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RESEARCH ARTICLE

Genetic and haplotype analysis of phytoplasma disease on sesame and insights into transmission biology and leafhopper interactions

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Abstract

The predominance of leafhopper species such as *Orosius albicinctus, Hishimonus phycitis* and *Amrasca biguttula biguttula* poses a significant threat to sesame production in India and globally. Phytoplasma disease, caused by *Candidatus Phytoplasma* species, has become a major challenge for sesame farming, with the potential to reduce yields by up to 95 %. First documented in Myanmar, this disease has since spread to various regions worldwide, affecting sesame crops across Asia, Africa and North America. The current study examined the relationship between phytoplasma and leafhoppers in sesame as well as the phylogenetic differences in phyllody phytoplasma. Previous research identified phytoplasma subgroups 16SrI-B and II-C in eastern Uttar Pradesh, Bihar and Delhi, India. Notably, this study discovered the 16SrI-B subgroup in Tamil Nadu for the first time, marking significant progress in understanding phytoplasma diversity in the region. The use of virtual RFLP analysis in sequencing sesame phyllody phytoplasma highlights the importance of molecular techniques in characterizing these phytoplasma. The leafhoppers species complex, including *O. albicinctus, H. phycitis* and *A. bigutulla bigutulla* was identified using species-specific taxonomical traits and a mtCOI gene-based genetic study confirmed their identities. In greenhouse studies on disease transmission efficiency, *O. albicinctus* transmitted phyllody at rates ranging from 7.8 % to 84 %, while *H. phycitis* transmitted it at rates of 5.2 % to 74 %. Both species exhibited the highest transmission efficiency when three insects were placed on a single plant for 5 days of acquisition feeding, followed by one day of inoculation feeding. A comprehensive understanding of leafhopper-phytoplasma interactions through molecular studies supports effective monitoring, early detection and the development of integrated pest management strategies.

Keywords

phytoplasma; transmission; vectors; symptoms; mapping

Introduction

Sesame, *Sesamum indicum* (L.), from the Pedaliaceae family, is regarded as the first oilseed crop in human civilization, cherished for its highly aromatic odor, mellow flavor and nutritional value. Sesame seeds are an excellent source of carbohydrates (4.5–20.5 %), proteins (14.1–29.5 %), fiber (2.7–6.7 %),

vitamins (E-tocopherol and B1-thiamine), minerals and phytosterols (1). The seeds contain approximately 85 % monounsaturated essential fatty acids, primarily oleic and linoleic acids (33–63 %). Sesame oil has a long shelf life due to its high content of lipid-soluble lignans, mainly sesamol, sesamin and sesamolin (2). It is widely cultivated in tropical and subtropical regions, especially in Africa and Asia (3). In 2022, Sudan, India and Myanmar were the top global producers among the 69 countries growing sesame, yielding 12.3, 7.8 and 7.3 MM, respectively (4). However, sesame production in India falls short of meeting demand. Productivity is hindered by issues such as seed bursting, low harvest index, disease susceptibility and unpredictable plant behavior (5). One of the most significant challenges to sesame production is sesame phyllody, a disease caused by phytoplasmas of the species '*Candidatus Phytoplasma*'. This disease poses a serious threat to sesame production worldwide, particularly in major producing countries like India (6), with reported yield losses ranging from 80 % to 100 % (7). In recent years, phyllody has caused substantial losses for sesame farmers and has been a major reason for the reduction in cultivated areas. The disease manifests as yellowing, virescence, floral sterility and stem expansion in infected plants (8). Phyllody-causing phytoplasmas belong to the 16Sr groups, especially 16SrI and II, which have been reported in sesame from India. The 16SrI-B and 16SrII-D subgroups are prevalent in North India, while 16SrII is found in South India. This suggests that regional variations exist in the prevalence and distribution of phytoplasma subgroups affecting sesame in India (9).

The cell-wall-less phytoplasma resides in the plant phloem and can infect healthy plants through seed transmission, vegetative propagation and insect vectors (10, 6). Insect vector-mediated transmission plays a crucial role in the horizontal spread of phyllody disease in sesame fields (11). Leafhoppers (Ciciadellidae: Hemiptera) have been identified as vectors of sesame phyllody phytoplasmas (6). Several species of leafhoppers are found worldwide harbouring sesame phytoplasma infections (10).

Globally, reports on sesame phyllody-transmitting leafhopper species vary, including *Hishimonus phycitis* Distant in India (6), *Neoaliturus haematoceps* (Mulsant & Rey) in Iran and Turkey (10), *Orosius albicinctus* Distant in Iran and India (14) and *Orosius cellulosus* (Lindberg) and *Orosius orientalis* (Matsumura) in Iran, Pakistan, Turkey and India (10, 12). Among these, *H. phycitis* and *O. albicinctus* are regarded as the primary vectors of sesame phyllody (6, 12, 13, 14) and have been found in several locations in India (15). Due to various factors in recent years, the complexity of pests and diseases has been changing in crops, including sesame, particularly in the tropical regions of India, such as Tamil Nadu. In 2022–2023, Tamil Nadu produced 0.34 lakh metric tonnes of sesame from 0.52 lakh ha of cultivated area, highlighting the region's importance in meeting global sesame demand (6). Previous research has shown that sesame phyllody is associated with various phytoplasma groups and subgroups and is spread by different leafhopper vectors. In this study, the phytoplasma responsible for sesame phyllody, identified as belonging to the 16SrI-B subgroup, was successfully transmitted through insect vectors. However, no evidence of seed transmission was found.

Identifying vectors is crucial for managing them and controlling the disease they spread. Leafhoppers, in particular, are extremely difficult to manage due to their small size, polyphagous habits, invasive nature, high reproductive rates and the lack of effective biological control methods through recognized natural enemies (6). This makes leafhoppers a critical species that must be regularly monitored, as they served as vectors for plant pathogens. The minute morphological differences between leafhoppers species pose a significant challenge to their identification at the species level. In this study, molecular characterization of leafhopper species predominantly present in sesame (*O. albicinctus, H. phycitis* and *A. biguttula biguttula*) was carried out, along with the assessment of their phylogenetic diversity, haplotype mapping and an investigation of phyllody transmission biology to confirm leafhopper-phytoplasma interactions in sesame.

Materials and Methods

Molecular analysis

Live leafhoppers and phyllody-diseased plant samples were collected from sesame-growing areas across various districts in Tamil Nadu, India and transported to the laboratory for molecular confirmation.

Leafhoppers

The collected leafhoppers, at both immature and adult stages were disinfected using a 3 % sodium hypochlorite (NaOCl) solution to sterilize their surfaces. After surface sterilization, the leafhoppers were rinsed 2–3 times with distilled water to ensure complete removal of any residual sodium hypochlorite, preventing interference with the DNA extraction process. Genomic DNA was then extracted using the Hot Sodium Hydroxide and Tris (HotSHOT) method (16). The purity and concentration of the extracted DNA were measured using a NanodropOne™ spectrophotometer (ThermoFisher Scientific, Waltham, MA, USA) and the samples were stored at -20 °C for future use. The mitochondrial cytochrome c oxidase subunit I (mtCOI) gene (658 bp) was amplified via polymerase chain reaction (PCR) using an Eppendorf Mastercycler™, (Hamburg, Germany) in a total reaction volume of 20 µL. Universal primers LCO 1490 and HCO 2198, obtained from BioServe (Telangana, India) (17, 18), were used, following the protocol (19). The amplified DNA products were separated on a 1 % agarose gel, stained with ethidium bromide (10 mg/mL), and quantified in NanodropOne™ at A260/280 nm to assess DNA concentration and purity.

The PCR products were purified using the PureLink™ PCR Purification Kit (ThermoFisher Scientific) and sequenced in both directions with double-pass Sanger dideoxy DNA sequencing, utilizing the mtCOI forward and reverse primers, at Biokart India Pvt. Ltd. (Bengaluru, India). The raw mtCOI sequences were manually edited

and annotated using the DNA MAN tool (Vrijenhoek, 1994). Reference sequences were retrieved from the National Centre for Biotechnological Information (NCBI) (Bethesda, MD, USA) and compared to determine sequence similarity. Species identity was confirmed through a homology search of the mtCOI gene using the BLASTn search engine (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The mtCOI sequences generated in the study were subsequently submitted to the NCBI GenBank database.

Sesame phyllody pathogen

Total DNA was extracted from the leaf tissues of sesame plants, both with and without phyllody symptoms, collected from the field using the method (20). DNA extracted from a glasshouse-grown, disease-free sesame plant was used as a negative control. Two sets of species-specific primers, P1 and P7 and R16F2n and R16R2, were employed in direct PCR and nested PCR to amplify the 16S rRNA region, including the 16S-23S intergenic spacer (ITS) region, tRNA-lle, and partial 23S rRNA region of the phytoplasma (21). After the PCR was completed, 5 µL of the PCR product were loaded onto a 1.0 % (w/v) agarose gel, stained with ethidium bromide and visualized under a UV transilluminator. The nested PCR product, a 1.25 kb amplicon, was purified using the Wizard^{R} SV Gel and PCR Clean-up System (Promega, USA).

Phylogenetic analyses

Sesame phyllody phytoplasma 16S rRNA and leafhopper mtCOI sequences were retrieved from the NCBI GenBank. These sequences were edited using BioEdit software (version 7.0.5.3) and aligned with the ClustalW algorithm (22, 23). A phylogenetic tree was then constructed in MEGA-X using the neighbour-joining method with a bootstrap value of 1000 iterations. The tree was subsequently coloured and visualized using the web-based iTOL software (24).

Virtual RFLP analysis

Virtual RFLP (Restriction Fragment Length Polymorphism) analysis was performed using the *i*PhyClassifier online tool to determine the subgroup affiliation of phytoplasmas based on DNA fragment patterns generated through *in silico* digestion with specific restriction enzymes. In this analysis, DNA fragments from phytoplasma samples were virtually digested with 17 distinct restriction enzymes: *Alu*I, *Bam*HI, *Bfa*I, *Bst*UI *(Tha*I*)*, *Dra*I, *Eco*RI, *Hae*III, *Hha*I, *Hin*fI, *Hpa*I, *Hpa*II, *Kpn*I, *Mbo*I *(Sau*3AI*)*, *Mse*I, *Rsa*I, *Ssp*I and *Taq*I. These enzymes cut DNA at specific recognition sites, producing fragment patterns of varying lengths. The selected enzymes are commonly used for phytoplasma 16S rRNA RFLP analysis (25, 26).

Genetic diversity assessment and haplotype mapping of sesame phyllody pathogen

The DNA Sequence Polymorphism software (DnaSP version 5.10) was used to import aligned sequences of 16S rRNA along with additional downloaded sequences to reconstruct haplotypes and generate the data file (23). The software analysed polymorphism information, haplotype distribution, variance, standard deviation of haplotype diversity and the number of haplotypes. Using this haplotype data file, the PoPART software version 1.6.1 (Population Analysis with Reticulate Trees, http://popart.otago.ac.nz) was employed to create a minimum spanning network (MSN) to illustrate the geographic and evolutionary connections between haplotypes (22).

Disease transmission studies

Insect source

Leafhopper nymphs and adults of each species were collected from the phyllody disease-free sesame fields using aspirators (a glass tube, 10 cm in length and 2 cm in diameter, attached to a 20 cm rubber tube). Randomly sampled leafhoppers from the field collections were PCR tested for the phytoplasma pathogen to confirm they were disease free. The collected leafhoppers were then released and confined to 30-day-old potted sesame (CO1) plants, which were kept in insect-proof cages under greenhouse conditions, allowing leafhoppers to multiply. The plants were regularly watered and fresh plants were introduced into the culturing cages as needed to sustain feeding and ensure continuous multiplication of the leafhoppers. The final instar nymphs from the culture were carefully aspirated into test tubes, released onto 30- to 35-day-old potted sesame plants, and kept in confined, insect-proof cages. Once the nymphs matured into adults, they were used for transmission studies.

Phytoplasma source

Phytoplasma-infected shoots (30–40 cm in length) were excised from disease-infected 60–65-day-old sesame (CO1) plants at the Department of Oil Seeds, TNAU, Coimbatore (11.0122° N, 76.9354° E). The cut ends of the shoots were sealed and transported to the laboratory, where they were placed in 500 mL glass bottles containing sterile water. These bottles were then maintained in acquisition cages (24.5 × 24.5 × 24.5 cm) for leafhopper acquisition feeding. The presence of phyllody was confirmed through PCR using 16S rRNA primers, as described in the previous section.

Experimental setup

Leafhopper species *O. albicinctus, H. phycitis* and *A. biguttula biguttula* were studied for their capability to transmit sesame phytoplasma in the greenhouse (30 ± 2.0 °C, L/D: 16:8 and a relative humidity of 70–80 %). Acquisition feeding periods of 1, 24 and 72 h, along with inoculation feeding periods of 30 min, 1 h and 24 h were selected (26). Freshly emerged adults (50 male and female) of each leafhopper species were released and confined to allow for acquisition feeding, with a 30 min buffer time provided for the leafhoppers to settle and feed in each test. After the recommended acquisition feeding, the leafhoppers were carefully removed from the acquisition cages and transferred to inoculation cages (45 × 45 × 45 cm^3, 96 × 26 mesh) containing clean, disease-free, 20-day-old sesame plants, prepared as detailed hereafter.

Sesame seeds (CO1) were sown in pots (45 cm diam. × 45 cm H) placed in insect-proof cages (65.5 × 65.5 × 65.5 cm). The germinated seedlings were thinned to one per pot and were maintained with regular applications of fertilizer and

water. The inoculation test plants in the cages were kept under greenhouse conditions. After the inoculation period, the insects were killed by spraying insecticide imidacloprid at a concentration of 0.3 mL/L. The plants in each cage were continuously monitored for symptom expression until the harvesting stage. Healthy sesame plants were maintained as controls and no insects were allowed to interact with them. Data were recorded on the number of days taken to develop phyllody symptoms and the percentage of phyllody transmission. After the transmission test, symptomatic sesame plants in the experimental cages were collected and subjected to further PCR analysis to detect the presence of phytoplasma.

Seed transmission

Ten phytoplasma-positive sesame plants of the CO1 variety with a disease index (PDI) of ≥ 85 % were randomly selected and marked at the Department of Oil Seeds, TNAU, Coimbatore, for seed transmission assessment studies. Seeds were harvested independently from each plant and fifty seeds were randomly selected from each seed lot. Simultaneously, seeds collected from phytoplasma-negative plants were used as negative controls. The collected seeds were sown in earthen pots (45 cm diam. × 45 cm H) and kept in insect-proof cages under greenhouse conditions. The seedlings were monitored for symptom development until the plants reached physiological maturity (75 days). Finally, two randomly selected seedlings per pot were subjected to PCR analysis for the phyllody pathogen using 16S rRNA-specific primer sets, as explained in the previous section.

Results and Discussion

Molecular analysis of the leafhopper species

These analyses involved 53 nucleotide sequences of leafhoppers targeted at the COI regions (Fig. 1). Among them, 45 sequences were gathered from the NCBI database representing populations from Israel, Tunisia, and several Indian states, including Tamil Nadu, Punjab, Haryana, New Delhi and Rajasthan.

Based on geographical differences, the sequences were grouped accordingly. The *H. phycitis* sequences from Tamil Nadu formed a cluster that included samples from Pudukkotai (PP124611), Thanjavur (PP124608), Coimbatore (PP124610) and Erode (PP124609). The remaining population from New Delhi (India) was scattered into subclades with other sequences, indicating no significant variance among all sequences.

The *O. albicinctus* sequences were divided into 3 minor clades: Tunisian, Indian and Israeli. The Tunisian population was well dispersed among the subclades in different clusters. Samples collected from Pudukkotai (OR229689) and Thanjavur (OR229688) were closely related to the New Delhi sequences. The sequences from the present study exhibited 99 % similarity with *O. albicinctus* (LN879006) (27). All the Indian sequences of *A. biguttula biguttula* formed a single cluster, with 2 sequences from Tamil Nadu being very closely related (acc. no. PP124614 and OR136458).

Identification of phytoplasmas through sequence analysis

The genetic diversity and distribution of the phytoplasmas associated with sesame phyllody are illustrated in Fig. 2. These analyses included 63 nucleotide sequences, each 1250 bp long, targeting the 16S rRNA region. The neighbour-joining tree revealed the divisions of clades, representing the genetic makeup and evolution. Sequences from Iran formed a distinct clade and served as an outgroup. In the phylogenetic analysis of phytoplasma sequences from different countries, most sequences from regions such as Egypt, Oman, the USA, Vietnam and Turkey clustered together within single clades, though exhibited different clustering behaviour, indicating unique genetic characteristics or evolutionary history compared to phytoplasmas from other countries. This distinctiveness may be attributed to geographical isolation, specific environmental conditions or the prevalence of different phytoplasma strains in Paraguay.

The observation that all the Indian sequences (from New Delhi, Assam, Tripura, Mizoram, Rajasthan, Nagaland, Kerala and Telangana) formed a single cluster closely related to Paraguayan sequences suggests several intriguing possibilities. There is a high degree of genetic similarity between the phytoplasmas from these regions, which could point to a common ancestry. This shared lineage may indicate historical migration patterns or the spread of a particular phytoplasma strain across various parts of India. The clustering of sequences from Tindivanam (THINDI-PP866671), Namakkal (NMK-PP866379) and Tiruppur (TRP-PP866380) into a single clade, closly related to the Tamil Nadu clone (S4-PP729606), suggests a genetic affinity and possible shared ancestry among these phytoplasma isolates. Similarly, the grouping of sequences from Sivagangai (SVG-PP866392), Madurai (MDU-PP866391) and Virudhachalam (VS-VRI-PP866670) in close relation to the Tamil Nadu clone (S2-PP721311) highlights a strong genetic connection among these phytoplasma isolates from different regions within Tamil Nadu.

The fact that sesame phyllody isolates from various locations within Tamil Nadu, including Erode (ERD-PP866293), Salem (SLM-PP866306), Ramanathapuram (RMD-PP866408), Pudukkottai (PDK- PP866407 & PDK-VAMBAN-OR880898) and Coimbatore (CBE-OR880562, TNAU-VS- PP865964 and CBE-THON- PP866203), share 99 % genetic identity suggests a high level of similarity among these isolates. Additionally, these isolates are clustered with the Tamil Nadu clone (S5-PP721312 and TNSeI-OR644124). This clustering pattern provides valuable insights into the genetic diversity, population structure and evolutionary dynamics of phytoplasmas across different continents and regions. The close genetic relationship between sequences from Dindigul (DIND-I-PP869181) and Kerala (Trissur2021-ON332489) as well as between Erode (ERD-I-PP869180) and Uttar Pradesh (Kushinagar 5 isolate, Accession No. KF728954), indicates genetic similarity between phytoplasmas from these specific regions in In-

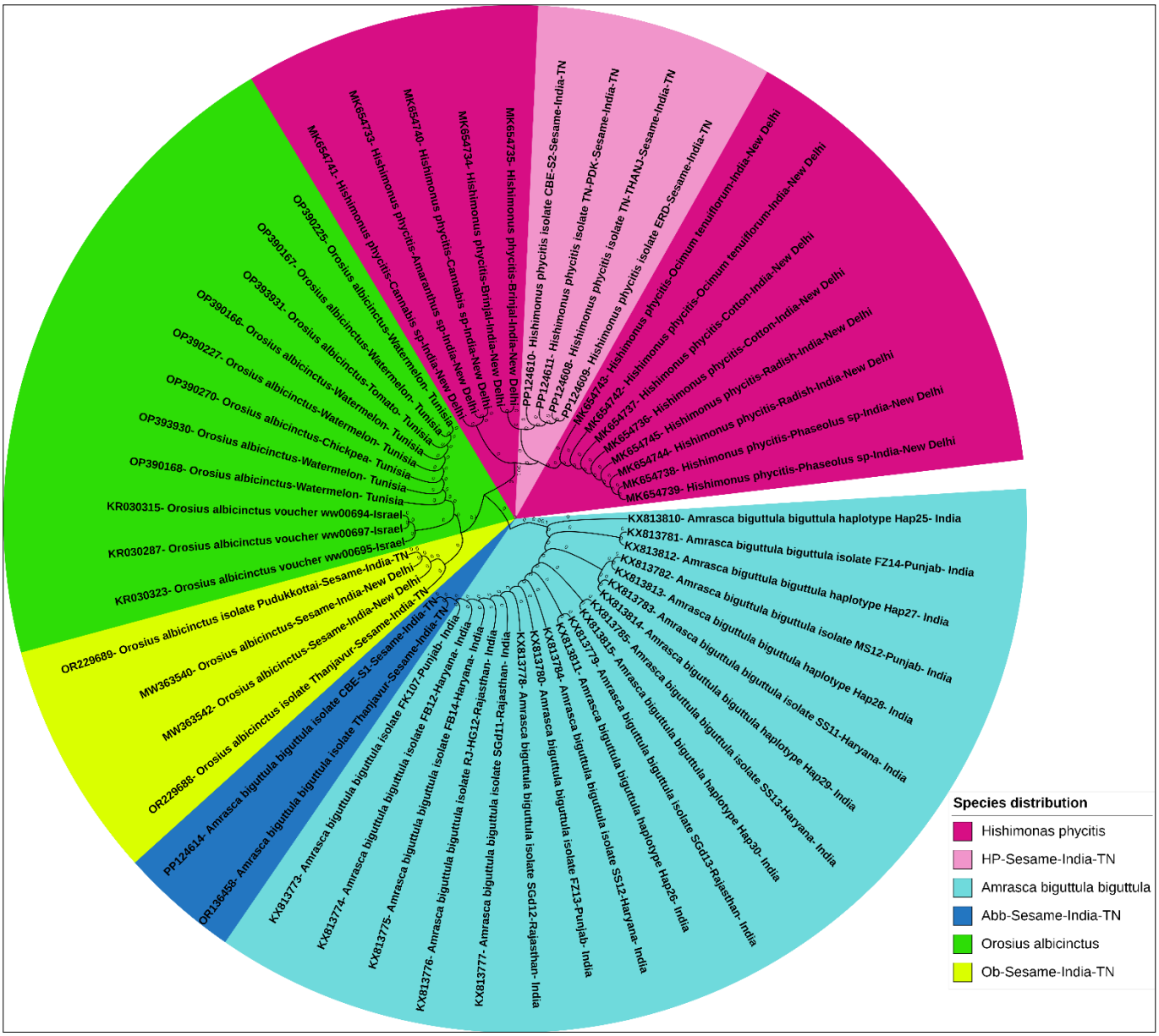

Fig. 1. Graphic representation of the neighbour-joining dendrogram. These analyses involved 53 nucleotide sequences of leafhopper complexes targeting the mtCOI regions. Out of this, 45 sequences collected from different Indian states, namely Tamil Nadu, New Delhi, Rajasthan, Punjab and Haryana (sky blue) and different parts of the world, namely Israel and Tunisia (fluorescent green), were included in the analysis. Eight sequences had been submitted to the NCBI and they were collected from different regions of major sesame-growing areas in Tamil Nadu, namely, Pudukkottai, Thanjavur, Coimbatore and Erode.

dia. Phylogenetic analysis also revealed that the sesame phyllody phytoplasma sequences from New Delhi (GKP-3 isolate, Accession No. KF744232) exhibited the highest level of similarity, approximately 99 %, to the sequences from Virudhachalam (VRI-OR880336).

The high level of similarity (around 99 %) observed between the sequences from Kerala (Trissur2021-ON332489), New Delhi (GKP-3 isolate, Accession No. KF744232) and Uttar Pradesh (Kushinagar 3 isolate, Accession No. KF728954) and those from Dindigul (DIND-I-PP869181), Erode (ERD-I-PP869180) and Virudhachalam (VRI isolate, Accession No. OR880336) in Tamil Nadu suggests that the sequences from Dindigul, Erode and Virudhachalam indeed belong to the 16SrI group of phytoplasma.

This study, for the first time, reports the presence of the 16SrI group in Tamil Nadu, located in Southern India. This conclusion is supported by comparisons with sequences from Uttar Pradesh, Kerala and New Delhi, which are known to belong to the 16SrI group of phytoplasma (6). Previous studies have already reported the presence of the 16SrII group in South India (9). This analysis is crucial for understanding the genetic relationships and evolutionary history of various phytoplasma strains. It aids in categorizing phytoplasma isolates into specific groups or subgroups based on genetic similarity, which in turn provides valuable insights into their distribution, host range and potential agricultural impact.

Virtual RFLP analysis

Virtual RFLP analyses were performed on 16S rRNA sequences from various phytoplasma isolates, including those from Dindigul, Erode Tiruppur, Coimbatore, Pudukkottai and Tindivanam. The RFLP patterns of the Dindigul (DIND-I-PP869181) and Erode (ERD-I-PP869180) isolated were similar to those of *'Candidatus Phytoplasma asteris'* (Genbank Acc. no. M30790), which is representative of 16Sr group I, subgroup B (Fig. 3a–b). On the other hand, the sequences from the Tiruppur, Coimbatore, Pudukkot-

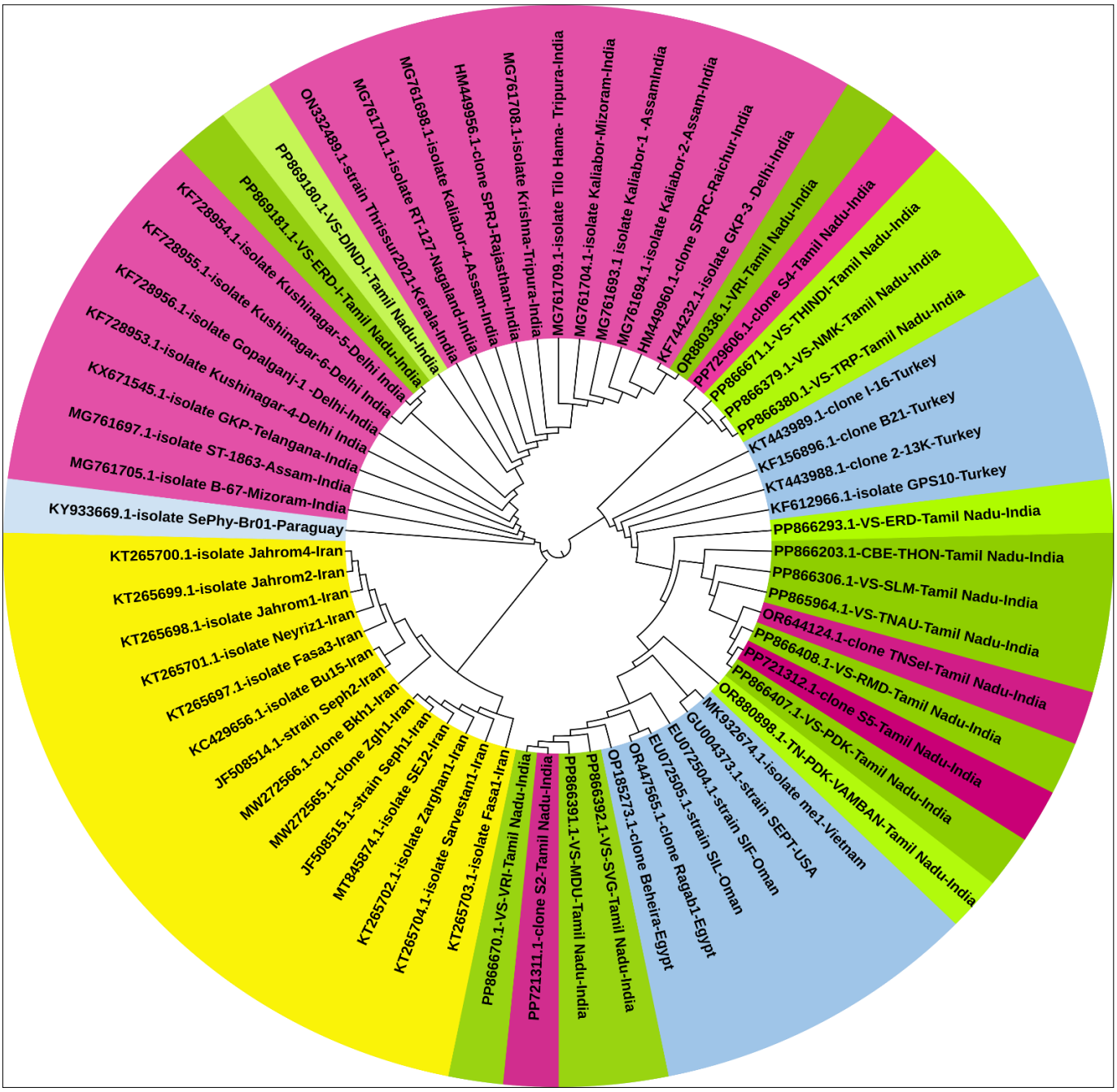

Fig. 2. A graphic representation of the dendrogram constructed by the neighbour-joining method.

tai and Tindivanam isolates displayed identical to those of *'Candidatus Phytoplasma aurantifolia'*.

The Tiruppur isolate (TRP-PP866380) showed 97.15 % identity with a phytoplasma sequence belonging to the 16Sr group II, subgroup N (GenBank accession: JF781309), with a similarity coefficient of 0.82 (Fig. 3c). The Coimbatore isolate (TNAU-VS-PP865964) exhibited 98.67 % identity with a sequence from 16Sr group II, subgroup D (GenBank accession: Y10097), with a similarity coefficient of 0.69 (Fig. 3d). The Pudukkottai isolate (PDK-PP866407) was 97.97 % identical to a sequence from the 16Sr group II, subgroup G (GenBank accession: EU099568), with a similarity coefficient of 0.29 (Fig. 3e). The Tindivanam isolate (THINDI-PP866671) showed 96.82 % identity with a phytoplasma sequence from the 16Sr group II, subgroup G (GenBank accession: JF781309), with a similarity coefficient of 0.70 (Fig. 3f).

In this study, all the Tamil Nadu isolates, except those from Dindigul and Erode are closely related to *'Candidatus Phytoplasma aurantifolia'* and belong to the 16Sr group II. The Dindigul and Erode isolates are part of the 16Sr group I and are closely related to *'Candidatus Phytoplasma asteris'*. This is the first report of 16Sr group I in Tamil Nadu (Dindigul and Erode), building on earlier findings from Kerala and other South Indian regions (28). The identification of 16Sr group I phytoplasma strains on sesame in these areas provides important context for the current discoveries in Tamil Nadu.

Haplotype identification

The MSN haplotype tree illustrates the global distribution of phytoplasma disease (Fig. 4a–b). A total of 127 haplotypes were identified from 864 sequences collected across 8 countries, showing low nucleotide and haplotype diversity. Each circle in the tree represents a distinct haplotype group, with the size of the circle proportional to the frequency of that haplotype. The lines connecting the cir-

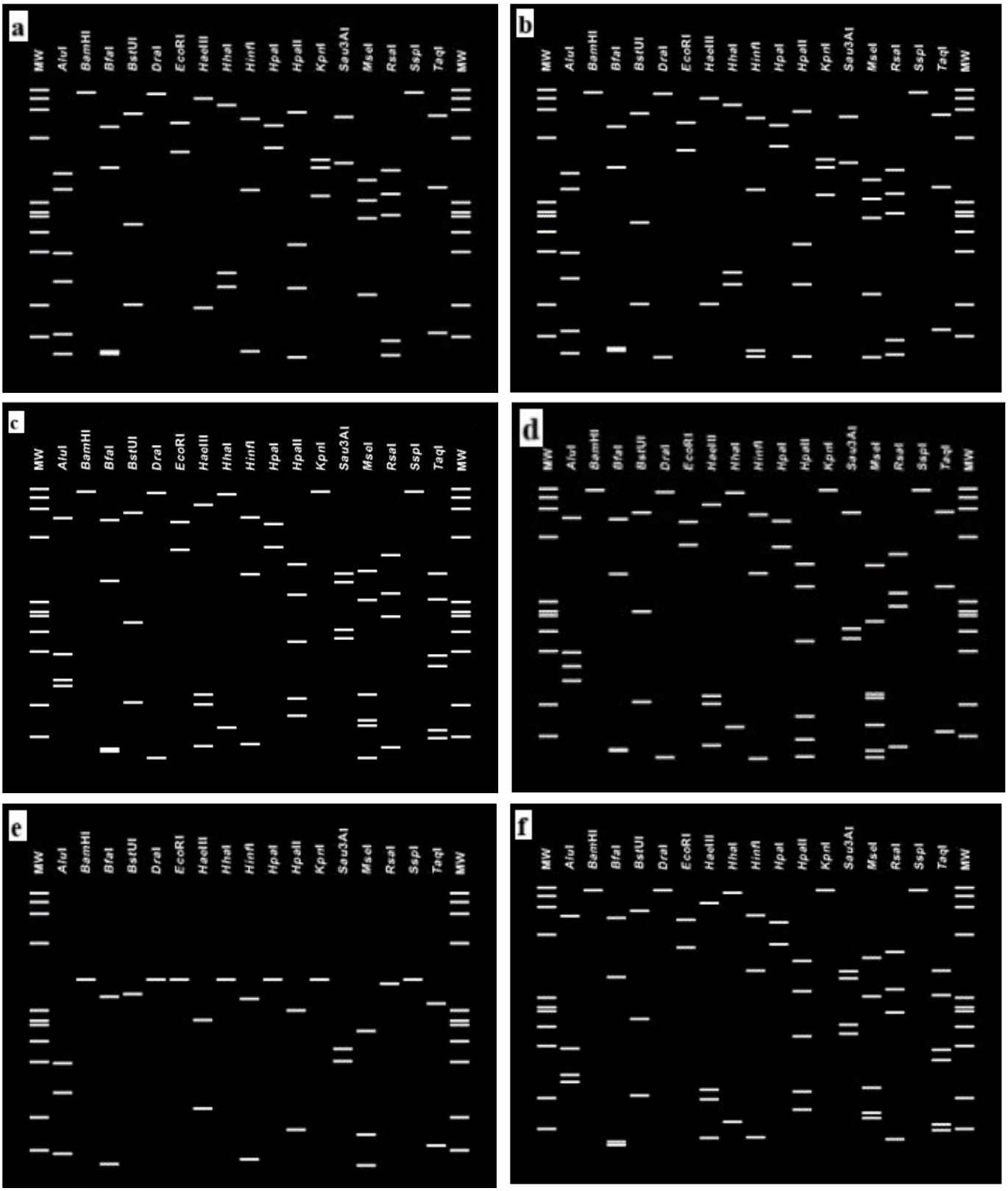

Fig. 3. Virtual RFLP profiles were generated with the program *i*PhyClassifier from *in silico* digestion of the R16F2n/R16R2 DNA fragments (100-bp DNA ladder) of the sesame phyllody phytoplasmas and selected representatives of groups of 16SrI and 16SrII: **a-b**) Dindigul (DIND-I-PP869181) and Erode (ERD-I-PP869180) were similar to *'Candidatus Phytoplasma asteris'* (Genbank accession: M30790) and belong to 16Sr group I, subgroup B; **c**) Tiruppur isolate (TRP-PP866380) belonging to 16Sr group II, subgroup N (GenBank accession: JF781309); **d**) Coimbatore isolate (TNAU-VS-PP865964) comes under 16Sr group II, subgroup D (GenBank accession: Y10097); **e**) Pudukkottai isolate (PDK-PP866407) belongs to 16Sr group II, subgroup G (GenBank accession: EU099568); **f**) Tindivanam isolate (THINDI-PP866671) belonging to 16Sr group II, subgroup G (GenBank accession: JF781309).

cles represent the mutations between each haplotype group, while the larger circles with multiple segments represent shared populations on a global scale. Each country's haplotypes are color-coded for differentiation. The Hap_13 emerged as the most predominant haplotype, comprising 42 sequences, including samples from Delhi (17), Arunachal Pradesh (4), Assam (7), Tripura (2), Manipur (2), Mizoram (2), Nagaland (2), Meghalaya (1), Telangana (1), Karnataka (1), Rajasthan (1), Raichur (1) and Egypt (1). Hap_2 was the second most common haplotype, with 21 sequences, including samples from Delhi (9), Turkey (3), Iran (7), Egypt (1) and Andhra Pradesh (1). Hap_10 was the

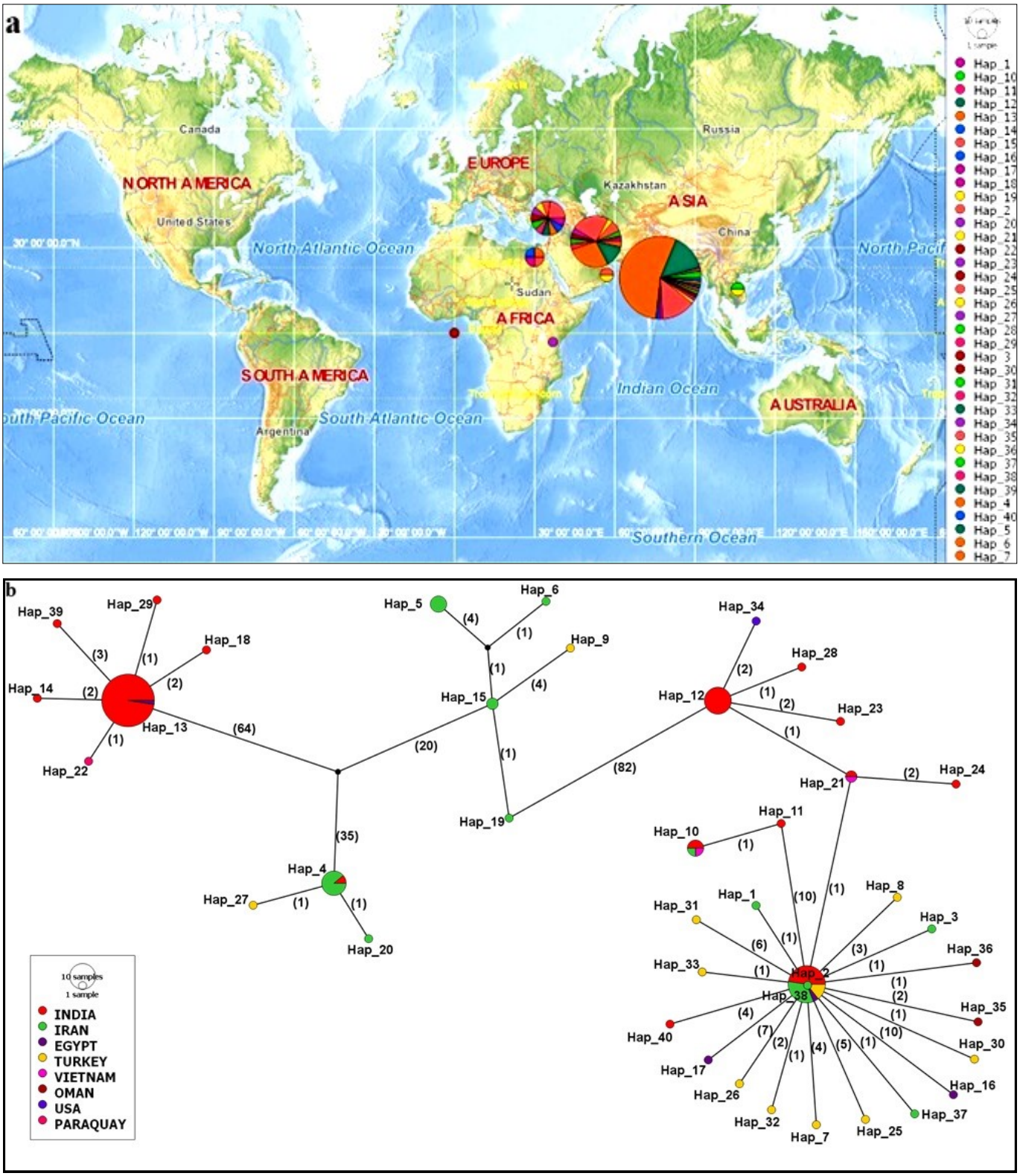

Fig. 4. a) Hieroglyph of the MSN haplotype network tree for the 16S ribosomal RNA region of *Candidatus Phytoplasma asteris* lineages reported worldwide; **b**) Each circle represents a haplotype group identified. The size of the circles is proportional to the frequency of the haplotypes. The line between each haplotype represents the mutations between each haplotype group.

third most prevalent haplotype, with sequence from Delhi (8), Karnataka (2) and China (1). Additional haplotypes Hap_4, Hap_5, and Hap_10, had 9, 4 and 4 sequences, respectively. In total, 8 haplotypes were identified globally, while 30 haplotypes were represented by only a single specimen each. Till now, no haplotype studies have been conducted specifically on the '*Candidatus Phytoplasma*' species, particularly regarding sesame phyllody.

Phyllody transmission by leafhoppers

The results of the transmission of sesame phyllody phytoplasma (Fig. 5a–d) through *O. albicinctus* are summarised in Table 1. All treatments (T1–T9) showed statistically significant difference compared to the control (T10). The percentage of phyllody transmission by *O. albicinctus* ranged from 7.8 % to 84 %, depending on the treatment combinations. The highest transmission rate of 84 % was observed in the treatment with 3 insects per plant + 5 DAF + 1 DIF (Days of Acquisition Feeding + Days of Inoculation Feeding). In contrast, the lowest transmission rate of 7.8 %

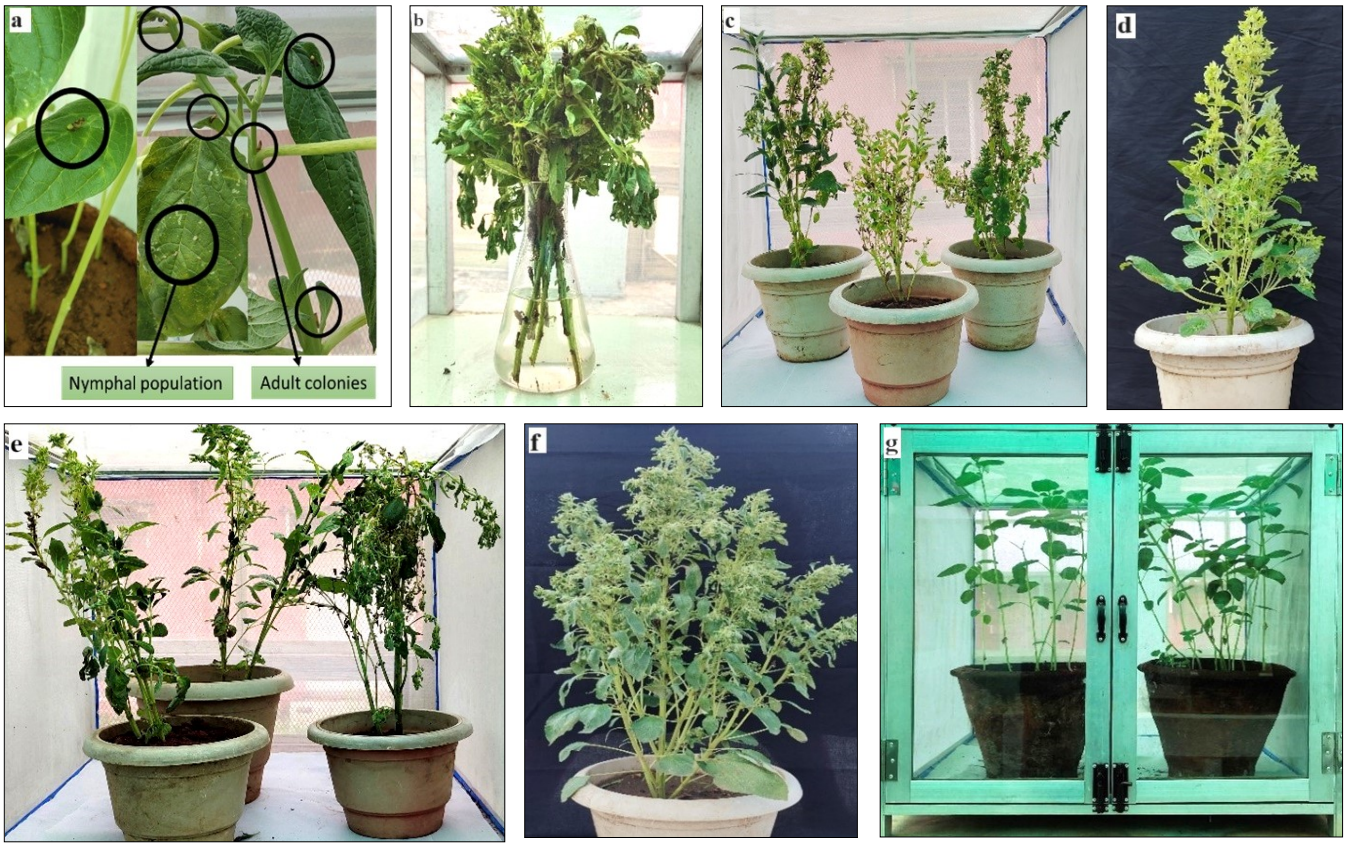

Fig. 5. Experimental transmission of sesame phyllody phytoplasma: **a**) leafhoppers were maintained in insect-proof cages; **b**) acquisition feeding hoppers were maintained in acquisition cages; **c**-**d**) symptoms developed by *O. albicinctus*; **e**-**f**) symptoms developed by *H. phycitis*; **g**) symptoms were not developed by *A. biguttula biguttula.*

was recorded in treatment T1.

An earlier study also reported a similar transmission rate of 84.26 % with 3 leafhoppers per plant (29). The data suggests that the percentage of phyllody transmission increased as the DAF increased (T5, T7 and T8). The time for the first appearance of phyllody symptoms in all treatments (T1–T9) ranged from 17 to 43 days (Table 1). The transmission rate was significantly influenced by the number of leafhoppers released per plant, the length of acquisition feeding period, and the inoculation feeding period (29). After seven days of acquisition and inoculation feeding by *O. albicinctus*, 60 % of the plants developed phyllody disease (30).

As evidenced by symptom development and nested PCR analysis, *H. phycitis* was also capable of transferring phytoplasma from diseased to healthy sesame plants, indicating its role as a vector for phyllody. In the *H. phycitis* transmission assays, the treatment with 3 insects/plant + 5 DAF + 1 DIF recorded 74 % of plants showing phyllody disease symptoms, which tested positive for phytoplasma in PCR analyses (Fig. 5e–f). Control plants did not exhibit any symptoms and were phytoplasma-negative in PCR studies. Previous studies have similarly reported transmission rates of over 80 % (31-34). Therefore, both *O. albicinctus* and *H. phycitis* are important vectors of phytoplasma in the tropical Tamil Nadu.

In contrast, transmission assays with *A. biguttula*

Table 1. Transmission of sesame phyllody disease by insect vectors, *O. albicinctus* and *H. phycitis.*

Treatments		Days required for the appearance of phyllody symptoms		Percent of phyllody transmission (%)	
		O. albicinctus	***H. phycitis***	***O. albicinctus***	***H. phycitis***
1 insects/plant + 1 DAF + 30 MIF	T1	43	47	7.8	5.2
1 insects/plant + 3 DAF + 1 HIF	T2	38	42	18	10.5
1 insects/plant + 5 DAF + 1 DIF	T3	30	34	21	14.3
2 insects/plant + 1 DAF + 30 MIF	T4	32	35	44	17
2 insects/plant + 3 DAF + 1 HIF	T5	31	30	57	25
2 insects/plant + 5 DAF + 1 DIF	T6	27	32	41	35
3 insects/plant + 1 DAF + 30 MIF	T7	25	28	66	49
3 insects/plant + 3 DAF + 1 HIF	T8	23	30	77	57
3 insects/plant + 5 DAF + 1 DIF	T9	17	19	84	74
No inoculation (Control)	T10	0	0	0	0

*__DAF__: Days of Acquisition Feeding; *__DIF__: Days of Inoculation Feeding, *__MIF__: Minutes of Inoculation Feeding; *__HIF__: Hour of Inoculation Feeding.

biguttula showed that the test plants did not develop the disease and were PCR-negative for phytoplasma (Fig. 5g), indicating that *A. biguttula biguttula* is not a vector for sesame phyllody disease (34).

Phyllody transmission by seeds

In the seed transmission studies, seedlings did not exhibit phyllody symptoms up to 75 days after germination and were PCR-negative for phytoplasma. These results suggest that sesame phyllody disease is not transmitted to the next generation through seeds of infected plants and is not seed-borne, as previously reported (33, 34).

Conclusion

Sesame phyllody poses a significant challenge in India's sesame-growing regions. Molecular data indicate that the sesame phyllody phytoplasma forms a unique clade with well-supported subclades worldwide, revealing minor genetic variations among global haplotypes that may enhance pathogen transmission. Transmission studies demonstrated a phyllody incidence ranging from 5.2 % to 84 %, depending on the leafhopper species and environmental conditions, with *O. albicinctus* and *H. phycitis* exhibiting the highest incidence rates. In contrast, *A. biguttula biguttula* did not transmit the phytoplasma. The identification of both 16Sr group I and II phytoplasma isolates in Tamil Nadu is a crucial finding, expanding our understanding of phytoplasma diversity in India. Enhanced surveillance and monitoring of sesame-growing regions for the presence and spread of phyllody disease is essential. This effort should include tracking both the phytoplasma strains and their insect vectors to detect new outbreaks early. Additionally, establishing and enforcing quarantine measures can help prevent the introduction and spread of new phytoplasma strains and vectors to unaffected regions.

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Authors' contributions

VS and MM: Wrote the manuscript. GS and MS: Designed the article and helped with revisions of the article. LK, MJ and KP: Analysed the data. All authors read and approved the final manuscript.

Compliance with ethical standards

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References

1. Shyu YS, Hwang LS. Antioxidative activity of the crude extract of lignan glycosides from unroasted Burma black sesame meal. Food Research International. 2002;35(4):357-65. http://dx.doi.org/10.1016/S0963-9969(01)00130-2
2. Anilakumar KR, Pal A, Khanum F, Bawa AS. Nutritional, medicinal and industrial uses of sesame (*Sesamum indicum* L.) seeds-an overview. Agriculturae Conspectus Scientificus. 2010;75(4):159-68.
3. Zech-Matterne V, Tengberg M, Van Andringa W. *Sesamum indicum* L. (sesame) in 2nd century BC Pompeii, Southwest Italy and a review of early sesame finds in Asia and Europe. Vegetation History and Archaeobotany. 2015;24:673-81. https://doi.org/10.1007/s00334-015-0521-3
4. Food and agriculture organization of the united nations [FAO]. Food and Agriculture Organization Corporate Statistical Database; 2022. Available at: http://faostat.fao.org/faostat 2022 [Accessed December 10, 2023]
5. Manjunatha N, Prameela HA, Rangaswamy KT, Palanna KB, Wickramaaracgchi WA. Phyllody phytoplasma infecting sesame (*Sesamum indicum* L.) in South India. Phytopathogenic Mollicutes. 2012;2(1):29-32. http://dx.doi.org/10.5958/j.2249-4669.2.1.005
6. Nabi S, Madhupriya, Dubey DK, Rao GP, Baranwal VK, Sharma P. Molecular characterization of '*Candidatus Phytoplasma asteris*' subgroup IB associated with sesame phyllody disease and identification of its natural vector and weed reservoir in India. Australasian Plant Pathology. 2015;44:289-97. http://dx.doi.org/10.1007/s13313-015-0345-8
7. Sahambi HS. Studies on seasmum phyllody virus: virus-vector relationship and host range. In: Plant Disease Problems, Proc. 1st Int. Symposium Plant Pathol., New Delhi, Indian Phytopathological Society; 1970:pp. 340-51.
8. Bertaccini A, Duduk B. Phytoplasma and phytoplasma diseases: a review of recent research. Phytopathologia Mediterranea. 2009;48(3):355-78. https://doi.org/10.14601/Phytopathol_Mediterr-3300
9. Singh V, Kumar S, Reddy NB, Naik MK, Bhat KV, Lakhanpaul S. Devastation of sesame (*Sesamum indicum* L.) crops in different agroclimatic zones of India by genetically diverse subgroups of phytoplasma. Crop Protection. 2016;86:24-30. https://doi.org/10.1016/j.cropro.2016.04.004
10. Akhtar KP, Dickinson M, Sarwar G, Jamil FF, Haq MA. First report on the association of a 16SrII phytoplasma with sesame phyllody in Pakistan. Plant Pathology. 2008;57(4):7-19. http://dx.doi.org/10.1590/1678-992x-2017-0140
11. Kirkpatrick BC. Mycoplasma-like organisms—plant and invertebrate pathogens. In: The Prokaryotes: A Handbook on the Biology of Bacteria: Ecophysiology, Isolation, Identification, Applications, NY: Springer New York; 1992: pp. 4050-67. https://doi.org/10.1007/b113360
12. Pathak DM, Pateland DR, Patel RR. Control of sesame phyllody caused by plo's. International Journal of Forestry and Crop Improvement. 2013;4(1):47-48.
13. Prasad SM, Sagambi HS. Sesamum phyllody-some new host records. Indian Phytopathology. 1982;35(1):159-60.
14. Salehi M, Esmailzadeh Hosseini SA, Salehi E, Bertaccini A. Genetic diversity and vector transmission of phytoplasmas associated with sesame phyllody in Iran. Folia Microbiology. 2017;62:99-109. https://doi.org/10.1007/s12223-016-0476-5
15. Viraktamath CA, Murthy HA. Review of the genera *Hishimonus Ishihara* and Litura Knight (Hemiptera: Cicadellidae) from the Indian subcontinent with description of new species. Zootaxa. 3785(2):101-38. https://doi.org/10.11646/zootaxa.3785.2.1

16. Sadhana V, Senguttuvan K, Murugan M, Manikanda Boopathi N, Sathiah N. First record of Bondar's nesting whitefly, *Paraleyrodes bondari* Peracchi (Hemiptera: Aleyrodidae), occurrence and infestation in the cotton ecosystem of Tamil Nadu. India Journal of Pharma Innovation. 2021;10(10S):1278-84.

17. Martini M, Lee IM. PCR and RFLP analyses based on the ribosomal protein operon. Phytoplasma: Methods and Protocols. 2013;5(2):173-88. https://doi.org/10.1007/978-1-62703-089-2_15

18. Xu YE, Wang Y, Dietrich CH, Fletcher MJ, Qin D. Review of Chinese species of the leafhopper genus *Amrasca Ghauri* (Hemiptera, Cicadellidae, Typhlocybinae), with description of a new species, species checklist and notes on the identity of the Indian cotton leafhopper. Zootaxa. 2017;4353(2):360-70. https://doi.org/10.11646/zootaxa.4353.2.7

19. Sambrook J, Russell DW. Molecular cloning: a laboratory manual. New York: Cold Spring Harbor; 2001.

20. Vrijenhoek R. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology. 1994;3 (5):294-99.

21. Zhang YP, Uyemoto JK, Kirkpatrick BC. A small-scale procedure for extracting nucleic acids from woody plants infected with various phytopathogens for PCR assay. Journal of Virological Methods. 1998;71(1):45-50. https://doi.org/10.1016/s0166-0934(97)00190-0

22. Wang K, Hiruki C. Molecular characterization and classification of phytoplasmas associated with canola yellows and a new phytoplasma strain associated with dandelions. Plant Disease. 2001;85(1):76-79. https://doi.org/10.1094/pdis.2001.85.1.76

23. Bandelt HJ, Forster P, Röhl A. Median-joining networks for inferring intraspecific phylogenies. Molecular Biology and Evolution. 1999;16(1):37-48. https://doi.org/10.1093/oxfordjournals.molbev.a026036

24. Librado P, Rozas J. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics. 2009;25 (11):1451-52. https://doi.org/10.1093/bioinformatics/btp187

25. Thompson JD, Higgins DG, Gibson TJ. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Research. 1994;1 (22):4673-80. https://doi.org/10.1093/nar/22.22.4673

26. Zhao Y, Wei W, Lee IM, Shao J, Suo X, Davis RE. Construction of an interactive online phytoplasma classification tool, i PhyClassifier and its application in analysis of the peach X-disease phytoplasma group (16SrIII). International Journal of Systematic and Evolutionary Microbiology. 2009;59(10):2582-93. https://doi.org/10.1099%2Fijs.0.010249-0

27. Vemana K, Rao GP, Kalita MK, Esmaeilzadeh-Hosseini SA, Azadvar M, Li Z. Update on phytoplasma diseases associated with oil seed crops in Asia. In: Phytoplasma Diseases of Major Crops, Trees and Weeds. Academic Press; 2023: pp. 105-24. http://dx.doi.org/10.1016/B978-0-323-91897-8.00006-X

28. Yadav A, Thorat V, Bhale U, Shouche Y. Association of 16SrII-C and 16SrII-D subgroup phytoplasma strains with witches' broom disease of *Parthenium hysterophorus* and insect vector *Orosius albicinctus* in India. Australasian Plant Disease Notes. 2015;10:1-5. https://doi.org/10.1007/s13314-015-0181-2

29. Thirumalaisamy PP, Amal TC, Pradheep K, Celia Chalam V, Anitha K. Phytoplasma association with phyllody of *Sesamum* species in India. Tropical Plant Pathology. 2023;48:594-603. http://dx.doi.org/10.1007/s40858-023-00603-7

30. Gogoi SH, Kalita MK, Nath PD. Biological characterization of sesamum phyllody disease in Assam, India. International Journal of Current Microbiology and Applied Sciences. 2017;6 (11):1862-75. http://dx.doi.org/10.20546/ijcmas.2017.611.222

31. Akhtar KP, Sarwar G, Abbas G, Asghar MJ, Sarwar N, Hamed M. Mungbean phyllody disease in Pakistan: symptomatology, transmission, varietal response and effects on yield characteristics. International Journal of Pest Management. 2012;58(2):139-45. http://dx.doi.org/10.1080/09670874.2012.669870

32. Dutta DS, Kalita MK, Nath PD. Detection, characterization and management of brinjal little leaf disease in Assam. Journal of Environmental Biology. 2022;43(3):460-67. http://dx.doi.org/10.22438/jeb/43/3/MRN-2002

33. Hemmati C, Moharramipour S, Askari Seyahooei M, Bagheri A, Mehrabadi M. Population genetic structure of *Hishimonus phycitis* (Hem.: Cicadellidae), vector of lime witches' broom phytoplasma. Journal of Agricultural Science and Technology. 2018;20(5):999-1012.

34. Nakashima K, Hayashi T, Chaleeprom W, Wongkaew P, Sirithorn P. Detection of DNA of phytoplasmas associated with phyllody disease of sesame in Thailand. Japanese Journal of Phytopathology. 1995;61(6):519-28. https://doi.org/10.3186/jjphytopath.61.519