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GENETIC DIVERSITY OF PHYTOPLASMAS INFECTING SESAME IN SOUTHERN INDIA

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ABSTRACT
Sesame phyllody disease poses a major threat to sesame cultivation in India. In a survey conducted in southern India (Tamil Nadu), 44 symptomatic sesame samples were collected from 20 districts covering five agro-climatic zones. Infected plants exhibited typical symptoms such as phyllody, witches' broom, and virescence, with disease incidence ranging from 5-87% at different crop stages. Lower disease incidence was observed during the early flowering stage in Coimbatore district, while higher incidence occurred during maturity in Cuddalore district. Molecular characterization and phylogenetic analysis of the samples consistently showed that they belonged to '*Candidatus* Phytoplasma australasiaticum', clustering with 16SrII-D subgroup sequences from the GenBank database. No significant diversity was identified within the collected samples based on *SecA* gene analysis, indicating a single phytoplasma lineage in Tamil Nadu. However, the minimum spanning haplotype network revealed considerable haplotype differentiation of Tamil Nadu sesame samples compared to other regions of India. Neutrality tests and demographic analyses showed negative Tajima's D and Fu's F values, suggesting a stable population structure and moderate gene flow within Tamil Nadu. Genetic differentiation analyses indicate a strong distinction between the Tamil Nadu population and other regions' populations. These findings highlight very low genetic diversity and moderate gene flow within phytoplasma populations infecting sesame in Tamil Nadu, underscoring the need for early, region-specific management strategies.

KEYWORDS: Sesame, phytoplasma, population diversity, 16SrII-D, haplotype diversity

INTRODUCTION
Sesame is an ancient oilseed crop cultivated by humankind, with the cultivation dating back to Harappan, Mesopotamian, Anatolian and Graeco-Roman worlds (Thirumalaisamy et al. 2023). Sesame is regarded as the 'Queen of oilseeds' for its excellent quality and quantity of the oil (53.3%) and protein (25%) in the seeds (Venkataravanappa et al. 2017; Bhaskar et al. 2025). It is rich in natural antioxidants such as sesamoline, sesamol and sesamin (Nabi et al. 2015). In India, sesame is grown in an area of 1.52 million hectare with a yield of 0.65 million tonnes and a productivity of 432.9 kg/hectare. India's production is lower than the global average of 510.8 kg/ha due to both abiotic and biotic factors (Bhaskar et al. 2025).
Among the biotic factors, phyllody disease associated with the presence of phytoplasmas is considered as one of the most destructive diseases affecting the sesame production. The disease, which is characterized by stunting of the plants and alteration of the floral parts into leafy structures bearing no capsule and seeds, is a serious threat to sesame production in various Asian and African countries (Akhtar et al. 2009; Ikten et al. 2011). In severe stages, the disease can cause significant yield loss from 80 to 100% in India (Gogoi et al. 2017; Boopathi et al. 2023).
Phytoplasmas are wall-less phloem-limited pathogenic bacteria placed under the class Mollicutes. They are mainly transmitted by phloem feeding insect vectors, particularly leafhoppers (Ikten et al. 2014). In India, phytoplasmas enclosed in several ribosomal groups have been reported associated with sesame phyllody. In Northern India and countries like Thailand, '*Candidatus* Phytoplasma asteris' is the most commonly observed phytoplasma in sesame. In Karnataka, Telangana and Maharashtra regions of India, '*Ca.* P. australasiaticum' (16SrII-A and 16SrII-D subgroups) was identified (Devanna et al. 2020). Not much study was carried out in the state of Tamil Nadu regarding the devastating incidence of phytoplasmas in sesame. Hence, this study was conducted to determine the molecular diversity of the phytoplasmas infecting sesame in Tamil Nadu. The classification of phytoplasmas is mainly based on the 16S rRNA gene sequencing and RFLP analysis. Several genetic markers like immunodominant protein (*imp*) and *SecA* genes have been also developed for differentiating the phytoplasma strains described in the present study (Siampour et al. 2013; Al-Abadi et al. 2016).
The molecular diversity of phytoplasmas infecting sesame in Tamil Nadu remains understudied. Therefore, the present study was conducted to investigate this diversity based on 16S rRNA, *imp*, and *secA* genes. The findings of this study will contribute to addressing existing research gaps in the genetic variability and population structure of sesame-infecting phytoplasmas.

MATERIALS AND METHODS

Survey and collection of phytoplasma infected sesame samples

An extensive survey was conducted for the collection of phyllody infected sesame samples in 20 major sesame growing districts of Tamil Nadu covering different agro-climatic zones such as, North-eastern, North-western, Western, Cauvery Delta and Southern zones. The survey was carried out from the period of January 2024 to July 2025. A total of 44 samples were collected across different districts of Tamil Nadu at various growth stages of crop. Diverse symptoms were documented in phytoplasma infected sesame plants during the survey. Detailed record of the sampling locations and crop growth staged were maintained. Both the asymptomatic and symptomatic samples were collected in triplicate from each field. All the samples were properly labelled and transported to the Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India and stored at -20ºC until further analysis.

DNA extraction and polymerase chain reaction (PCR) analysis

The collected symptomatic leaves were washed with tap water to remove the debris and the total genomic DNA from all the samples was extracted by a CTAB method (Ahrens and Seemüller 1992). DNA extracted from non-symptomatic samples was used as a negative control. Amplification of phytoplasma ribosomal DNA was performed with phytoplasma universal primer pairs P1/P7, followed by nested primer pairs R16F2n/R16R2 (Suhas et al. 2025). The PCR was performed for all the samples using an Applied Biosystems VeritiPro 96-Well Thermal Cycler (Thermo Fisher Scientific, USA). The 10 µl PCR mixture consisted of 5 µl Takara Master mix, 2 µl double sterile distilled water, 1 µl forward primer (10 pM/µl), 1 µl reverse primer (10 pM/µl) and 1 µl template DNA (~100 ng DNA). The PCR product of the PCR was diluted at 1:20 with double-sterile distilled water prior to nested PCR. The products from nested PCR were separated on gel electrophoresis using 1% agarose gel stained with ethidium bromide and visualized under UV light in the gel documentation unit (UVITEC Cambridge).

All 44 DNA samples that tested positive through nested PCR analysis were subsequently subjected to amplification of the *SecA* gene by direct and semi-nested PCR assays using *SecA*for1, *SecA*for2 and *SecA*rev3 primers (Hodgetts et al. 2008). PCR products were diluted at 1:20 with double-sterile distilled water prior to semi-nested PCR and were visualized as described above. All the samples were amplified for *imp* gene using the primers ImpF and ImpR (Siampour et al. 2013). PCR products were visualized as described above. The details of the primers and PCR conditions for all the primer sets were described in Table 1&2.

Multiple sequence alignment and phylogenetic analysis based on 16S rRNA, *secA* and *imp* genes

The PCR amplicons corresponding to the 16S rRNA, *secA* and *imp* genes from 44 phytoplasma-positive samples collected across 20 districts of Tamil Nadu were subjected to Sanger sequencing at Medauxin, Bangalore, India using the respective primers employed for PCR amplification. The resulting nucleotide sequences were subjected to NCBI BLASTn algorithm, edited, assembled and aligned prior to further analysis.

For phytoplasma classification, the 16S rRNA gene (1250 bp) sequences were subjected to *in silico* RFLP comparison using an online tool, iPhyClassifier (https://plantpathology.ba.ars.usda.gov/cgi-bin/resource/iphyclassifier.cgi). The virtual RFLP profiles were used to determine the 16Sr groups and subgroups of the phytoplasma isolates (Zhao et al., 2009). All the sequences were subsequently deposited in DNA Data Bank of Japan (DDBJ) and the corresponding accession numbers were obtained.

Phylogenetic relationships among the phytoplasma isolates were inferred using the neighbour-joining method implemented in MEGA version 12. Evolutionary distances were estimated using the Tamura 3 substitution model. The branch support of the phylogenetic trees was assessed by bootstrap analysis with 1000 replicates (Kumar et al., 2024).

Table 1: Primers employed in the study

Sl. No.	Primers	PCR method	Primer sequence	Amplicon size	Target gene	References
1.	P1	Direct	AAGAGTTTGATCCTGGCTCAGGATT	~1800 bp	16SrRNA	Deng and Hiruki, 1999
2.	P7		CGTCCTTCATCGGCTCTT			
3.	R16F2n	Nested	TGACGGGTGTGTACAAACCCCG	~1250 bp		Gundersen and Lee, 1996
4.	R16R2		GAAACGACTGCTAAGACTGG			
5.	ImpF	Direct	ATGAATCACAAAGAAATTTTTTAC	482 bp	*imp*	Siampour et al., 2012
6.	ImpR		TTATGATAATTTTAAATCTG			
7.	SecAfor1	Semi-nested	GARATGAAAACTGGRGAAGG	519 bp	*SecA*	Hodgetts et al., 2008
8.	SecAfor2		GAYGARGSWAGAACKCCT			
9.	SecArev3		GTTTTRGCAGTTCCTGTCATNCC			

Table 2: PCR cycling conditions for the primers in the present study

Sl. No.	Steps	P1/P7	R16F2n/R16R2	ImpF/ImpR	SecAfor1/SecArev3 &SecAfor2/SecArev3
1.	Initial denaturation	94ºC for 5 mins	94ºC for 2 mins	94ºC for 2 mins	94ºC for 2 mins
2.	Denaturation	94ºC for 1 min	94ºC for 1 min	94ºC for 45 secs	94ºC for 30 secs
3.	Annealing	55ºC for 30 secs	55ºC for 2 mins	53ºC for 1 min	53ºC for 1 min
4.	Extension	72ºC for 2 mins	72ºC for 3 mins	72ºC for 1:30 mins	72ºC for 1:30 mins
5.	Final extension	72ºC for 10 mins	72ºC for 10 mins	72ºC for 10 mins	72ºC for 15 mins
6.	No. of cycles (Steps 2 to 4)	35	35	40	35

Minimum spanning haplotype network of sesame phytoplasmas

The haplotype inference and network construction were performed in PopArt v1.7 using minimum spanning algorithm (Misra et al., 2024). This study was carried out to determine the relationship among the phytoplasmas infecting sesame in Tamil Nadu and other states of India and around the world. The network incorporated 44 sequences of study isolates collected from Tamil Nadu, 25 sequences from different states of India and 15 sequences from around the world (Table 5). The resulting network was evaluated for haplotype frequency, geographical distribution and mutation of sesame phytoplasma strains around the world.

Genetic diversity and population-genetic structure

The population genetic diversity indicators, such as the number of segregating sites (S), average number of pairwise nucleotide differences (K), number of haplotypes (H), haplotype diversity (Hd), and nucleotide diversity (π), both generally and for each individual country was determined using DnaSPv6. Neutrality indices like Tajima's D, Fu and Li's F, Fu's Fs, and Ramos-Onsins and Rozas' R2 were calculated in DnaSPv6 to evaluate selective neutrality. DnaSP was also used to calculate a number of Wright's F statistics, estimating pairwise genetic differences (Fst), average number of pairwise nucleotide differences (Kxy), nucleotide substitution per site (Dxy) and net nucleotide substitution per site (Da) and other parameters (Misra et al., 2024). A total of 84 secA sequences from India and Thailand were included in the demographic analysis. Analysis of molecular variance (AMOVA) was performed in RStudio software to assess the genetic structure within and among populations (Misra et al., 2024).

RESULTS

Survey and sample collection

During the survey conducted in 2024 – 2025, a total of 44 symptomatic sesame samples were collected from 20 districts of Tamil Nadu, representing five major agro-climatic zones, North-eastern, North-western, Western, Cauvery Delta and Southern zones. The sesame crop exhibited typical phyllody and witches' broom symptoms in all the fields. Characteristic symptoms such as, phyllody (conversion of floral parts into green leaf like structures), witches' broom (reduction in leaf size and appearance of dense growth of shoots on the top of the stem with short internodes) and virescence (greening of floral parts) were observed (Fig. 1). The incidence of sesame phyllody varied considerably across the surveyed locations and crop growth stages. The percent disease incidence (PDI) ranged from 5 to 87% across different surveyed fields, with the highest incidence of 87% recorded in Cuddalore and the lowest incidence of 5% recorded in Coimbatore district of Tamil Nadu (Table 3). Based on the crop growth stage, PDI ranged from 5% to 16% during early flowering, 18% to 38% during flowering, 33% to 62% during capsule formation and 45% to 87% during maturity (Table 4).

Figure 1: Symptomatology of sesame phytoplasma observed during the survey in Tamil Nadu **(a)** Phyllody **(b)** Virescence **(c) & (d)** Witches' broom symptoms



(a)



(b)



(c)



(d)

Table 3: Characteristics of the phytoplasma infected sesame samples collected from different districts of Tamil Nadu, India

Sl. No.	Agro-climatic zones of Tamil Nadu	District	Sample	Year of collection	Stage of the crop	Per cent Disease incidence (%)	DDBJ accession numbers		
							16S rRNA	*secA*	*imp*
1.	North-eastern zone	Ranipet	Ran1	2024	Flowering	38%	LC903116	LC898031	LC897987
2.		Tiruvallur	Tir1	2025	Early flowering	15%	LC897801	LC898034	LC897990
3.		Vellore	Ban1	2024	Flowering	23%	LC897802	LC898035	LC897991
4.		Tiruvannamalai	Tvm1	2025	Capsule formation	43%	LC897806	LC898039	LC897995
5.		Tiruvannamalai	Tvm2	2025	Flowering	23%	LC897807	LC898040	LC897996
6.		Villupuram	Svill1	2024	Flowering	25%	LC897819	LC903117	LC898008
7.		Villupuram	Svill2	2024	Capsule formation	47%	LC897820	LC903118	LC898009
8.		Cuddalore	Vri1	2024	Flowering	37%	LC897817	LC898050	LC898006
9.		Cuddalore	Vri2	2025	Maturity	87%**	LC897818	LC898051	LC898007
10.		Tindivanam	Tnd1	2024	Capsule formation	54%	LC897828	LC903126	LC898017

11.		Tindivanam	Tnd2	2024	Flowering	25%	LC897810	LC898043	LC897999
12.	North-western zone	Dharmapuri	Dpi1	2024	Flowering	13%	LC897803	LC898036	LC897992
13.		Dharmapuri	Dpi2	2024	Capsule formation	43%	LC897804	LC898037	LC897993
14.		Dharmapuri	Dpi3	2024	Flowering	21%	LC897805	LC898038	LC897994
15.		Dharmapuri	Dpi4	2024	Early flowering	13%	LC903113	LC898028	LC897984
16.		Krishnagiri	Kri1	2024	Flowering	23%	LC897812	LC898045	LC898001
17.		Krishnagiri	Kri2	2024	Flowering	34%	LC897813	LC898046	LC898002
18.		Krishnagiri	Kri3	2024	Flowering	16%	LC897814	LC898047	LC898003
19.		Salem	Slm1	2024	Maturity	45%	LC897825	LC903123	LC898014
20.		Salem	Slm2	2024	Flowering	22%	LC897826	LC903124	LC898015
21.		Salem	Slm3	2024	Capsule formation	40%	LC897827	LC903125	LC898016
22.		Namakkal	Sn1	2024	Maturity	63%	LC897832	LC903130	LC898021
23.		Namakkal	Sn2	2025	Flowering	20%	LC897824	LC903122	LC898013
24.	Western zone	Coimbatore	Cbe1	2024	Maturity	65%	LC903111	LC898026	LC897982
25.		Coimbatore	Cbe2	2025	Maturity	67%	LC897800	LC898033	LC897989
26.		Coimbatore	Cbe3	2024	Early flowering	5%*	LC897821	LC903119	LC898010
27.		Coimbatore	Cbe4	2025	Flowering	24%	LC897822	LC903120	LC898011
28.		Erode	Ero1	2024	Maturity	65%	LC897823	LC903121	LC898012
29.		Karur	Ka1	2025	Capsule formation	43%	LC897799	LC898032	LC897988
30.		Karur	Ka2	2025	Maturity	55%	LC897833	LC903131	LC898022
31.		Dindigul	Dnd1	2025	Capsule formation	33%	LC903114	LC898029	LC897985
32.		Dindigul	Dnd2	2025	Early flowering	16%	LC903115	LC898030	LC897986
33.		Tiruppur	Tpr1	2024	Capsule formation	45%	LC897815	LC898048	LC898004
34.		Tiruppur	Tpr2	2024	Flowering	18%	LC897816	LC898049	LC898005
35.	Cauvery Delta zone (CDZ)	Trichy	Tri1	2024	Capsule formation	62%	LC897808	LC898041	LC897997
36.		Trichy	Tri2	2025	Capsule formation	55%	LC897809	LC898042	LC897998
37.		Trichy	Tri3	2024	Maturity	62%	LC897811	LC898044	LC898000
38.		Pudukottai	Pud1	2025	Flowering	27%	LC897829	LC903127	LC898018
39.		Pudukottai	Pud2	2025	Capsule formation	43%	LC897834	LC903132	LC898023
40.		Thanjavur	Tj1	2025	Capsule formation	47%	LC897835	LC903133	LC898024
41.		Thanjavur	Tj2	2025	Flowering	27%	LC897836	LC903134	LC898025
42.	Southern zone	Madurai	Mdu1	2024	Flowering	33%	LC897830	LC903128	LC898019
43.		Madurai	Mdu2	2024	Capsule formation	56%	LC897831	LC903129	LC898020
44.		Madurai	Mdu3	2025	Maturity	63%	LC903112	LC898027	LC897983

*Lower disease incidence observed in Coimbatore district during early flowering stage

**Highest disease incidence observed in Vriddhachalam region of Cuddalore district during maturity stage

Table 4: Per cent Disease Incidence (PDI) based on different crop stages

Samples	Early flowering	Flowering	Capsule formation	Maturity
No. of samples	4	18	13	9
Range of PDI (%)	5-16	18-38	33-62	45-87
Cumulative PDI (%)	12.25	24.94	47	63.5

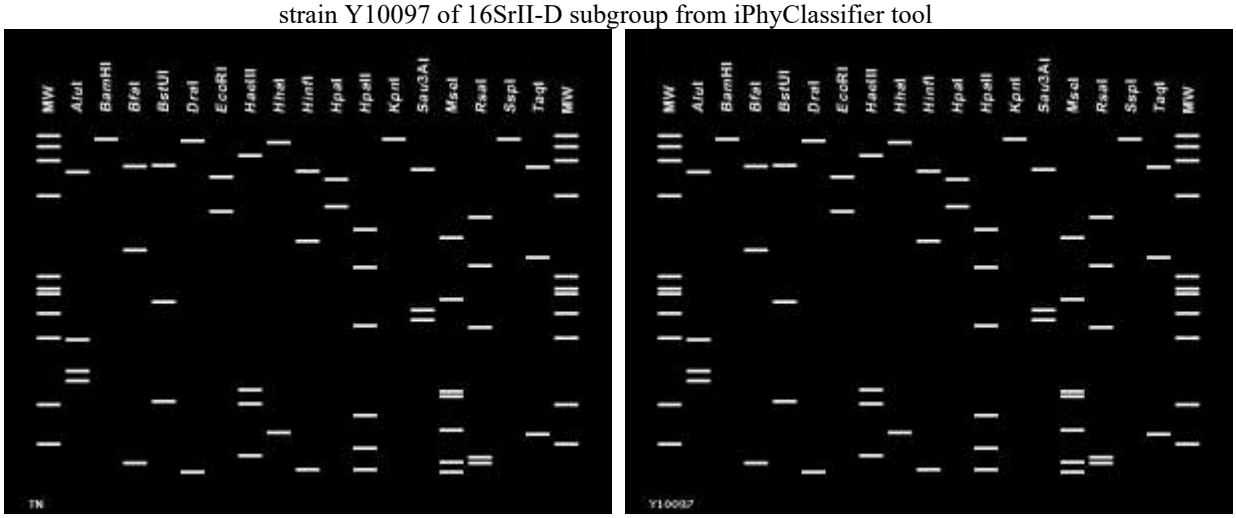
Molecular characterization of sesame phytoplasmas

PCR analysis of 44 symptomatic sesame samples was carried out on 16S rRNA, *secA* and *imp* genes. All the samples showed amplification for the universal primers, P1/P7 followed by nested PCR with R16F2n/R16R2 (~1250 bp) primers which confirms the presence of phytoplasmas in the collected samples. On the other hand, the asymptomatic samples showed no amplification for the universal primers in nested PCR. Further, to amplify the *secA* gene, *SecA*for1/ *SecA*rev3 semi-nested with *SecA*for2/*SecA*rev3 primer set were used and all the samples were amplified at an expected amplicon size of ~480 bp. Amplification of *imp* gene using the primers, ImpF/ImpR yielded an amplicon size of ~420 bp for all the samples.

16S rRNA, *secA* and *imp* genes sequence analysis

Sequence analysis of the 16S rRNA, *secA*, and *imp* genes from all 44 samples revealed >98% identity with '*Candidatus* phytoplasma australasiaticum' sequences available in the NCBI database (Table 3). Virtual RFLP analysis of the 16S rRNA gene sequences using the *i*PhyClassifier online tool assigned all the phytoplasma isolates to 16SrII-D subgroup ('*Ca.* Phytoplasma australasiaticum'). All the isolates generated identical virtual restriction profiles consistent with the reference strain associated papaya yellow crinkle disease (GenBank accession number Y10097) with similarity coefficients of ≥0.97 (Fig. 2).

Figure 2: Restriction profiles of **(a)** TN isolates from the present study and **(b)** phytoplasma representative strain Y10097 of 16SrII-D subgroup from iPhyClassifier tool



(a) **(b)**

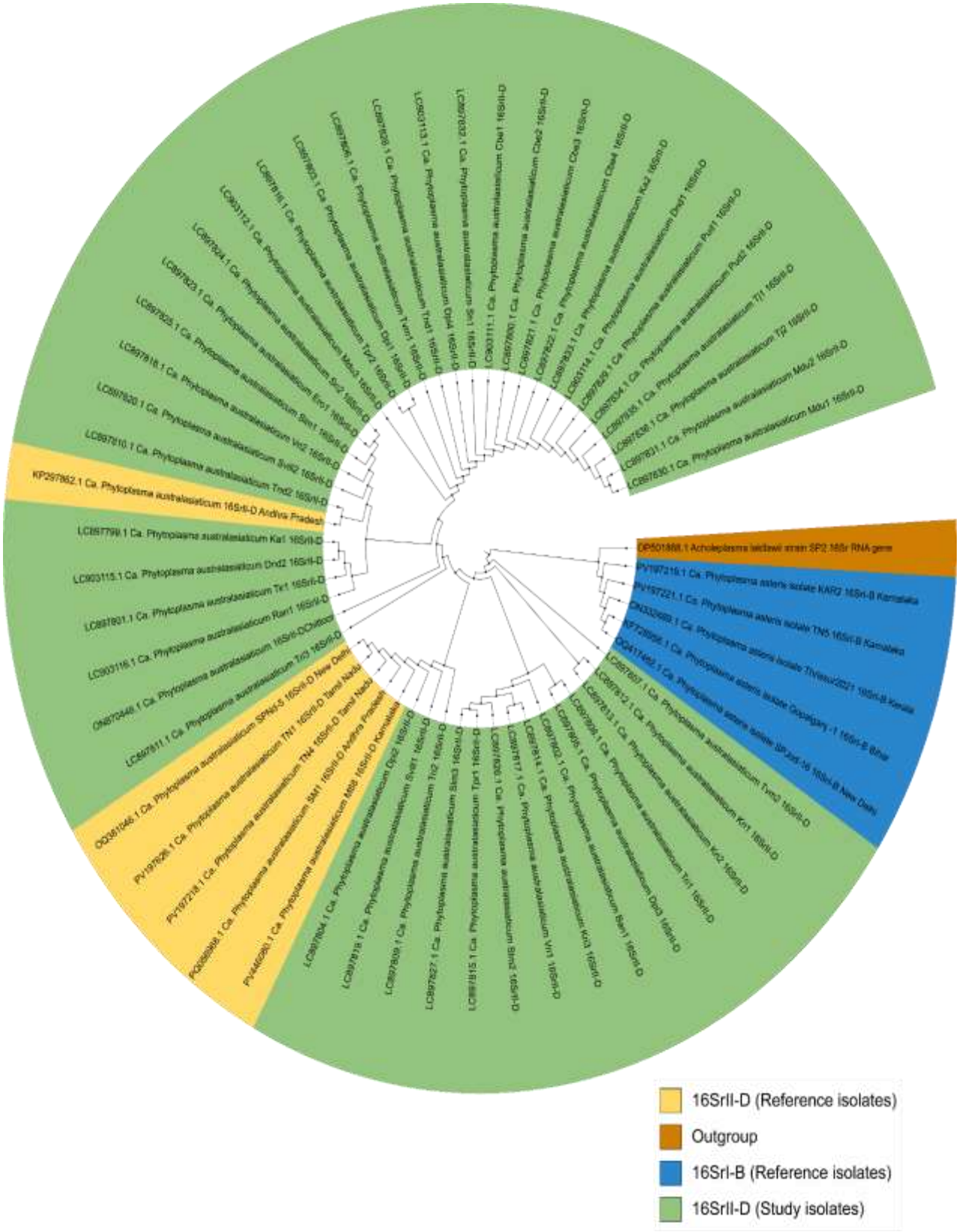
Phylogenetic analysis

In the phylogenetic analysis of 16S rRNA, all 44 phytoplasma isolates (GenBank accession numbers PV197218-PV197626) obtained in the present study clustered within the 16SrII-D group together with reference isolates of '*Ca.* Phytoplasma australasiaticum' reported from different regions. The study isolates showed close phylogenetic relationships with sesame phytoplasmas previously reported from Tamil Nadu, Hyderabad, Chittoor, Karnataka, New Delhi and Andhra Pradesh. The reference phytoplasma sequences belonging to 16SrI-B subgroup formed a distinct cluster and *Acholeplasma laidlawii* was included as outgroup. The clustering of all the study isolates with the 16SrII-D reference sequences was consistent with the results obtained using the *i*PhyClassifier online tool, further confirming the identity of the study isolates as '*Ca.* Phytoplasma australasiaticum' (Fig. 3).

Phylogenetic analysis based on *secA* and *imp* gene sequences were carried out to further resolve the relationships among the phytoplasma isolates. In the phylogenetic analysis of *secA* gene, all the isolates obtained from the present study clustered within 16SrII group together with reference phytoplasma sequences retrieved from NCBI GenBank database. The study isolates showed close relationship with 16SrII sequences reported from Tamil Nadu, Rajasthan, Tirupati, Thailand, Gujarat, Uttar Pradesh and Raipur. The reference sequences belonging to 16SrI group of phytoplasmas formed a separate distinct cluster. The *secA* sequences of '*Ca*. Phytoplasma asteris' and '*Ca.* Phytoplasma mali' belonging to 16SrI and 16SrX groups, respectively, were positioned distantly from the 16SrII group of phytoplasmas and was included as outgroups (Fig. 4).

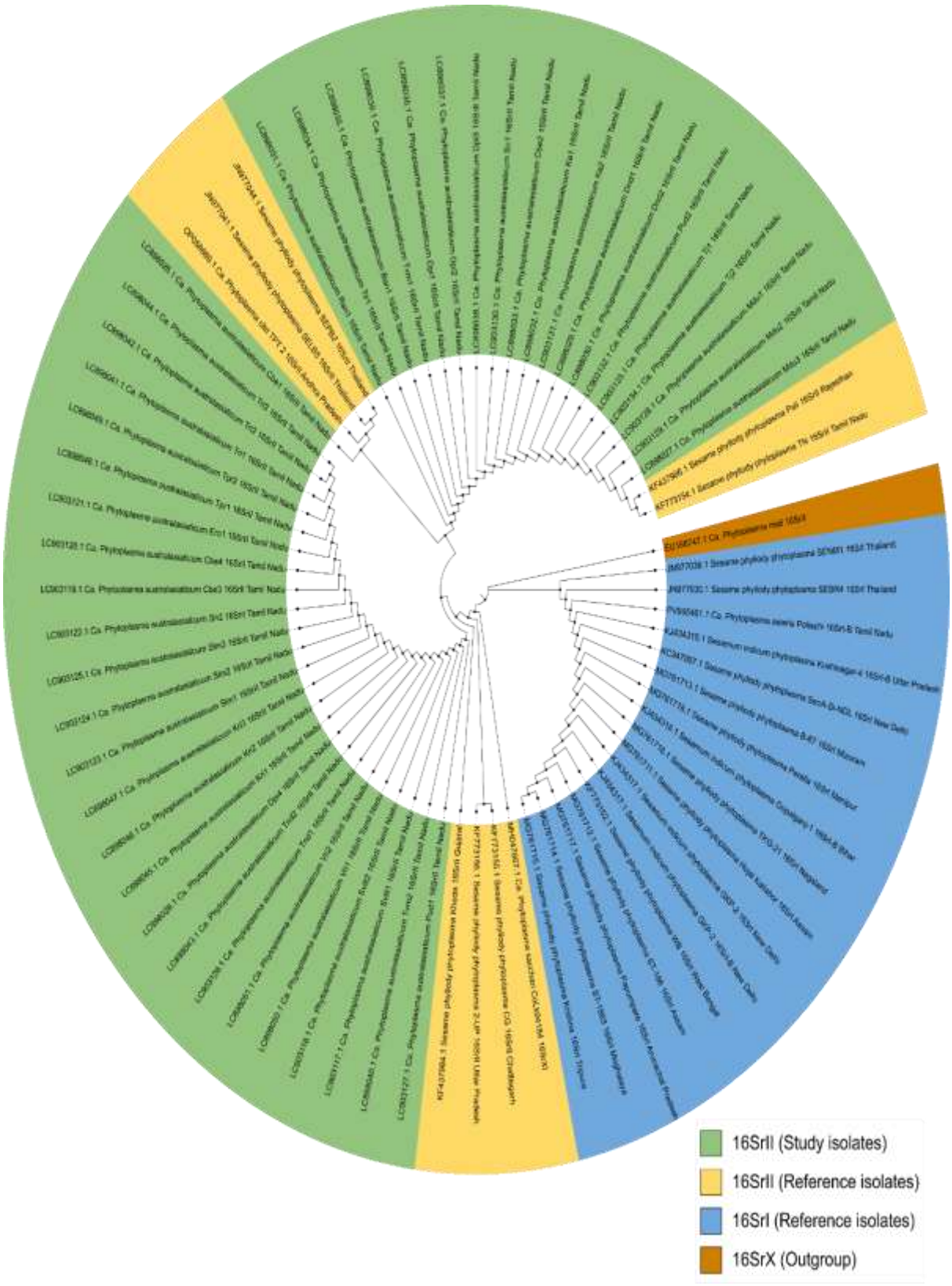
Further confirmation was obtained through phylogenetic analysis of the *imp* gene. All the sequences obtained from the present study clustered within the 16SrII-D subgroup together with reference phytoplasmas associated with mango, chickpea, *Corynandra viscosa*, *Parthenium hysterophorus*, alfalfa, papaya, bitter gourd, *Cyanthillium cinereum* and chayote retrieved from NCBI database. Reference sequences belonging to 16SrII-A and 16SrII-B subgroups formed separate clusters from the study isolates, while the reference sequence belonging to 16SrIII was included as an outgroup (Fig. 5). The phylogenetic analyses showed that the study isolates exhibited minimal sequence divergence and consistently clustered together in the 16S rRNA, *secA* and *imp* gene phylogenies.

Figure 3: Phylogenetic analysis of the 16S rRNA region of phytoplasmas infecting sesame



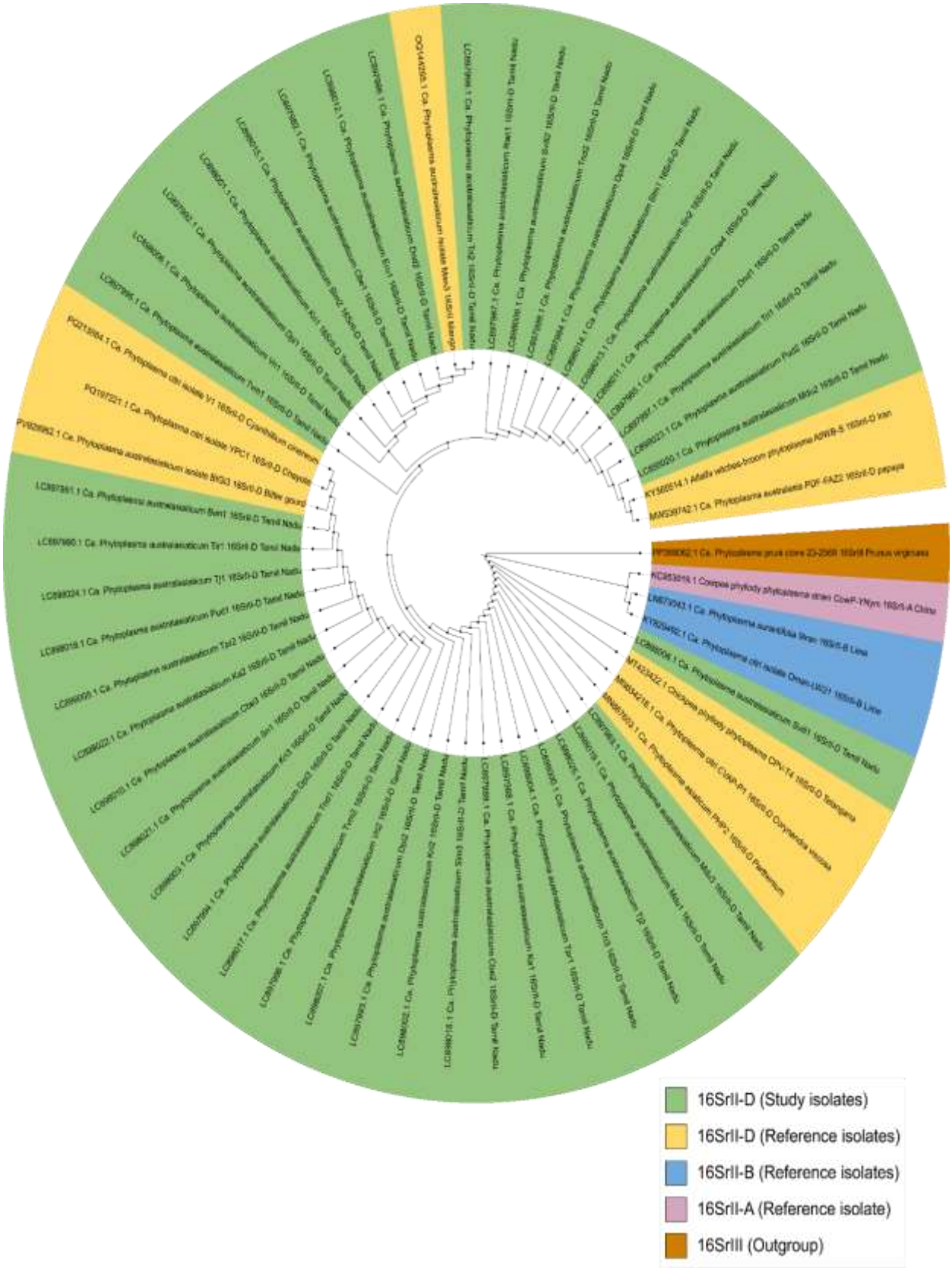
Phylogenetic analysis performed using the neighbour-joining method with evolutionary distances computed by the maximum composite likehood method. Bootstrap support values were calculated with 1000 bootstrap replicates. Sequences belonging to 16SrI group and *Acholeplasma laidlawii* sequences were included as outgroups

Figure 4: Phylogenetic analysis of phytoplasmas infecting sesame based on *SecA* gene sequences



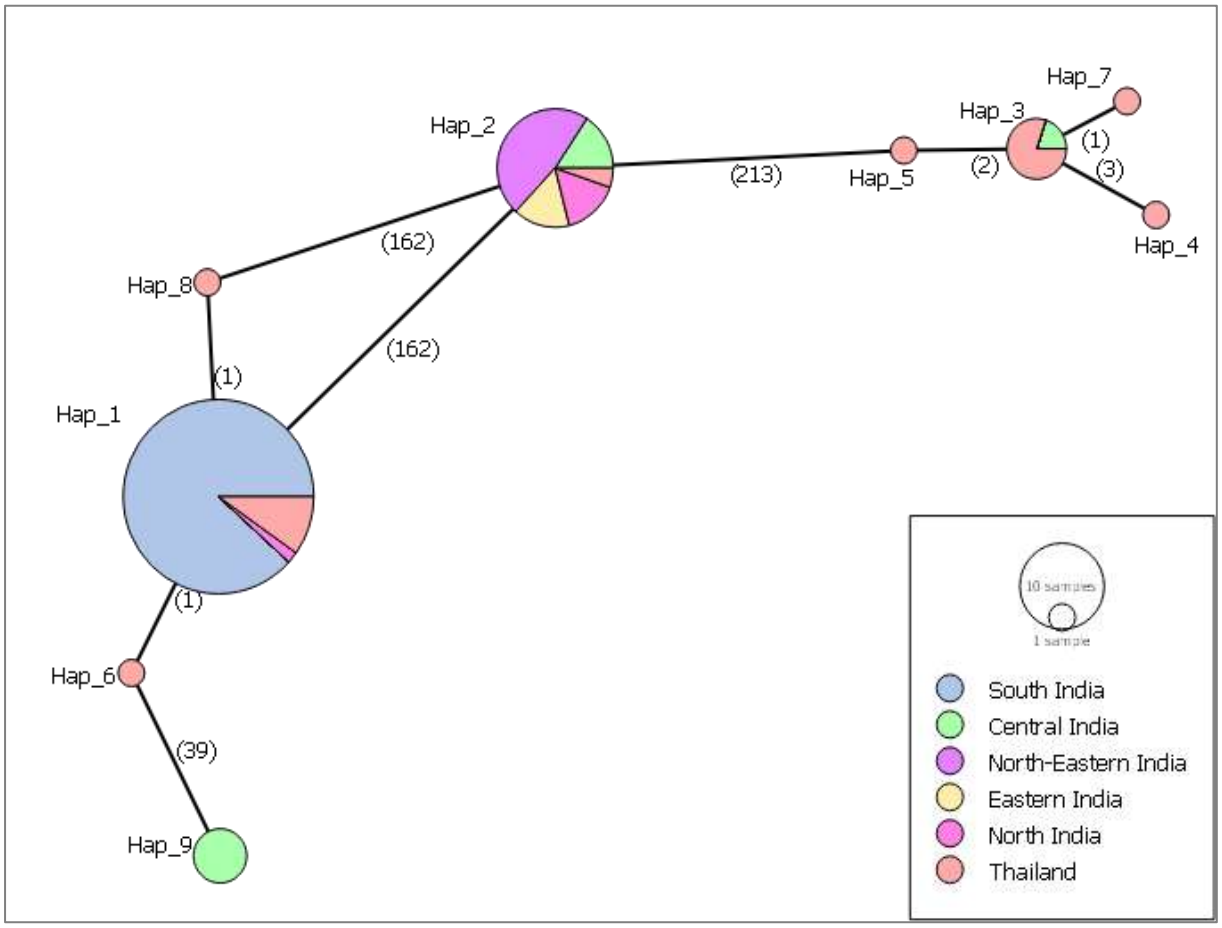
Phylogenetic analysis performed using the neighbour-joining method with evolutionary distances computed by the tamura-3 parameter substitution model. Bootstrap support values were calculated with 1000 bootstrap replicates. Sequences belonging to 16SrI group and 16SrX sequences were included as outgroups

Figure 5: Phylogenetic analysis of phytoplasmas infecting sesame based on *imp* gene sequences



Phylogenetic analysis performed using the neighbour-joining method with evolutionary distances computed by the tamura-3 parameter substitution model. Bootstrap support values were calculated with 1000 bootstrap replicates. Sequences belonging to 16SrIII sequence was included as outgroup

Figure 6: Minimum spanning haplotype network constructed based on *SecA* gene sequences of phytoplasmas-infecting sesame



Each circle represents unique haplotype (n=9) and the size of the circles corresponds to the number of sequences per haplotype. Each colour represents different geographic region of the samples. Lines connecting each haplotype indicate mutational steps.

Minimum spanning haplotype network

A minimum spanning haplotype network was constructed using the *secA* sequences obtained in the present study along with representative sequences retrieved from the NCBI GenBank database (Table 5&6). Nine haplotypes (Hap_1 to Hap_9) were identified. The size of each node represents the number of sequences in a haplotype, while the colours in each node represent samples from different geographical regions (Fig. 6). Haplotype 1 was the most common, comprising 51 sequences and was mainly represented by isolates from South India (Tamil Nadu) along with a few sequences from other regions and Thailand. Haplotype 2 was the second largest haplotype with 19 sequences from North India, Central India, Eastern India, and widely from North-Eastern states (Table 6).

Haplotype 3 contained five sequences, mainly from Thailand along with one sequence from Central India. Haplotype 4 to haplotype 8 were represented by singletons from Thailand, forming peripheral branches in the network which indicates additional variation among the Thailand isolates. Haplotype 9 encompassed four sequences, all originating from central India, from the states of Uttar Pradesh, Chhattisgarh and Madhya Pradesh (Table 6).

The network also showed clear differences in the number of mutational steps among the haplotypes. The close relationship between each haplotype is explained by the number of mutational steps between them. Hap_6 and Hap_8 were closely related to Hap_1 (1 step), while Hap_9 was separated from Hap_6 by 39 steps. Similarly, Hap_7 (1 step), Hap_5

(2 steps) and Hap_4 (3 steps) were closely associated with Hap_3. Hap_1 and Hap_2 were separated by 162 mutational steps, while Hap_2 and Hap_5 were separated by 213 steps, indicating greater genetic differentiation among the major haplotype groups. The network showed the predominance of H1 and H2, together with several less frequent and singleton haplotypes. The occurrence of shared haplotypes across geographically distant regions indicated close genetic relationships among some phytoplasma strains, whereas the large number of mutational steps separating some haplotypes revealed considerable genetic differentiation within the overall dataset.

Population genetics and demographic analysis

Population genetic analysis of the *secA* gene included 84 sesame-associated phytoplasma sequences. The dataset contained a total of 287 polymorphic sites and 368 mutations from all the sequences. Nucleotide diversity (π) varied among geographical populations and was highest in Thailand (0.32995), followed by Central India (0.31410) and North India (0.19773). South India showed very low nucleotide diversity (0.00218), while no nucleotide variation was observed in the North-Eastern and Eastern Indian populations. The average number of pairwise nucleotide differences (k) was highest in Thailand (151.419), followed by Central India (134.750) and North India (87.000) (Table 7).

Haplotype diversity (Hd) was highest in Central India (0.893), followed by Thailand (0.848), South India (0.529) and North India (0.500). Thailand contained the highest number of haplotypes (8), followed by Central India (5), South India (3) and North India (2). North-Eastern and Eastern India were each represented by a single haplotype and showed no haplotype diversity.

Neutrality tests also varied among the populations. Tajima's D was positive in South India (1.81614), Central India (1.03320) and Thailand (2.47667), with significant positive values recorded for Thailand and the overall dataset (2.12433). North India showed a negative but non-significant Tajima's D value (−0.87377). Fu's Fs was positive in all polymorphic populations, with values of 0.433, 10.983, 11.674 and 17.415 for South India, Central India, North India and Thailand, respectively. Fu and Li's D was significantly negative in North India, whereas the overall dataset showed significant positive values for both Fu and Li's D and Fu and Li's F. The raggedness index ranged from 0.1263 in Central India to 0.7500 in North India (Table 7). Considering all the neutrality tests across the dataset, there is no strong evidence for sudden population expansion, because of the positive Fu's F and Tajima's D and low R2 values. But, the results may imply population subdivision, population bottlenecks or balancing selection shaping population genetic structure of phytoplasma across the analysed sequences.

Table 5: List of all *SecA* gene sequences of sesame phytoplasma retrieved from the NCBI GenBank database for diversity analysis

Sl. No.	NCBI accession number	State	Country	Region in India	Year	References
1.	KF773154	Tamil Nadu (TN)	India	South India	2014	Priya,M. and Rao,G.P. (Unpublished)
2.	KT335272	Uttar Pradesh (UP)		Central India	2016	Singh,M. and Rao,R.P. (Unpublished)
3.	KF773156, KF773153, KF437987				2014	Priya,M. and Rao,G.P. (Unpublished)
4.	KJ434315, KJ434314				2014	Nabi et al., 2015
5.	KF773155	Chattisgarh (CG)			2014	Priya,M. and Rao,G.P. (Unpublished)
6.	KF437985	Madhya Pradesh (MP)			2013	
7.	KC347007	New Delhi (NDL)		North India	2013	Priya,M., Rao,G.P. and Kumar,A. (Unpublished)
8.	KJ434316, KJ434317				2014	Nabi et al., 2015
9.	KF437986	Rajasthan (RJ)			2013	Priya,M. and Rao,G.P. (Unpublished)
10.	KJ434319, KJ434318	Bihar (BR)		Eastern India	2014	Nabi et al., 2015
11.	KF773152	West Bengal (WB)			2014	Priya,M. and Rao,G.P. (Unpublished)
12.	MG761718	Manipur (MN)		North-eastern India	2018	Priya,M., Gogoi,A.K., Kalita,M.K., Rao,G.P. and Baranwal,V.K. (Unpublished)
13.	MG761717	Arunachal Pradesh (AR)				
14.	MG761716	Nagaland (NL)				
15.	MG761715	South Tripura (TR)				
16.	MG761714	Meghalaya (ML)				
17.	MG761713	Mizoram (MZ)				

18.	MG761712, MG761711, MG761710	Assam				
19.	JN977030, JN977031, JN977032, JN977033, JN977034, JN977035, JN977036, JN977037, JN977038, JN977039, JN977040, JN977041, JN977042, JN977043, JN977044	-	Thailand	-	2012	Klinkong,S., Reanwarakorn,K. and Panthong,N. (Unpublished)

Table 6: Various haplotypes of sesame phytoplasma identified in the present study based on *SecA* gene

Sl. No.	Haplotype	Number of sequences	GenBank accession (Region/country)
1.	Hap_1	51	LC898031, LC898034, LC898035, LC898039, LC898040, LC903117, LC903118, LC898050, LC898051, LC903126, LC898043, LC898036, LC898037, LC898038, LC898028, LC898045, LC898046, LC898047, LC903123, LC903124, LC903125, LC903130, LC903122, LC898026, LC898033, LC903119, LC903120, LC903121, LC898032, LC903131, LC898029, LC898030, LC898048, LC898049, LC898041, LC898042, LC898044, LC903127, LC903132, LC903133, LC903134, LC903128, LC903129, LC898027, KF773154 (TN, South India) KF437986 (RJ, North India) JN977043, JN977042, JN977040, JN977044, JN977041 (Thailand)
2.	Hap_2	19	KC347007, KJ434317, KJ434316 (NDL, North India) KF773153, KF437987, KJ434315 (UP, Central India) KF773152 (WB, Eastern India) KJ434319, KJ434318 (BR, Eastern India) MG761718 (MN), MG761717 (AR), MG761716 (NL), MG761715 (TR), MG761714 (ML), MG761713 (MZ), MG761712, MG761711, MG761710 (AS) (North Eastern India) JN977037 (Thailand)
3.	Hap_3	5	KT335272 (UP, Central India) JN977035, JN977034, JN977033, JN977032 (Thailand)
4.	Hap_4	1	JN977031 (Thailand)
5.	Hap_5	1	JN977036 (Thailand)
6.	Hap_6	1	JN977039 (Thailand)
7.	Hap_7	1	JN977030 (Thailand)
8.	Hap_8	1	JN977038 (Thailand)
9.	Hap_9	4	KF773156, KJ434314 (UP, Central India) KF773155 (CG, Central India) KF437985 (MP, Central India)

Table 7: Genetic diversity parameters and neutrality tests of sesame phytoplasma based on *SecA* gene

Sl. No.	Name of statistical data	South India	Central India	North-eastern India	Eastern India	North India	Thailand	Total data estimates
1.	Number of sequences (n)	45	8	9	3	4	15	84
2.	Number of segregating sites (S)	0	282	0	0	162	278	287
3.	Nucleotide diversity, Pi	0.00218	0.31410	0.0000	0.0000	0.19773	0.32995	0.22774
4.	Average number of pairwise nucleotide differences, k	0.529	134.750	0.000	0.0000	87.000	151.419	92.918

5.	Number of haplotypes (h)	3	5	1	1	2	8	9
6.	Haplotype diversity (Hd)	0.529	0.893	0.0000	0.0000	0.500	0.848	0.581
7.	Tajima's D	1.81614*^{NS}	1.03320**^{NS}	NP	NA	-0.87377**^{NS}	2.47667**^{S}	2.12433*^{S}
8.	Ramos-Onsins and Rozas' R2	0.1319	0.1335	NP	NP	0.1341	0.1314	0.1313
9.	Raggedness statistic r	0.2836	0.1263	NP	NP	0.7500	0.1351	0.3388
10.	Average number of differences, K	0.0000	129.928	0.0000	0.0000	81.000	134.61905	92.91796
11.	Number of polymorphic (segregating) sites, S	1	294	0	0	174	316	287
12.	Total number of mutations, Eta	2	357	0	0	174	387	368
13.	Total number of singleton mutations, Eta(s)	1	182	0	NA	NA	123	6
14.	Variance of haplotype diversity	0.00084	0.00736	0.0000	0.0000	0.07031	0.00508	0.00261
15.	Fu Fs statistic	0.433	10.983	NP	NA	11.674	17.415	85.555
16.	Strobeck's S statistic	0.684	0.001	NP	NA	0.001	0.000	0.000
17.	Fu and Li D test statistic FLD	-1.80985**^{NS}	-0.67961**^{NS}	0.0000**^{NS}	NA	-0.87377***^{S}	-0.31474**^{NS}	2.25571***^{S}
18.	Fu and Li F test statistic FLF (P > 0.10)	-0.89432**^{NS}	-0.31055**^{NS}	0.0000**^{NS}	NA	-0.93972**^{NS}	0.54251**^{NS}	2.64102***^{S}

NS, not significant; *, 0.05<P<0.10; **, P>0.10; **S**, significant; *, P<0.05; **, P<0.01; ***,P<0.02; **NP** – No polymorphism; **NA** – No analysis due to less sequences.

Gene flow and genetic differentiation indices of sesame phytoplasmas

Pairwise genetic differentiation analysis was performed on pairwise comparisons in DnaSPv6. The analysis revealed significant differences among the phytoplasmas infecting sesame from different geographical regions. South India showed strong differentiation from Central India (Nst = 0.34503; Fst = 0.40874), with Dxy and Da values of 0.26930 and 0.11008, respectively. Also, high genetic differentiation and divergence were observed between South India and both Eastern and North-Eastern India, with Nst and Fst values of 1.000 and Dxy and Da values of 0.39706 (Table 8).

The comparison between the isolates of South and North India revealed considerable genetic divergence (Dxy=0.29779; Da=0.19853) and very high genetic differentiation (Nst=0.66667; Fst=0.66667) while the differentiation with Thailand was comparatively moderate (Nst = 0.44233; Fst = 0.43846). Among the other populations, Central India and North India showed the lowest differentiation (Nst = 0.01863; Fst = 0.04907; Dxy = 0.27183; Da = 0.01334), indicating a close genetic relationship. Central India and Thailand also showed

relatively low differentiation (Fst = 0.13462), whereas Eastern and North-Eastern Indian populations showed greater differentiation from Thailand (Fst = 0.61683). Based on the Fst values, very low or no differentiation was observed between Eastern India and North India, North India and North-Eastern India, and Eastern India and North-Eastern India; however, these populations showed little or no within-population variation (Table 8).

Table 8: Gene flow and genetic differentiation indices of sesame phytoplasma among population

POPULATION 1	POPULATION 2	Hs	Ks	Kxy	Gst	DeltaSt	Gamma St	Nst	Fst	Dxy	Da
South India	Central India	0.08309	19.61186	109.87500	0.62566	0.03332	0.44200	0.34503	0.40874	0.26930	0.11008
South India	Eastern India	0.00000	0.00000	162.00000	1.00000	0.04653	1.00000	1.00000	1.00000	0.39706	0.39706
South India	North India	0.02222	6.61225	121.50000	0.65331	0.03349	0.73370	0.66667	0.66667	0.29779	0.19853
South India	North-eastern India	0.00000	0.00000	162.00000	1.00000	0.11029	1.00000	1.00000	1.00000	0.39706	0.39706
South India	Thailand	0.19677	33.65476	119.86670	0.30330	0.05243	0.40512	0.44233	0.43846	0.29379	0.12882
Central India	Eastern India	0.58163	94.49351	111.25000	0.20213	0.05290	0.20700	0.36897	0.41605	0.27267	0.11345
Central India	North India	0.63393	113.61900	110.90630	0.09368	0.02580	0.09879	0.01863	0.04907	0.27183	0.01334
Central India	North-eastern India	0.31319	61.14286	111.25000	0.30845	0.06644	0.33630	0.36897	0.41605	0.27267	0.11345
Central India	Thailand	0.79424	132.98760	152.85000	0.09677	0.03690	0.11026	0.12975	0.13462	0.37463	0.05043
Eastern India	North India	0.33333	46.28571	40.50000	0.00297	0.01215	0.12500	0.00000	0.00000	0.09926	0.00000
Eastern India	North-eastern India	0.00000	0.00000	0.00000	1.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
Eastern India	Thailand	0.78707	112.18250	175.66670	0.19667	0.07683	0.23040	0.55956	0.61683	0.43056	0.26558
North India	North-eastern India	0.11111	24.92308	40.50000	0.01318	0.01057	0.18750	0.00000	0.00000	0.09926	0.00000
North India	Thailand	0.80127	123.33080	161.71670	0.09854	0.05582	0.16902	0.31765	0.33334	0.39636	0.13213
North eastern India	Thailand	0.55095	84.13690	175.66670	0.31206	0.12965	0.40248	0.55956	0.61683	0.43056	0.26558

Note: Hs – Genetic diversity within populations; **Ks** – Average number of nucleotide substitutions within populations; **Kxy** - Average proportion of nucleotide differences between populations; **Gst** - Genetic differentiation index based on the frequency of haplotypes; **DeltaSt –** Absolute genetic differentiation index; **GammaSt** – Standardized genetic differentiation Index; **Nst –** Nucleotide differentiation statistic; **FST** - Wright's F-statistics, pairwise genetic distance; **Dxy** - The average number of nucleotide substitutions per site between populations; **Da** - Number of net nucleotide substitutions per site between populations; **NS** - not significant ($p < 0.05$).

AMOVA further showed that 67.2% of the total genetic variation occurred among populations and 32.8% was distributed within populations. The variance components were 0.1167 among populations and 0.0569 within populations. The overall F_{ST} value was high (0.6721) and statistically significant ($p < 0.001$), indicating strong genetic differentiation among the geographical populations (Table 9). The pairwise genetic differentiation and AMOVA results indicated geographical structuring among phytoplasma populations infecting sesame. The South Indian isolates were more differentiated from the Eastern, North-Eastern and North Indian populations, whereas closer genetic relationships were observed between Central India and North India and between Central India and Thailand.

Collectively, the gene flow and genetic differentiation analysis of the sesame phytoplasma demonstrated that the South Indian (Tamil Nadu) phytoplasma population was genetically distinct from all other populations, with greater divergence observed against Eastern and North-eastern India populations. Furthermore, the consistantly high Fst, Gst and Nst values shows the strong population structure and minimal gene flow among the studied geographic populations.

Table 9: AMOVA analysis of population-genetic structure of *Candidatus* Phytoplasma infecting sesame based on the *secA* gene

Source of variation	Degree of freedom (df)	Sum of squares (SSD)	Variance components	Percentage of variation	Phi-statistic (F_{ST})	*p*-value
Among populations	5	6.690	0.1167	67.2	0.6721	<0.001

Within populations	78	4.441	0.0569	32.8		
Total	83	11.132	0.1737			

Discussion

A survey conducted across 20 districts covering five agro-climatic zones showed a widespread prevalence of phyllody disease in Tamil Nadu. Disease incidence ranged from 5 to 87% in the surveyed locations in Tamil Nadu and varied considerably in different locations and crop growth stages. Earlier studies also reported varied sesame phyllody incidences, ranging from 12–35% and 15–35% (Nabi et al., 2015; Un Nabi et al., 2015) in North India , 49-68% in South India (Thirumalaisamy et al., 2023) and up to 100% in Iran (Salehi et al., 2017). Severe phytoplasma infection in sesame has also been associated with yield losses reaching 100% under highly favourable disease conditions. Also, previous reports suggest the phytoplasma incidence in sesame can impose upto 100% yield loss in severe stages (Gogoi et al., 2017; Venkataravanappa et al., 2017).

In the present study, the lowest disease incidence was observed during early flowering (5-16%) and increased gradually during flowering (18-38%), capsule formation (33-62%) and maturity (45-87%). This pattern clearly indicates a stage-wise increase in disease incidence as the crop reached maturity. The higher disease incidence recorded at maturity may reflect that the pathogen established during the earlier growth stages, exhibited more pronounced symptoms as the crop reached maturity.

The symptoms observed during the survey were similar to those reported earlier in sesame and other oilseed crops, particularly, phyllody, witches' broom and virescence (Bertaccini and Duduk, 2009; Un Nabi et al., 2015; Thirumalaisamy et al., 2023; Bhaskar et al., 2025). The presence of these symptoms across the surveyed locations was similar to those reported earlier for sesame phyllody.

Molecular detection of phytoplasma in the symptomatic sesame samples using multiple markers such as 16S rRNA, *secA* and *imp,* provided strong evidence for the association of '*Candidatus* Phytoplasma australasiaticum' with sesame phyllody in Tamil Nadu. Further, the use of additional markers, including *secA* and *imp* strengthened the molecular characterization, overcoming the limitations of 16S rRNA gene sequences in detecting variability among groups (Al-Abadi et al., 2016). Virtual RFLP analysis of the 16S rRNA gene sequences using *i*PhyClassifier confirmed the presence of 16SrII-D subgroup across all surveyed locations indicating a consistent phytoplasma population associated with sesame phyllody in Tamil Nadu.

Globally, sesame-infecting phytoplasmas have been reported from several 16Sr groups and subgroups, showing considerable diversity in the phytoplasma populations associated with sesame phyllody. These include 16SrVI in Turkey (Sertkaya et al., 2007), 16SrI-B in Myanmar (Win et al., 2010), 16SrII-A group in Taiwan (Tseng et al., 2014), 16SrII-D and 16SrIX-C subgroups in Turkey (Ikten et al., 2014), 16SrII-D, 16SrVI-A, and 16SrIX-C subgroups in Iran (Salehi et al., 2017), 16SrII-A and 16SrVI-A subgroups in southeastern Iran (Asghari Tazehkand et al., 2017), 16SrI-B in Paraguay (Ganem et al., 2019), and group 16SrII in Egypt (Ahmed et al., 2022; Mohamed et al., 2024). The 16SrII-D subgroup has also been reported in sesame from Oman (Khan et al., 2007), supporting its wider geographical distribution.

Previous studies have reported that phytoplasmas-infecting sesame in India have been associated with multiple groups, including 16SrI-B and 16SrII-C subgroups from Uttar Pradesh (Nabi et al., 2015) and 16SrI-B subgroup from West Bengal, Bihar and Delhi (Nabi et al., 2015), 16SrI group from Karnataka and North-Eastern India (Manjunatha et al., 2012) and 16SrII-C from Uttar Pradesh, Madhya Pradesh, Chhattisgarh and Rajasthan (Rao and Nabi, 2015). Furthermore, 16SrII-D has been reported from Telangana, Haryana province, and Andhra Pradesh (Suhas et al., 2025); 16SrII-A and 16SrII-D subgroups from Telangana, Karnataka and Maharashtra (Devanna et al., 2020). These reports show that sesame phyllody is associated with several phytoplasma groups; however, 16SrII, particularly 16SrII-D, occur frequently in India and other countries, supporting the present findings from Tamil Nadu.

Phylogenetic analysis based on 16S rRNA, *secA* and *imp* gene sequences showed consistent clustering pattern of all the study isolates within the 16SrII-D subgroup. Other groups like 16SrII-C and 16SrI-B formed distinct clades separate from the 16SrII-D isolates. A uniform clustering of 16SrII-D sequences was also observed in the *secA* and *imp* phylogenies. As 16S rRNA is highly conserved, the additional markers such as *secA* and *imp,* helped to distinguish closely related phytoplasma strains more clearly. A similar multilocus approach using 16S rRNA, *secA* and *imp* markers was used by Misra et al. (2024) for studying the diversity and genetic relationships of *Ca.* Phytoplasma aurantifolia isolates infecting various crops. The consistent clustering across all three gene phylogenies along with virtual RFLP analysis further supports the placement of the Tamil Nadu isolates within the 16SrII-D subgroup.

The *secA*-based haplotype diversity analysis revealed nine haplotypes among the phytoplasma sequences. Hap_1 and Hap_2 were the major haplotypes and were distributed across different geographical regions. Hap_1 included a diverse set of sequences mainly from South India and a few sequences from across North India and Thailand, whereas hap_2 included sequences from North, Central, Eastern, North-eastern and western zones of India and only one sequence from Thailand. Hap_3 mainly comprised sequences from Thailand and Central India, while Hap_9 was restricted to Central India. The occurrence of common haplotypes across different geographical regions indicates close genetic relatedness among the phytoplasma populations. Such widespread distribution may be influenced by movement of insect vectors, availability of favourable hosts and possible exchange of infected

planting materials (Misra et al., 2024). On the other hand, Hap_4 to Hap_8 exclusively represented singleton sequences from Thailand, indicating higher haplotype variation among the Thailand isolates included in the analysis.

Pilet et al. (2019) reported geographically distinct haplotypes of '*Ca.* Phytoplasma palmicola' across African countries, while Kong et al. (2022) observed both widely distributed and region-specific haplotypes in Paulownia witches'-broom phytoplasma populations in China. Mehle et al. (2022) also identified several multilocus haplotypes of '*Ca.* Phytoplasma solani' that differed in their geographical distribution. In contrast, palm lethal decline phytoplasmas in Florida showed very low genetic variation, with a single dominant haplotype over several years (Soto et al., 2021). Similar results were observed in the present study, where most of the Tamil Nadu isolates were grouped within Hap_1, suggesting a relatively homogeneous phytoplasma population with limited sequence variation and low genetic diversity in the region.

The *secA*-based population genetic analysis showed apparent differences in diversity among the geographical populations. Haplotype diversity was highest in Central India, whereas nucleotide diversity was highest in Thailand, whereas Eastern and North-Eastern India showed no detectable polymorphism in the analysed sequences. South India showed very low nucleotide diversity ($\pi = 0.00218$), indicating a largely conserved phytoplasma population with limited sequence variation. Similar geographical structuring of phytoplasma populations has been reported in '*Ca.* Phytoplasma palmicola', Paulownia witches'-broom phytoplasma and '*Ca.* P. solani', where genetic diversity differed considerably among regions (Pilet et al., 2019; Kong et al., 2022; Mehle et al., 2022). The low diversity in South India was also reflected by the dominance of a common haplotype, indicating a closely related phytoplasma population in this region.

The neutrality tests did not provide strong evidence for recent population expansion in most regions. Tajima's D was positive in South India, Central India and Thailand and Fu's Fs was also positive in the polymorphic populations. In South India, Fu and Li's D and F values were negative but not significant, which was not strong enough to support recent population expansion or selection. Although some regions showed low R2 and raggedness values, the overall results suggest stable or geographically structured phytoplasma populations rather than recent population expansion. Similar patterns were also reported in other phytoplasma populations of *Ca.* Phytoplasma palmicola in Mozambique and Africa (Bila et al., 2015; Pilet et al., 2019). The absence of genetic variability in Eastern and North-Eastern India should, however, be interpreted cautiously because of the limited number of sequences available from these regions.

The pairwise genetic differentiation analysis showed clear differences among sesame-associated phytoplasma populations from different geographical regions. South India showed strong differentiation from Eastern and North-Eastern India, with Fst values of 1.00, followed by North India (0.67), Thailand (0.44) and Central India (0.41). The higher Dxy and Da values in these comparisons also indicated greater genetic distance between the populations belonging to different geographical regions. In contrast, Central and North India showed low differentiation (Fst = 0.049), while Central India and Thailand also showed comparatively lower differentiation (Fst = 0.135), suggesting closer genetic relationships. The South Indian population which consists of the study isolates showed strong genetic differentiation from most other regions, suggesting that the phytoplasma population in South India is comparatively distinct. Similarly, Pilet et al. (2019) reported three geographically distinct populations of '*Ca.* Phytoplasma palmicola' across Africa, while Kong et al. (2022) observed clear regional differences among paulownia witches'-broom phytoplasma populations in China. These findings support the geographical differentiation observed among the sesame phytoplasma populations in the present study.

AMOVA further supported the population differentiation, with 67.2% of the genetic variation occurring among populations and 32.8% within populations. The higher variation among populations indicates clear genetic differentiation across geographical regions. In Tamil Nadu, sesame-infecting phytoplasmas showed low sequence variation and were mainly represented by a common haplotype, indicating a genetically similar population within South India. Similar population structure has been reported in other phytoplasma populations. Al-Abadi et al. (2016) reported low genetic differentiation among '*Ca.* Phytoplasma aurantifolia' populations associated with witches' broom disease of lime, whereas Pilet et al. (2019) observed clear genetic differences among geographical regions in '*Ca.* P. palmicola' causing coconut lethal yellowing disease in Africa. Rossi et al. (2019) also reported genetic variation in flavescence dorée phytoplasmas associated with grapevine yellows.

Conclusion

The current study offers deeper understanding on the distribution and genetic diversity of the phytoplasma populations infecting sesame in Tamil Nadu state of India. Survey across different district of Tamil Nadu revealed typical phyllody, witches' broom and virescence symptoms with the highest disease incidence of 87% in Cuddalore district which is an hotspot region for sesame phyllody disease. Molecular characterization and phylogenetic analysis of the collected samples using 16S rRNA, *secA* and *imp* genes confirmed that all the sequences belonged to '*Ca.* P. australasiaticum'. Further, the minimum spanning haplotype network based on *secA* gene revealed nine distinct haplotypes with Haplotype 1 comprises all the South Indian strains. Demographic and genetic differentiation analysis demonstrated extermely low genetic diversity and strong differentiation of

South Indian strains when compared with all other populations. This leads to the understanding that the Tamil Nadu strains showed single phytoplasma lineage with moderate genetic flow and very low genetic diversity within populations. Stage dependent increase in disease incidence was also established from the study which stresses the importance of early detection and early management strategies to control the disease. These findings enhance the understanding of sesame phytoplasmas spread and support future surveillence and management efforts in South India.

Author contributions
Eswaran Priyadharshini: Data curation, Formal analysis, Investigation, Methodology, Software, Writing – original draft. **Maddi Sandhya**: Formal analysis, Methodology, Software. **Theerthagiri Anand**: Formal analysis, Validation, Visualization, Writing – review & editing. **Manickam Sudha:** Formal analysis, Validation, Visualization, Writing – review & editing. **Angamuthu Mahalingam**: Formal analysis, Methodology, Software, Writing – review & editing. **Muthurajan Raveendran:** Software, Resources, Validation, Visualization, Writing – review & editing. **Govindasamy Senthilraja**: Conceptualization, Funding acquisition, Investigation, Project administration, Resources, Supervision, Writing – review & editing.

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Data availability
All datasets generated for this study are included in the article.

Declarations
The authors declare there is no conflict of interest.

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