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Eggplant Little Leaf-Associated Phytoplasma Detection in Seedlings under Insect-Proof Conditions

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Abstract: Eggplant, or brinjal, is one of the most consumed and important tropical solanaceous vegetable crops grown worldwide. Little leaf is a disease associated with the presence of phytoplasmas especially widespread in brinjal in India. To clarify the epidemiology of this disease, a verification of its transmission through seeds to seedlings and their progeny derived from symptomatic mother plants was performed. Brinjal seeds field-collected in the Dharwad district of Karnataka State, India, were sowed in a greenhouse under insect-proof conditions. DNA was extracted from seedlings and their progeny and from symptomatic plant samples collected in the field. The first- and second-generation seedlings obtained *under these conditions were tested at various time points after germination by amplification of the 16S rRNA gene of phytoplasmas. The amplicons obtained were subjected to restriction fragment length polymorphism (RFLP) analysis and sequencing for the identification of detected phytoplasmas. Ribosomal groups 16SrI, 16SrII, 16SrIII, 16SrV, 16SrVI, and 16SrXII were identified. Moreover, a number of fruits produced from the first-generation seedlings showed precocious seed germination, and the young seedlings resulted as phytoplasma-positive. The seed transmission of phytoplasmas in eggplants for two subsequent generations highlights the risk of additional sources of infection of the disease represented by asymptomatic and infected seedlings in the presence of insect vectors. The seed transmission could explain the continuous presence of epidemic outbreaks of phytoplasmas in brinjal cultivations in several cultivation areas.

Keywords: disease management; eggplant; DNA extraction; RFLP analysis; nested PCR amplification; sequencing; phylogeny

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1. Introduction

Eggplant, or brinjal (*Solanum melongena* L., Solanaceae), is among the most important solanaceous vegetable crops cultivated in tropical and subtropical regions of the world [1]. The edible varieties derive from the wild African species *Solanum incanum*. In India, the area under brinjal cultivation is 730,000 ha, and the total production is about 128,008,000 MT, with a productivity of 17.5 MT/ha. The major brinjal-producing states are West Bengal, Odisha, Gujarat, Madhya Pradesh, and Bihar. West Bengal has the largest share of area (163,150 ha) and production (3027.75 MT) [2]. The crop is affected by the presence of several infectious diseases and pests, among which the little leaf is a phytoplasma-associated disease causing a yield loss of up to 100%, especially in India [3].

Phytoplasmas, which belong to the class *Mollicutes*, are bacterial pathogens that reside in plants' sieve tubes and insects' hemolymph. They lack the cell wall and have a pleomorphic shape; surrounded by a single membrane, they are 200–800 nm in diameter and possess a small genome of about 680–1600 kb. The difficulties in cultivating phytoplasmas in pure culture [4,5] have limited the use of classical taxonomic methods for their identification. A restriction fragment length polymorphism (RFLP) of amplified 16Sr RNA gene-based approach developed in the 1990s allows the detection and identification of phytoplasmas

and the assignment of them to ribosomal groups and subgroups [6]. The phytoplasmas are classified based on the 1500 bp sequence of the 16S rRNA gene sequence as '*Candidatus* Phytoplasma' species, having more than 98.65% identity on this sequence to previously identified '*Ca*. Phytoplasma' species [7]. Phytoplasmas were detected in over 1000 plant species associated with a variety of symptoms and diseases in agricultural, horticultural, ornamental, trees, and weed species [8]. Among these diseases, brinjal little leaf is especially spread in the Indian subcontinent, where it is associated with small yellow leaves, reduced length of stem internodes, bushy appearance, flower phyllody, and virescence [9]. The infection is initially observed in one branch and, later, the entire plant shows symptoms. Starting from the early disease stages, the infected plants are often shorter in size and have a greater number of branches, roots, and leaves than healthy plants. In severe epidemics, the flower parts are deformed, leading the plants to be sterile; in these cases, the infected plants do not bear fruits or fruiting is rare and yield losses can approach 100%.

Phytoplasmas enclosed in six ribosomal groups and diverse subgroups were reported to infect brinjal worldwide. These are 16SrI, 16SrII-D, 16SrIII-J, 16SrIII-U, 16SrVI-A and 16SrVI-D, 16SrIX-C, and 16SrXII-A; out of these subgroups, 16SrVI-D and 16SrII-D are those mainly detected infecting brinjal plants in eight states of India [10]. The frequent association of floral abnormalities and fruit malformations with the presence of phytoplasmas has led to the belief that seeds from infected plants are not viable and do not germinate. However, advancements in research have shown that such seeds are germinating and phytoplasmas can also be transmitted through seeds [5], especially in case of late infection time in the plant growth cycle. Phytoplasma transmission by seeds was reported in alfalfa, tomato, sesame, carrot, petunia, and corn [5,11–13].

This study aimed to verify the presence of seed transmission of phytoplasmas from symptomatic eggplant (brinjal) mother plants collected in the southern part of an Indian farmers' field to clarify the epidemiological role of infected seedlings in the dissemination of brinjal little leaf disease. The experiments were carried out by testing seedlings germinated under an insect-proof greenhouse. The phytoplasma presence in young plants was verified at different growth periods and the progeny from these plants was also tested. The identification of detected phytoplasmas was achieved by restriction fragment length polymorphism and sequence analyses on the 16S rRNA gene.

2. Materials and Methods

2.1. Seed Collection and Germination

Eggplant seeds belonging to the cultivar Manjari were collected from a farmer's field (15°33′53.8″ N 74°55′51.1″ E) in Garag village, Dharwad Taluk, in the Dharwad district of Karnataka state, India. The seeds originated from two eggplant fruits, designated as 1 and 2, obtained from symptomatic plants displaying little leaf symptoms (Figure 1) sown in a field in February 2021. Subsequently, in May 2021, the seeds were harvested and transferred to cold storage; after three months, they were put in a germination chamber for 3–8 days with conditions of 16 h of light at 20 °C during daytime and 8 h of dark at 30 °C at night. A total of 471 out of 516 seeds germinated and 240 seedlings (30 per batch from 8 batches, 4 per each fruit) were transplanted to pots in an insect-proof greenhouse. The cultivation was performed under a temperature range of 20 °C as the minimum and 25 °C as the maximum (±2 °C); there was no supplementary light, the relative humidity was maintained at 70%, and regular irrigation was conducted every three/four days. The potting soil was a commercial mixture of blonde and Irish peat added with bark humus, coconut fiber, pumice (3–8 mm/7–12 mm), and volcanic lapilli (5–10 mm). Initially, 25 seeds were transplanted, and after three days, 5 more seeds from each batch were transplanted, for a total of 30 germinated seeds per batch. To differentiate the first 25 transplanted seedlings from the next 5 transplanted ones, the former ones were labeled from 1 to 25, i.e., M1, M2 to M25, and the latter 5 were labeled in alphabetical order from A to E for each batch, i.e., M1.A, M1.B to M1E.

(**a**) (**b**) (**c**)

Figure 1. Brinjal plants showing little leaf symptoms in the fields where the seeds were collected (**a**,**b**) and seedlings in insect-proof greenhouse at 61 (**top**) and 68 (**bottom**) days after planting (**c**).

2.2. DNA Extraction

Molecular analyses were carried out on the seedlings at various times (50, 61, 68, and 76 days after transplanting, DAT) to check the presence and identity of phytoplasmas. A few plants produced fruits that were harvested after attaining maturity for the verification of phytoplasma presence in the second-generation seedlings. The fruits were asymptomatic, but after dissecting, it was possible to verify that several seeds were pre-germinated inside. However, in the same fruits, other seeds were either partially germinated or not germinated. DNA extraction was carried out from symptomatic plants from the same field where the seeds were produced from selected seedling plants belonging to the eight batches grown under greenhouse conditions and from the hypocotyl parts of the seeds produced in the same conditions. DNA was extracted by a CTAB method [14] from 1 g of fresh leaf midribs from mother plants and seedlings, mixing equal amounts of leaf veins from each plant in the batch, while for the hypocotyl tissues, the DNA was extracted after the mother tissues were carefully discarded.

2.3. Amplification Conditions

PCR and nested PCR were carried out with 1 µL of the extracted DNA diluted 1:30 with sterile distilled water (=30–50 ng) using the primers R16F2n/R16R2 [15] and R16(I)F1/R1 [16], as well as fU5/rU3 [17] and 16R758f (=M1)/16S1232r (=M2) [18]. Samples devoid of the DNA template were added in each reaction as a negative control; no positive control was used, to avoid carry-over contamination. Selected phytoplasmas from the EPPO-QBank collection (https://qbank.eppo.int/phytoplasmas/, accessed on 3 February 2024) were amplified in separate reactions as positive controls to be used in RFLP analyses. For the PCR assays, 35 cycles were performed for 1 min at 94 °C, 2 min at 55 °C, and 3 min at 72 °C (10 min for the last cycle). A volume of 1 µL of the amplified products was diluted 1:30 in sterile distilled water and added to the reaction mix for the nested PCR using the same cycle, with an annealing temperature of 50 °C. The PCR mix in a volume of 25 µL contained 0.5 µL of each primer, 12.5 µL of the Master mix (My TaqTM red mix, Bioline, TN, USA), and 10.5 µL of distilled sterile H_2O. All the PCR products (6 µL aliquots) were analyzed by electrophoresis on 1% agarose gel, followed by ethidium bromide staining, and were documented with a digital camera using a bench top UV transilluminator at 312 nm.

2.4. Phytoplasma Identification by RFLP Analyses and Phylogeny

RFLP analysis was performed on the amplicons containing about 300 ng of DNA from nested PCR assays using *Tru1*I and *Tsp509*I fast enzymes (Fermentas, Vilnius, Lithuania)

for 10 min at 65 °C, adding the corresponding digested amplicons from the phytoplasma strains. The restriction products were examined with vertical electrophoresis in 6.7% polyacrylamide gel, visualized and documented as described above. To confirm the phytoplasma identity, selected amplicons from the 16S rRNA gene were directly sequenced in both directions with the respective primer forward and reverse at the Macrogen Company (Amsterdam, The Netherlands). The BLAST-aligned and manually verified sequences were compared with nucleotide sequences of selected '*Ca*. Phytoplasma' species from the NCBI GenBank database. Molecular phylogenetic analysis by the maximum likelihood method based on the Jukes–Cantor model was performed [19]. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach and then selecting the topology with the superior log likelihood value. Evolutionary analyses were conducted in MEGA7 [20]. The same phylogenetic analyses were carried out to characterize one of the two phytoplasmas detected in the field-collected symptomatic brinjal plants after the amplification and direct sequencing of *secA* and *leu* genes [21,22].

3. Results

Seed Germination

Brinjal seeds from two fruits harvested from a symptomatic eggplant that exhibit typical little leaf symptoms (Figure 1) were used. The germination percentage and survival of seeds after transplanting were approximately 91%. The majority of the transplanted seedlings survived, and plants grew without little leaf symptoms (Table 1 and Figure 1).

Table 1. Germination percentage of seeds from the two fruits and survival rate of transplanted seedlings (1–4 are from fruit 1 and 5–8 are from fruit 2).

Seed Batches	1	2	3	4	5	6	7	8	Average
Germinated seeds (%)	92	92	93	89	93	81	95	98	91.63%
Transplanted seedlings (%)	97	90	93	90	97	90	87	80	91.00%

Even if the greenhouse conditions were challenging to grow eggplants, the transplanted seedlings developed successfully. Fifty days after transplanting, the plants exhibited irregular size, but after sixty-one days, no symptoms of little leaf were observed; at sixty-eight days, some of the seedlings showed leaf mottling, and after seventy-six days, yellowing was observed in some leaves. The plants produced flowers of normal purple color and immature fruits were, as expected, purple, white, and round, and matured showing the typic slight golden yellow color. Several of these fruits were, however, holding pre-germinated seeds (Figure 2).

The DNA extractions and amplifications were performed on the eggplant seedling batches tested at different time intervals (i.e., at 50, 61, 68, and 76 days after transplanting) and on hypocotyl parts of the germinated seeds, and the samples collected in the field provided positive results for phytoplasma detection. Molecular testing was performed by different PCR schemes with the above-reported primer pairs. The best methodology enabling the detection and identification of phytoplasmaswas the nested PCR assays using R16F2n/R2, fU5/rU3, R16(I)F1/R1, and M1/M2 primer pairs. The results obtained with the different primer pairs were consistent when applied to the same samples and allowed the identification of the same phytoplasma in repeated tests. The negative controls used in each PCR and nested PCR reaction always resulted as negative.

The RFLP analysis on the amplicons obtained from eggplant tissues and positive controls identified the presence of phytoplasmas belonging to ribosomal groups 16SrI, 16SrII, 16SrIII, 16SrV, 16SrVI, and 16SrXII (Figure 3). In the brinjal samples collected in the original fieldphytoplasmas belonging to groups 16SrIII and 16SrVI were identified.

(**a**) (**b**) (**c**)

Figure 2. Brinjal fruits produced in the insect-proof greenhouse from seedlings collected from phytoplasma-infected mother plants: (**a**) brinjal plant with fruits and (**b**,**c**) dissected eggplant with germinated seeds inside the fruit.

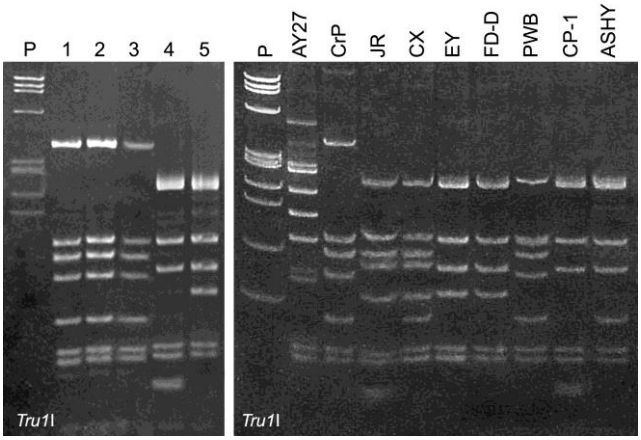


Figure 3. RFLP profiles of 16S rDNA in 6.7% polyacrylamide gels of selected amplicons from DNA samples amplified with fU5/rU3 primers in nested reaction from eggplant phytoplasmas and phytoplasma controls digested with *Tru1*I. Samples from eggplant on the left from 1 to 5. On the right, profiles of phytoplasma controls from EPPO-Qbank collection: AY27; 16SrI-B; CrP, 16SrII-C; JR, 16SrIII-H; CX, 16SrIII-A; EY, 16SrV-A; FD-D, 16SrV-D; PWB, 16SrVI; CP-1, 16SrVI-A; ASHY, 16SrVII-A. P, marker phiX174 *Hae*III digested with fragment sizes in base pairs from top to bottom of 1353; 1078; 872; 603; 310; 281; 271; 234; 194; 118; and 72.

After the sequencing and alignment of the 16S rRNA gene amplicons, the 16SrIII phytoplasmas resulted as 99.32% identical to '*Candidatus* Phytoplasma pruni' (GenBank accession number JQ044392), with five SNPs. The 16S rRNA genes of the 16SrVI phytoplasmas showed 99.41% identity to '*Ca*. P. trifolii' (GenBank accession number AY390261) with three SNPs (Figure 4).

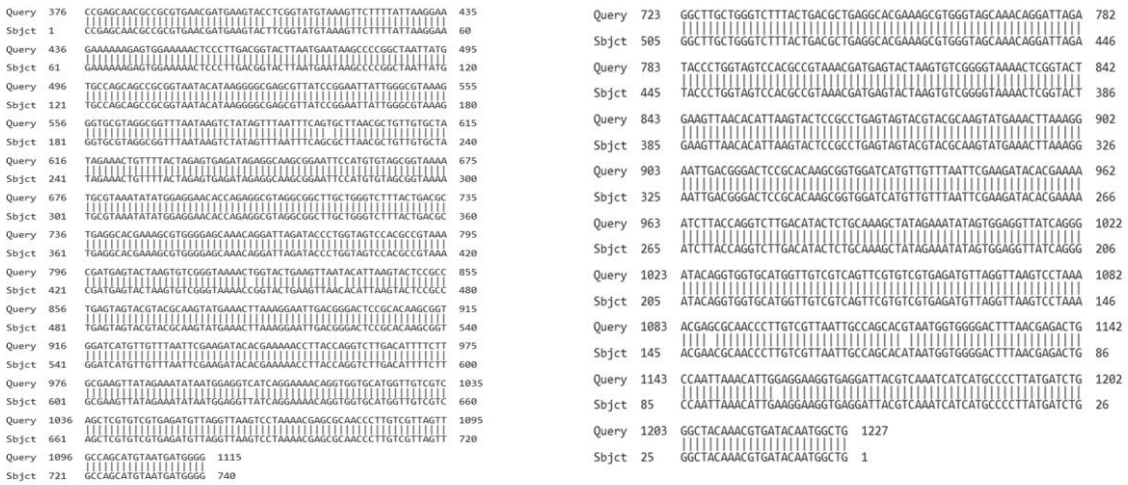


Figure 4. Alignments of the 16S rRNA gene sequences of the 16SrIII phytoplasma to '*Ca*. P. pruni' (**a**) and of 16SrVI phytoplasma to '*Ca*. P. trifolii' (**b**), showing the SNPs detected.

Among the phytoplasmas detected in eggplants, the phylogeny on the 16S rRNA genes confirmed the clustering of 16SrII phytoplasmas with '*Ca*. P. aurantifolia', 16SrIII with '*Ca*. P. pruni', 16SrVI with '*Ca*. P. trifolii', and 16SrXII with '*Ca.* P. solani' (Figure 5). For the phytoplasmas in groups 16SrI and 16SrV, the sequencing produced multiple peak readings, making the results unsuitable for interpretation.

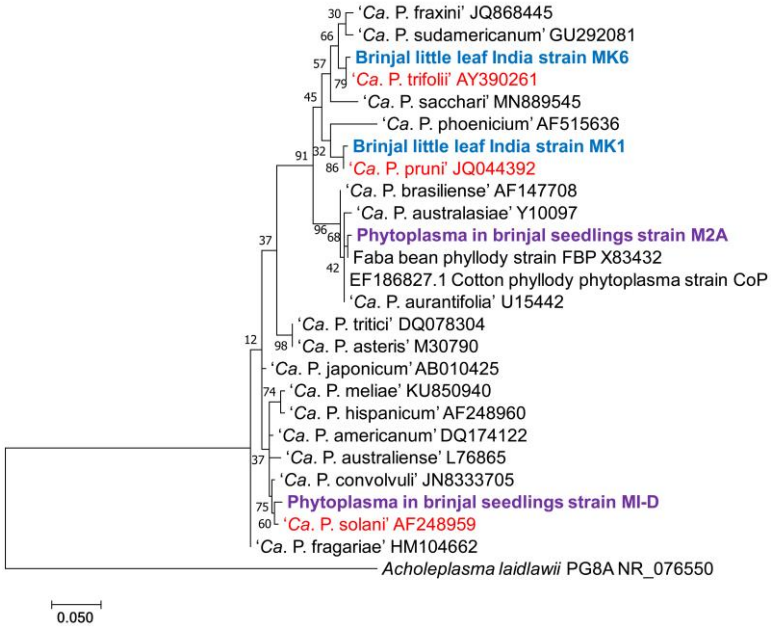
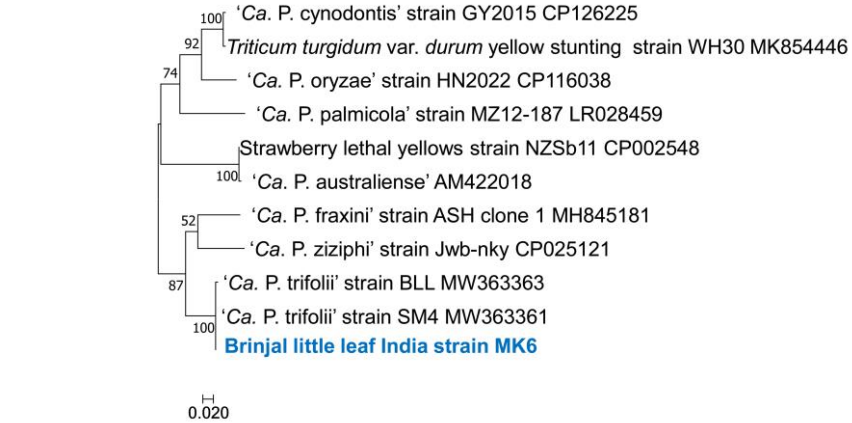


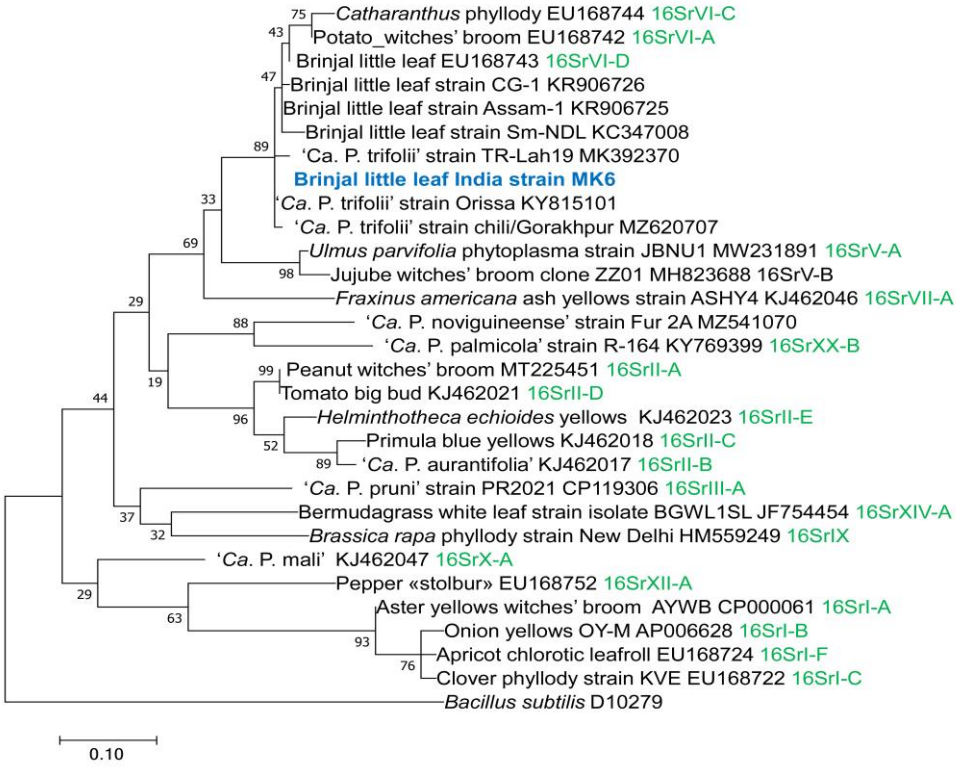
Figure 5. Phylogenetic analysis by maximum likelihood method. The percentage of trees in which the associated taxa clustered together is shown next to the branches. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 26 nucleotide sequences. *Acholeplasma laidlawii* is used as an outgroup to root the tree; in blue, the sequences of phytoplasma strains identified in the mother plants; in purple, the ones identified in seedlings; and in red, the reference strains used for classification [7].

Furthermore, the *leuS* and *secA* genes from the strain of '*Ca*. P. trifolii' detected in the field-collected brinjal plants were amplified, and after sequencing, resulted as 100% and 99.76%, respectively, identical to sequences of '*Ca*. P. trifolii' strains detected in phytoplas-

mas from India from brinjal (GenBank accession number MW363363) and neem (GenBank accession number MW363361) for the *leu* gene and to phytoplasma strains detected in chili (GenBank accession number MZ620707) and in potato (GenBank accession number EU168742) in India and in cabbage in Turkey (GenBank accession number KY815101) for the *secA* gene (Figure 6).



(**a**)



(**b**)

Figure 6. Phylogenetic analysis by maximum likelihood method in (**a**) sequences from 11 phytoplasma *leu* genes and in (**b**) from 29 *secA* genes from various '*Ca.* Phytoplasma' species available in GenBank. The percentage of trees in which the associated taxa clustered together is shown next to the branches. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. No outgroup is available for the *leu* gene, while for the *secA* gene, the *Bacillus subtilis* sequence is the outgroup. The phytoplasma strain studied (in blue) is from the symptomatic brinjal mother plants. In green 16S ribosomal group affiliation of the phytoplasma strains.

Molecular analyses carried out in the seedlings showed samples positive for phytoplasma presence. In particular, phytoplasmas in groups 16SrI, -II, -V, -VI, and -XII were

detected in first-generation seedlings, while 16SrI and -XII groups were only identified as present in the second-generation seedlings (Table 2). The 16Sr RNA gene sequenced from '*Ca*. P. solani' strains showed 99.25% identity with six SNPs to the reference strain (GenBank accession number AF248959). The 16SrII phytoplasmas (1016 nucleotides) shared 99.00% identity with '*Ca*. P. aurantifolia' (GenBank accession number U15442) (six SNPs and four GAPs). However, the sequence of this phytoplasma also showed 99.40% identity to the faba bean phyllody strain, subgroup 16SrII-C (GenBank accession number X83432), with three SNPs and one GAP. The phytoplasmas in ribosomal groups 16SrI and 16SrXII resulted as present in both generations of seedlings (Table 2).

Table 2. Summary of results from phytoplasma detection in the first- and second-generation eggplant seedlings grown under an insect-proof greenhouse (the yellow background highlight the detection of identical phytoplasmas in first and second generation seedlings).

Seedling Batch	Phytoplasma	Second-Generation Seedling Batch	Phytoplasma
1-4 M1.B	16SrI	M1.B(N/G), M1.B(SM), M1.B(SM)	16SrI
M3.D, M2.5, M8.18	16SrII	M1.A (N/G)	16SrI
M2-M20	16SrV	M1.C(G)	16SrI
M3.B	16SrVI	M3.D(G)	16SrXII
M1.D, M2.A	16SrXII	M5.A(G)	16SrXII

4. Discussion

Phytoplasma-associated diseases in eggplants are mainly reported in Asia, America, and Africa. However, the identified phytoplasmas are different mainly according to their geographic distribution. In detail, phytoplasmas identified in the 16SrI group were detected in Japan, Bangladesh, and India [23–25]; in the 16SrII-D in Oman, Egypt, and India [26–29]; in the 16SrIII-B, -J and -U in Brazil [30,31]; in the 16SrVI-A in Turkey [32]; in the 16SrVI-D in India [28,33]; in the 16SrIX-C in Iran [34]; and the 16SrXII-A in Russia and Turkey [35,36].

The most recent reports of brinjal little leaf outbreaks in India, with an incidence between 8 and 30%, were reported in three districts of Uttar Pradesh during 2015 and 2016 [37]; however, the yield losses for the disease in this crop very often reach 100% in India [10]. In Telangana; Tamil Nadu and Tirupati; Andhra Pradesh; New Delhi; Uttar Pradesh; Maharashtra and Chhattisgarh; Kerala; Mysore; and Dharwad, South Gujarat, the phytoplasmas associated with brinjal little leaf were identified as belonging to the 16SrVI group, mainly the 16SrVI-D subgroup [38–42]. However, in Andhra Pradesh, 16SrII-D phytoplasmas were also identified [43]. Kumar et al. [25] reported little leaf disease associated with 16SrI phytoplasmas in the brinjal fields of Bihar. A recent phytoplasma survey in various brinjal production areas of Hoshangabad in Central India resulted in the first report of a group of 16SrV phytoplasmas [44]. In Assam, the identified brinjal little leaf phytoplasma belongs to the 16SrVI group, and a phytoplasma of the same group was also detected in the leafhopper *Hishimonus phycitis*, suggesting its role as a vector of this phytoplasma in eggplants [45]. The fields (Dharwad district) where the seeds were collected were severely affected by this phytoplasma disease, which is a major concern for the farmer's economic revenue losses in India. Two phytoplasmas were identified, and one (16SrIII group) was never reported before in eggplants in India, while the 16SrVI group has already been reported as one of the main phytoplasma associated with this diseases in Indian eggplant cultivations [38–42].

This research extends the information for growing conditions and provides detailed molecular features of phytoplasmas detected in eggplant seedlings and pregerminated seeds under insect-proof greenhouse, confirming reports on seed transmission in other solanaceous crops [5,11,12,46]. The transmission of phytoplasmas by seed was, for a long time, considered a controversial issue. Some researchers argued that it was not possible due to the lack of a direct connection between the phloem system and the embryos [47]. The

frequent association of flower abnormalities and fruit malformations with the presence of phytoplasmas led researchers to believe that seeds from infected plants were not viable and would not germinate. However, phytoplasmas are potentially able to reach other organs connected to the phloem, as they are pleomorphic and have a small size that allows them to pass through the pores of the phloem and be transported by the flow of assimilates and are generally high in concentration in the floral structures of herbaceous host plants [5]. Cordova et al. [48] suggested that the flowers become necrotic, and fruits cannot become ripe in the initial stages of diseased coconut palms. However, in coconuts, the germination rate was higher for seeds from diseased plants, at 72.1%, compared to healthy plants, which had a germination rate of 57.6% [49]. A study found that apricot seeds from plants infected with European stone fruit yellows phytoplasmas showed a germination rate of 9.4% and very low viability (21.6%) [50].

The phytoplasma transmission through seeds was first reported in alfalfa from Oman [5]. Later on, phytoplasma seed transmission was demonstrated in rapeseed, tomato, and corn [5]. A similar research study was carried out on carnations (*Dianthus* spp.) [51], tomatoes (*Solanum lycopersicum* L.), and (Swingle) *Citrus aurantifolia* [52]. These studies found that a low percentage of the plants derived from phytoplasma-infected infected mother plants tested positive for phytoplasma presence. The identification of phytoplasmas in pea plants (*Pisum sativum* L.) germinated and grown in a protected environment and produced from seeds derived from "stolbur" infected plants was also reported [53]. Satta [4] reported that sesame plant seedlings from phytoplasma-infected mother plants exhibited the presence of phytoplasma groups 16SrI, 16SrII, and 16SrXII-A. Chung and Jeong [11] reported that in *Petunia hybrida*, phytoplasmas were detected in seedlings infected with "stolbur" (16SrXII-A). In Turkey, corn seeds infected by '*Ca*. P. cynodontis', sown in an insect-proof greenhouse, produced plantlets positive for the same phytoplasma, indicating its seed transmission [54]. Tomato samples collected in Southern Italy were used to verify seed transmission in the field. Phytoplasmas enclosed in groups 16SrI and 16SrXII were identified in both mother plants and seedlings [12].

The identified phytoplasmas belong to the groups 16SrI (aster yellows), 16SrII (peanut witches' broom), 16SrIII (X-disease), 16SrV (elm yellows), 16SrVI (clover proliferation), 16SrXII ("stolbur") and are reflecting the phytoplasmas identified so far in this plant species. The phytoplasmas most frequently identified in eggplants showing little leaves in India belong to the 16SrVI-D and 16SrII-D subgroups [10,55] and this result is confirmed by the detection of both phytoplasmas in first-generation seedlings. However, other ribosomal groups are associated with the little leaf of brinjal throughout the world; in particular, several subgroups and variants were recently reported from Iran by using nucleotide sequence comparisons and virtual RFLP analyses. In particular, several subgroups, including 16SrI, 16SrII-D and 16SrII-V, 16SrIII-J and 16SrIII-U, 16SrIX-C, 16SrVI-A, and 16SrXII-A, and molecular variants related to the 16SrII-D, 16SrVI-A, and 16SrIX-C subgroups were identified in eggplants showing diverse symptoms [56].

As eggplant is a tropical crop, the growth of the seedlings was quite challenging in the greenhouse, which made it not possible to appreciate the presence of symptoms. The percentages of germination and the survival of seeds after transplanting were above 90%, suggesting no interference in the process from the phytoplasma presence. Satta [4] states that germination percentage is variable according to the precocity of the infection in mother plants. Differences in the germination percentage from seeds produced from infected mother plants are reported as 72.1% for corn seeds from the diseased plants compared to 57.6% for the corresponding healthy plants [57]. However, an irregular growth rate is observed during the early stages of the seedlings' growth. The ribosomal groups of phytoplasmas detected in the early growth stages are not always the same as those identified in later stages, probably due to both the sampling not always being homogeneous and the testing using plant batches. However, this study has revealed that phytoplasmas in ribosomal groups 16SrI and 16SrXII were also detected in the eggplant

progeny of second-generation seedlings, confirming a similar result reported for tomato seeds [12,53].

Though the phytoplasma transmission rate reported in other crop species is relatively low (1–3%) [5], it can lead to serious outbreaks in the presence of insect vectors. This will also affect the natural environment, making this type of phytoplasma transmission of considerable concern for food and environmental safety. The reported presence of a second-generation infection by seeds increases this spreading risk and emphasizes the importance of acquiring comprehensive knowledge on phytoplasma seed transmission. This is of utmost relevance to develop effective management strategies and mitigate the impact of phytoplasma-associated diseases.

Worldwide phytoplasma diseases produce significant yield losses in all main solanaceous crops, especially in countries with not yet advanced agricultural technologies. In brinjal the average loss is 40%; in tomato, 60%, in pepper, up to 93% in potato, 30–80% and in cucumber it may reach 100% [56,57]. The most recorded disease is the potato "stolbur" reported from 1950 to the 1960s, associated with severe epidemics across Europe. Severe outbreaks are described in the Czech Republic, Hungary, and Romania, with yield losses of 30 to 80% [35,57]. However, limited studies have been performed in India and worldwide about losses associated with phytoplasma infection in both horticultural and agricultural crops such as brinjal (eggplant), sesame, sugarcane, areca nut, and coconut palms. The first demonstration of the seed transmission of phytoplasmas in eggplants for two subsequent generations highlights the risk of additional sources of infection represented by asymptomatic and infected seedlings. Under field conditions, this may also contribute to explaining the epidemic spread of phytoplasmas in brinjal cultivations in India and in other growing areas of the world.

5. Conclusions

As eggplant is a tropical crop, the growth of the seedlings was somewhat challenging in the greenhouse, which made it difficult to evaluate the presence of typical symptoms. Phytoplasmas belonging to five ribosomal groups were found in the first-generation seedlings from infected mother plants. This study indicates that phytoplasmas in several ribosomal groups are successfully transmitted to eggplant progeny for two growing cycles. The transmission of phytoplasmas through seeds is a critical issue because seeds from diseased plants can negatively impact the quality and quantity of brinjal cultivations. As the research has demonstrated, most seeds grow viable and germinate, thereby promoting the spread of the phytoplasma infection. Although the transmission percentage reported in other crop species is not high (1–3%) [5], it can cause an outbreak by insect vectors, which spreads the disease in the surrounding natural and agricultural environments, causing epidemics in agricultural and landscape environments. Additional studies are, therefore, needed to understand the phytoplasma persistence from seedlings to mature plants. The epidemiological role of phytoplasma seed transmission is basic knowledge to be considered for the efficient planning of management strategies for healthy, high-yield, and quality agricultural and horticultural production with increasing environmental safety.

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