

## COMPARITIVE PHYLOGENETIC ANALYSIS OF PASSIFLORA BASED ON PROTEIN MARKER CHLOROPLAST EXPRESSED GLUTAMINE SYNTHETASE (ncpGS) AND RIBOSOMAL PROTEIN S4 (rpS4)

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### ABSTRACT

The phylogenetic analysis and evolutionary study of tropical genus Passiflora (passifloraceae) are reviewed using data from protein marker glutamine synthetase expressed in chloroplast (ncpGS) with comparison of ribosomal protein (rpS4). Glutamine synthetase gene shows a good resolution for phylogenetic reconstruction in combination with ribosomal protein rpS4. We observed a striking overall correlation between phylogenetic positions in different species of Passiflora.

**Keywords-** Passiflora, phylogenetic analysis, Glutamine synthetase, ribosomal protein, evolutionary study, chloroplast

### 1. INTRODUCTION

Passifloraceae family comprises genus Passiflora as largest one, this genus consists of approximately 465 species. The distribution is abundant, mainly found in tropical regions [1]. Most of these species are herbaceous; some are shrubs or trees, many found as vines. Passiflora species are cultivated as ornamentals and for their pharmacological importance also [2].

The common name of plant passiflora is "Passion flower" it also has a historical background, as the morphology of flower resembles with cruciform symbol of Christ hence the flower named as Passion flower [3].

From last few years varied analytical studies conducted for phylogeny based on a range of chloroplast and mitochondrial markers [4, 5]. The cytoplasmic sequence reflects a uniparental inheritance in evolution when analyzed [6]. We have used combination of nuclear and ribosomal

markers for analyzing the phylogenetic relation, by various Bioinformatics tools [7].

Glutamine synthetase is a chloroplast expressed nuclear gene (ncpGs), it is a part of small nuclear multigene family (3-5 genes), which has been characterized in a broad range of taxa [8]. This gene has been reported chloroplastic isozymes and cytosolic enzymes in many flowering plants, in monocots and legumes. The role of glutamine synthetase is in the assimilation of ammonium (soil) and in the reassimilation of the ammonium after the metabolic process [9, 10].

This gene was chosen mainly because of its slower evolutionary rate. The analysis was conducted by comparative analysis and revealed within 9 selected species of Passiflora.

**2. MATERIALS AND METHODS-**

In this particular part the 9 selected Passiflora species had been elucidated for phylogenetic analysis based on marker gene cytosol expressed glutamine synthetase (ncpGS) and ribosomal protein S4 (rpS4). Both sequences and other databases are collected from NCBI ( NATIONAL CENTRE FOR BIOTECHNOLOGY

INFORMATION) through site www.ncbi.nlm.nih.gov. These sequences were edited and multiple alignments had been performed by using clustal W, GenBee, etc. Bioinformatics tools. The neighbor joining tree and dendrograms, cluster algorithm trees, topological algorithm trees with bootstrap values were executed.

**3. RESULTS- TABLE 1:**

**LIST OF SPECIES OF *Passiflora* USED IN THIS STUDY**

NAMES OF SPECIES	COLLECTION DATABASE	NCBI ACCESION NUMBERS	
		ncpGS	rpS4
P. spruce	NCBI DATABASE	AAR05567.1	ABA01297.1
P. elagans	NCBI DATABASE	AAR05498.1	AAP88993.1
P. caerulea	NCBI DATABASE	AAR05472.1	AAP88982.1
P. foetida	NCBI DATABASE	AAR05501.1	AAP88979.1
P. alata	NCBI DATABASE	AAR05455.1	AAP89011.1
P. quadrangularis	NCBI DATABASE	AAR05551.1	AAP89010.1
P. gabrielliana	NCBI DATABASE	AAR05505.1	AAP89007.1
P. edulis	NCBI DATABASE	AAR05494.1	AAP88992.1
P. cincinnata	NCBI DATABASE	AAR05477.1	AAP88990.1

**TABLE 2.1(Distance Matrix Table )**

For protein marker Glutamine synthetase (ncpGS) in following selected species-

	1	2	3	4	5	6	7	8	9
1 P.sprucei	0.000	0.057	0.025	0.229	0.034	0.067	0.145	0.222	0.073
2 P.elegans	0.057	0.000	0.115	0.136	0.124	0.000	0.021	0.179	0.000
3 P.caerulea	0.025	0.115	0.000	0.278	0.000	0.124	0.199	0.271	0.131
4 P.foetida	0.229	0.136	0.278	0.000	0.287	0.146	0.068	0.000	0.154
5 P.alata	0.034	0.124	0.000	0.287	0.000	0.118	0.192	0.265	0.131
6 P.quadrangularis	0.067	0.000	0.124	0.146	0.118	0.000	0.014	0.172	0.000
7 P.gabrielliana	0.145	0.021	0.199	0.068	0.192	0.014	0.000	0.097	0.029
8 P.edulis	0.222	0.179	0.271	0.000	0.265	0.172	0.097	0.000	0.187
9 P.cincinnata	0.073	0.000	0.131	0.154	0.131	0.000	0.029	0.187	0.000

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TABLE 2.2(Distance Matrix Table -)

For protein marker ribosomal protein S4 (rpS4) in following selected species-

	1	2	3	4	5	6	7	8	9
1 <i>P.sprucei</i>	0.000	0.032	0.018	0.025	0.036	0.040	0.029	0.032	0.029
2 <i>P.elegans</i>	0.032	0.000	0.032	0.025	0.036	0.040	0.021	0.032	0.029
3 <i>P.caerulea</i>	0.018	0.032	0.000	0.025	0.036	0.040	0.029	0.032	0.029
4 <i>P.foetida</i>	0.025	0.025	0.025	0.000	0.030	0.034	0.023	0.025	0.023
5 <i>P.alata</i>	0.036	0.036	0.036	0.030	0.000	0.024	0.033	0.036	0.033
6 <i>P.quadrangularis</i>	0.040	0.040	0.040	0.034	0.024	0.000	0.038	0.040	0.038
7 <i>P.gabrielliana</i>	0.029	0.021	0.029	0.023	0.033	0.038	0.000	0.029	0.026
8 <i>P.edulis</i>	0.032	0.032	0.032	0.025	0.036	0.040	0.029	0.000	0.029
9 <i>P.cincinnata</i>	0.029	0.029	0.029	0.023	0.033	0.038	0.026	0.029	0.000

PHYLOGENETIC TREES OBTAINED FROM PHYLOGENY PROGRAMS AS FOLLOWS-

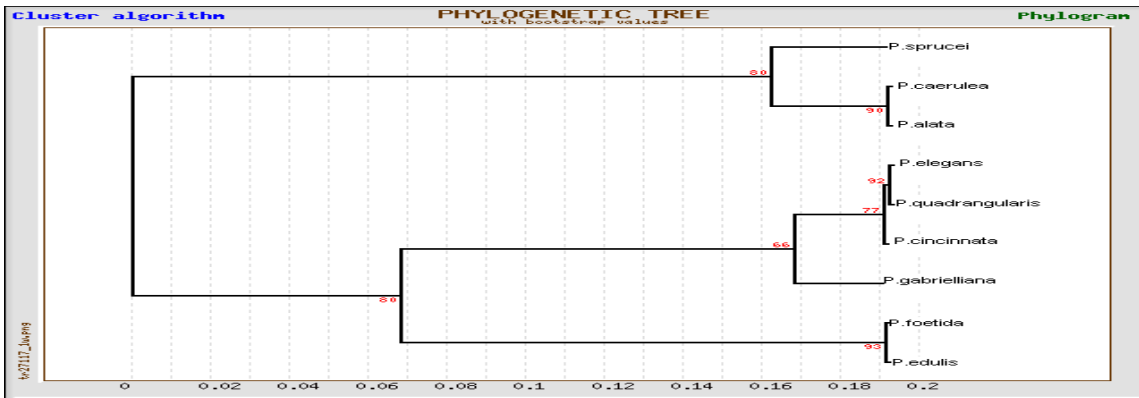


Fig1. The Phylogram based on marker protein chloroplast expressed glutamine synthetase (ncpGS).

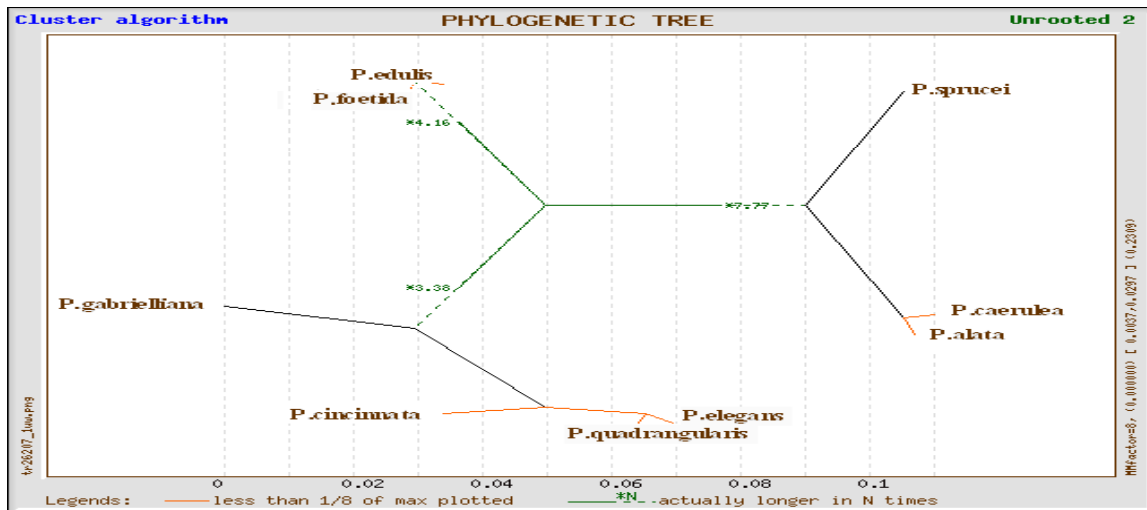


Fig2. The phylogram based on marker protein ribosomal protein S4 (rpS4)

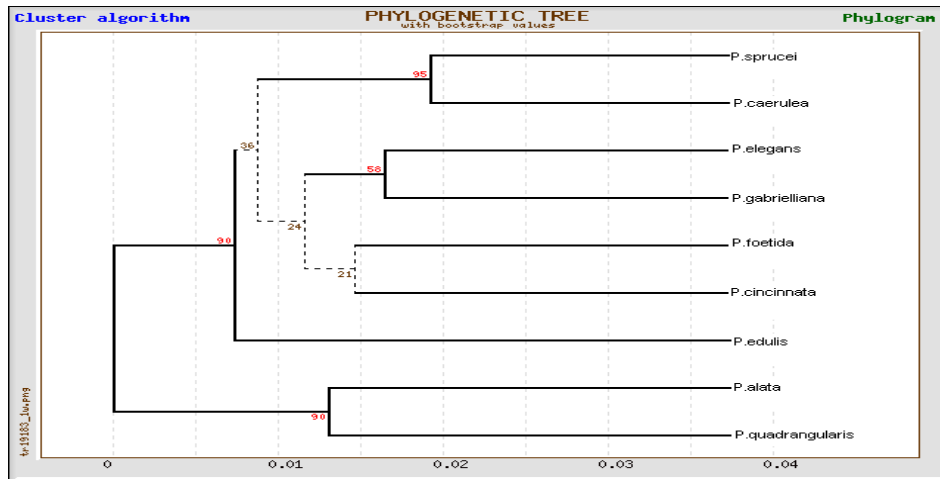


Fig3. An Unrooted Phylogentic tree based on marker protein Chloroplast expressed glutamine synthetase (ncpGS).

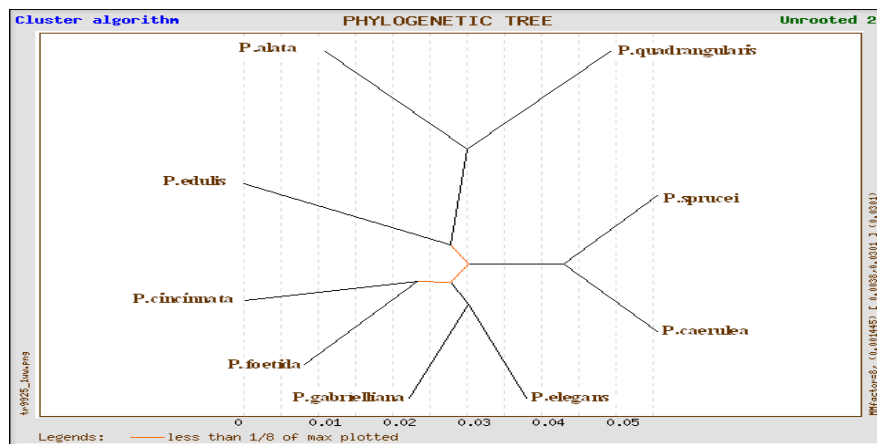


Fig 4. An Unrooted Phylogenetic tree based on marker protein ribosomal pritein S4 (rpS4).

**RESULTS BASED ON GLUTAMINE SYNTHETASE (ncpGS)-**

REFIGNED ALIGNMENT, power 132.48

Similarity percent 86.0

DRAFT SOURCE ALIGNMENT, power 132.48

Similarity percent 86.0

6 BEST LOCAL ALIGNMENTS (SUPERMOTIFS)

LOCAL SUPERMOTIF number 1, power 36.22

LOCAL SUPERMOTIF number 2, power 5.97

LOCAL SUPERMOTIF number 3, power 3.86

LOCAL SUPERMOTIF number 4, power 3.74

LOCAL SUPERMOTIF number 5, power 3.51

LOCAL SUPERMOTIF number 6, power 3.51

**RESULTS BASED ON RIBOSOMAL PROTEIN S4 (rpS4)-**

REFIGNED ALIGNMENT, power 271.29

Similarity percent 76.6

DRAFT SOURCE ALIGNMENT, power 271.29

Similarity percent 76.6

BEST LOCAL ALIGNMENTS (SUPERMOTIFS)

LOCAL SUPERMOTIF number 1, power 60.17

LOCAL SUPERMOTIF number 2, power 27.24

LOCAL SUPERMOTIF number 3, power 3.5

#### 4. DISCUSSION

Still some questions remained unresolved to focus some light on these we had performed a phylogenetic analysis based on two molecular markers which are chloroplast expressed nuclear gene glutamine synthetase (ncpGS) and ribosomal protein (rpS4). Although the analyses presented here are based on limited number of species, this study brings enhancing results for phylogenetic relationships among closely related species.

From all the phylogenetic trees and multiple alignments based on protein marker chloroplast expressed glutamine synthetase (ncpGS) it found that the *P. alata* and *P. caerulea* shows much similarity and they are closely related with 99 as Bootstrap value, and in the same manner the *P. cincinnata* and *P. elegans* also shows similar origin or evolved from same ancestors having 100 as Bootstrap value.

The other species like *P. foetida* and *P. edulis* shows 99 as a Bootstrap value; while the species like *P. cincinnata* and *P. quadrangularis* does not show much evolutionary relationship hence they are not much related with each other having 60 as Bootstrap value.

In the same manner Phylogenetic analysis based on marker ribosomal protein S4 (rpS4), shows that *P. sprucei* and *P. caerulea* having

most similarity with 95 as Bootstrap value. Following *P. alata* and *P. quadrangularis* shows a slightly lower evolutionary relationship with 90 as Bootstrap value. The *P. elegans* and *P. gabrielliana* shows 58 as Bootstrap value and the least related species are *P. foetida* and *P. cincinnata* with 21 as Bootstrap value.

#### 5. SUMMARY AND CONCLUSION-

The present investigation entitiled “The Comparative Phylogenetic Analysis of *Passiflora* based on markers chloroplast expressed protein glutamine synthetase (ncpGS) and ribosomal protein S4 (rpS4)” was conducted using various BIOINFORMATICS TOOLS and evolutionary relationships among closely related species had been evaluated.

The recent popularity of genus *Passiflora*, because of its edible species, has attracted the attention not only of taxonomists but also of cytogeneticists. Of the 400 known species of *Passiflora*, about 50 to 60 bear edible fruits. Probably all these are indigenous to the tropical and subtropical world where passion fruit is grown, because of its high fruit quality is by far more popular in Australia, New Zealand, Brazil and S. Africa. In India it is known as most important ornamental plant and also for its edible fruits passion flower commonly known as “*Krishna Kamala*” means flower of Lord Krishna.

The *Passiflora* plant already had been evaluated for phylogenetic analysis

based on various markers like ribosomal internal transcribed spacers (ITS), plastid trn L- trnF spacers, cytosolic glutamine synthetase protein, chloroplast expressed glutamine synthetase (ncpGS) and mitochondrial matK marker .In this analysis we have evaluated the comparative phylogeny based on combined markers i.e. glutamine synthetase (ncpGS) and ribosomal protein S4 which had resulted in a striking overall correlation between selected *Passiflora* species. This suggests that both proteins had evolved in these species through the process of evolution, and some of them don't show much relatedness this may occurred because gene loss through various mutational processes like transitions, transversions, duplications. The variation may occur because of high divergence in habitats of these species; adaptation resulted in the gene loss. Hence this analysis helped to check evolutionary relationship among closely related *passiflora* species.

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