid biosynthesis-related gene were identified in some medicinal plant of Lamiacea family. However, flavonol synthase (FLS) have not been phylogeny and evolutionary divergence analyzed in different *Saliva* species. In this study, *Arabidopsis thaliana* FLS gene (NCBI accession NM_120951) was select and used as a query sequence for performing a BLASTN search. Six sequences query which was found by NCBI platform was selected for further analysis. Phylogenetic trees of full length sequences of different FLS gene of *Salvia* genus were constructed by the Maximum Likelihood method using MEGA software. This study may be the reference value for the genetic relationship among different FLS gene in *Salvia* genus species. This variation provides us the possibility the design the primer to achieve us to find the exact FLS gene and also showed the evolutionary divergence between *salvia* species.

Keywords: Medicinal Plant, *Salvia* genus, Phylogeny, Evolutionary Divergence

INTRODUCTION

One of the most important herbal families with wide variety of medicinal plants is Lamiaceae family which cover 236 genera and 6900 to 7200 species (Fidan *et al.,* 2019). *Salvia* species is the most known genus which contains medicinal potentialities used for its antioxidative, antibacterial, antidiabetic and antitumor effects. (Bekut *et al*., 2018). Essential oils of *Salvia* genus presence more than 100 active compounds. It has the high value of flavones like 6-hydroxyluteolin, apigenin, luteolin and methyl-flavones (Kuete *et al*., 2017). Studies need to focus on the main genes in flavonoid biosynthesis which can further become a basis for phytomedicines.

Flavonoids is one of the main group of secondary metabolites which are distributed in the plant kingdom. Flavones, flavanones, flavanols, flavonols, isoflavones, aurones, anthocyanins, and proanthocyanidins (PA, tannins) are the important flavonoids (Deng and Lu, 2017). Flavonoids showed anti-oxidation, anti-inflammation, anti-cancer, anti-mutagenesis, anti-bacterial and anti-viral activity (Kumar and Pandey, 2013). The enzymes catalyzing flavonoid biosynthesis have been analyzed in various plant species, such as *Arabidopsis thaliana* (Saito *et al*., 2013), *Glycine max* (Dastmalchi *et al*., 2015), and *Brassica napus* (Auger *et al*., 2010). Flavonol synthase (FLS) acts the main role in the flavonoid biosynthetic pathway (Cheng *et al*., 2014). According to NCBI platform, 29 genes were recorded for flavonol synthase (FLS). Among 22 gene, 4 gene in *Arabidopsis thaliana*, 3 gene in *Dendrobium catenatum*, 2 gene in *Malus domestica* and 13 genes from other Taxa. In current study characterization of the main flavonoids gen: flavonol synthase (FLS) gene was analyzed in *Salvia* genes and showed the distance between genes in different species of *Salvia* genus.

MATERIAL AND METHODS

Specific information of the FLS gene of different genus was obtained using the NCBI-Gene platform. A total of 22 species gene information was retrieved, including 22 known species information FLS gene of *Arabidopsis thaliana* with ID number: 830765 were selected for further analysis.

Phylogenetic trees of full length sequences of different FLS gene of *Salvia* genus were constructed by the Maximum Likelihood method and Tamura-Nei model (Tamura and Nei, 1993) using Molecular Evolutionary Genetics Analysis Version 11 (MEGA version 11 http://www.megasoftware.net, Tamura *et al*., 2021).

Furthermore, estimates of evolutionary divergence between sequences were analyzed by the Maximum Composite Likelihood model (Tamura *et al*., 2004). This analysis involved 6 nucleotide sequences. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 1394 positions in the final dataset. Evolutionary analyses were conducted in MEGA11 (MEGA version 11 http://www.megasoftware.net, Tamura *et al*., 2021).

RESULTS AND DISCUSSION

The current study provided basic bioinformatics information to understand the genetic diversity of FLS gene in the *Salvia* genus. FLS gene have been studied in various plant species, such as *A. thaliana* (Wilmouth *et al*., 2002), *S. miltiorrhiza* (Deng *et al*., 2018), and *P. granatum* (Ben-Simhon *et al*., 2015). Among 22 FLS genes, four genes in *A. thaliana*, were define in NCBI platform. NCBI's RefSeq of FLS1 gene of *A. thaliana* (NCBI accession NM_120951) was select and used as a query sequence for performing a BLASTN search. BLASTN was done against all nucleotide database in *Saliva* (taxid: 21880) organism. Total of 100 putative non redundant FLS gene identified by performing BLASTN search against all database. Among 100 sequence query, 6 sequences with high coverage was selected for further analysis (Table 1). The sequence with the lower E-value sequence represented the query sequence matches the database sequence and more significant. 6 sequence queries showed the exact or predicted FLS gene of *Salvia* genus.

Table 1. Information of all 6 gene sequence of FLS.

No	Accession	Scientific Name	Query Cover	E value	Accession length
1	MH 447675.1	*Salvia miltiorrhiza*	99%	3E-143	1008
2	MH 447674.1	*Salvia miltiorrhiza*	98%	7E-124	972
3	XM 048104578.1	*Salvia hispanica*	99%	9E-138	1164
4	XM 048127980.1	*Salvia hispanica*	95%	1E-120	1265
5	XM 048105844.1	*Salvia hispanica*	68%	1E-63	674
6	XM 042193621.1	*Salvia splendens*	98%	3E-122	1279

According to MEGA software, 6 different genotypes of genes were analyzed and the genetic distance was calculated using the highest log likelihood method constructed a phylogenetic tree of the *Salvia* genus. The results showed that the FLS gene sequences from six different *Salvia* species was divided into 4 branches and belonged to different groups. The first group containing four sequences from three species (*S. splendes, S. hispanica, S. miltiorrhiza*) which separated in three branches *S. splendes* belonged to the parallel classification with FLS1 of *S. hispanica* in the first branch. It means the detected gene of *S. splendes* may be FLS1 in our study. Furthermore FLS1, 2 and 3 genes of *S. hispanica* was placed in different branches which means the differences of these three genes. FLS3 was placed very close FLS1 gene of *S. miltiorrhiza* branch that means these two gene was very similar to each other. FLS2 of two species (*S. hispanica* and *S. miltiorrhiza*) was in second branch with the high coverage value of BLAST and high length of sequence, whereas the second group included low mean value with low length of sequence. This phylogeny analysis

shows that the *S. splendes* and *S. hispanica*1 genes were similar than *S. hispanica*3 and *S. miltiorrhiza* genes. As previouse analysis of Deng *et al*., (2018) FLS1 is mainly expressed in flowers, while expression of FLS2 showed the highest levels in flowers and leaves. It means FLS gene in *Salvia splendens* (XM 042193621.1) is expressed in flower. This variation provides us the possibility the design the primer to achieve us to find the exact FLS gene which effect all FLSs in flower, leave and root of *Salvia* genuse.
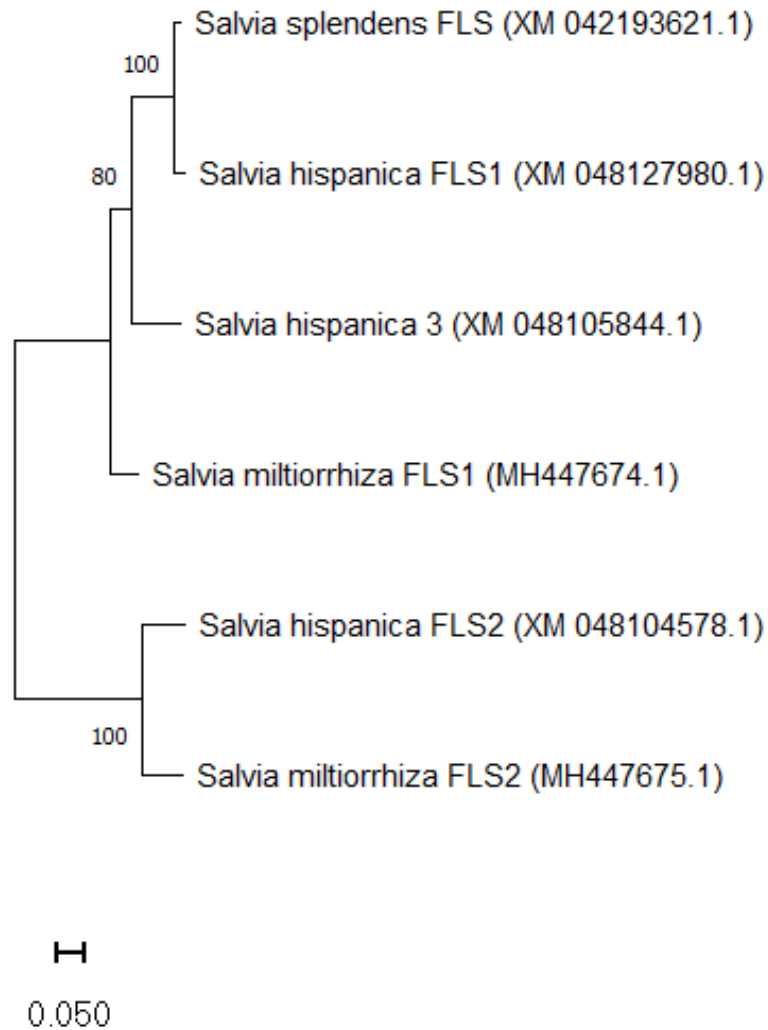

Figure 1. phylogeny analysis for 6 FLS gene sequence of *Salvia* genus by using the Maximum Likelihood method and Tamura-Nei mode in MEGA software.

Estimates of evolutionary divergence between sequences were detected using the Maximum Composite Likelihood model (Table 2, Tamura *et al*., 2004). This analysis involved 6 nucleotide sequences. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 1394 positions in the final dataset. Evolutionary analyses were conducted in MEGA11 (MEGA version 11 http://www.megasoftware.net, Tamura *et al*., 2021). Acording to anallysis the high evolutionary divergence was detected between *Salvia splendens* FLS (XM 042193621.1) and

Salvia hispanica FLS2 (XM 048104578.1), *Salvia hispanica* FLS1 (XM 048127980.1) and *Salvia hispanica* FLS2 (XM 048104578.1), *Salvia miltiorrhiza* FLS2 (MH447675.1) and *Salvia hispanica* 3 (XM 048105844.1). Also the low evolutionary divergence was identify between *Salvia hispanica* FLS2 (XM 048104578.1) and *Salvia miltiorrhiza* FLS2 (MH447675.1), *Salvia splendens* FLS (XM 042193621.1) and *Salvia miltiorrhiza* FLS1 (MH447674.1). This evolutionary divergence help us to design the polimorphic primers in further research which can be use all FLSs genes in flower, leave and root of *Salvia* genuse.

CONCLUSION

According to the results, the variation FLS genes in *salvia* species showed the differences among the sequence and also protein followed by mechanism of flavonoids makes. These variations are useful for primer design and find the level of gene expression in different *salvia* species. The high level of gene expression can be concluded the high amount of flavonoid in species for special purposes in breeding programs. Evolutionary divergence between species showed the assessment of the genetic and phylogenetic relationships between *salvia* genus which separated two cluster of FLS genes in studied genus. the Furthermore, some of the above species could be used directly as a good source for drug industries.

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Table 2. Estimates of evolutionary divergence between sequences. The number of base substitutions per site from between sequences are shown.

	Salvia splendens FLS (XM 042193621.1)	*Salvia hispanica* FLS1 (XM 048127980.1)	*Salvia miltiorrhiza* FLS1 (MH447674.1)	*Salvia hispanica* FLS2 (XM 048104578.1)	*Salvia miltiorrhiza* FLS2 (MH447675.1)	*Salvia hispanica* 3 (XM 048105844.1)
Salvia splendens FLS (XM 042193621.1)						
Salvia hispanica FLS1 (XM 048127980.1)	0.0377					
Salvia miltiorrhiza FLS1 (MH447674.1)	**0.1566**	0.1658				
Salvia hispanica FLS2 (XM 048104578.1)	**0.5568**	**0.5449**	0.4352			
Salvia miltiorrhiza FLS2 (MH447675.1)	0.4610	0.4677	0.4505	**0.1436**		
Salvia hispanica 3 (XM 048105844.1)	0.1693	0.1848	0.1732	0.4433	**0.5006**	

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