Eur J Plant Pathol (2021) 161:195–205
https://doi.org/10.1007/s10658-021-02314-8

# Molecular diversity of phytoplasmas associated with eggplant phyllody disease in Iran

**Mohammad Salehi · Seyyed Alireza Esmaeilzadeh-Hosseini · Elham Salehi · Assunta Bertaccini**

Accepted: 10 June 2021 / Published online: 19 June 2021


**Abstract** During 2015–18, surveys were conducted in the main eggplant growing areas of Iran and in all areas phytoplasma-type symptoms were observed. A total of 350 symptomatic eggplant plants were collected and tested for the phytoplasma presence on 16S rDNA. Diversity of the detected phytoplasmas was verified by molecular analyses, dodder and graft transmission on experimental test plants. Phytoplasmas were detected in all symptomatic samples and, by using nucleotide sequence comparisons and virtual restriction fragment length polymorphism analyses of 16S rDNA, six subgroups including 16SrII-D and -V, 16SrIX-C and -I, 16SrVI-A and 16SrXII-A and molecular variants related to 16SrII-D, 16SrVI-A, 16SrIX-C subgroups were identified. Based on symptomatology in dodder and graft inoculated eggplant and periwinkle plants, the phytoplasmas enclosed in the identified subgroups were differentiable. Collectively, based on the results of the present study and considering the reported presence of phytoplasmas belonging to the same ribosomal subgroups in other crops, eggplant fields play an important role in the epidemiology of other diseases associated with these phytoplasmas in Iran.

**Keywords** *Solanum melongena* · 16SrII-D · 16SrII-V · 16SrVI-A · 16SrXII-A · 16SrIX-C · 16SrIX-I

M. Salehi · E. Salehi
Plant Protection Research Department, Fars Agricultural and Natural Resources Research and Education Centre, AREEO, Zarghan, Iran

S. A. Esmaeilzadeh-Hosseini (✉)
Plant Protection Research Department, Yazd Agricultural and Natural Resources Research and Education Centre, AREEO, Yazd, Iran
e-mail: phytoplasma.iran@gmail.com

A. Bertaccini
Department of Agricultural and Food Sciences, Alma Mater Studiorum, University of Bologna, Bologna, Italy

Phytoplasmas are associated with different destructive plant diseases worldwide (Bertaccini et al., 2014) and are transmitted mainly by leafhoppers, however they could be disseminated also by propagation materials and in several cases by seeds (Satta et al., 2019). Phytoplasma presence is associated with symptoms of yellowing, discoloration, witches' broom, dwarfing, virescence, and phyllody. More than 1000 plant species from different plant families are reported as affected by phytoplasmas (Bertaccini & Duduk, 2009; Lee et al., 2000) and among them, vegetables growing in the major production areas worldwide, are infected by phytoplasmas belonging to numerous ribosomal groups (Kumari et al., 2019). In particular, eggplant (*Solanum melongena* L.) was reported as infected with strains belonging to 16SrI in Japan, Bangladesh and India (Kelly et al., 2009; Kumar et al., 2012; Lee et al., 1998; Okuda et al., 1997), 16SrII in Oman, Egypt and India (Al-Subhi et al., 2011; Omar & Foissac, 2012; Yadav et al., 2016), 16SrIII in Brazil (Amaral-Mello et al., 2011; Barros et al., 1998), 16SrVI in India, Turkey and Bangladesh (Azadvar & Baranwal, 2012; Sertkaya et al., 2007; Siddique et al., 2001), and 16SrXII in

Romania and Southern Russia (Ember et al., 2011). Eggplant, with harvested areas of 5312 ha, yield of 5419 kg/ha and a production of 5510 tons (FAOSTAT, 2018), is widely cultivated in Iran where the average production ranks the country as fifth in global production. Formerly the association of a 16SrIX-C phytoplasma with eggplant phyllody in Roodan (Hormozgan province of Iran) was reported (Tohidi et al., 2015). The present work reports genetic diversity of phytoplasmas associated with eggplant phyllody disease in several cultivation areas of Iran.

During 2015–2018, sampling of eggplant phyllody was carried out in the major eggplant growing areas of Fars (Khafr, Fassa, Firooz Abad, Sarvestan, Darab), Yazd (Abarkooh), Zanjan (Zanjan), Kerman (Sirjan), Khorasan Razavi (Mashhad), Bushehr (Bushehr, Kangan, Dashtestan) and Hormozgan (Roodan, Bandar Abbas) provinces of Iran (Fig. 1). In each area, five eggplant fields were randomly selected, and sampling was carried out at five points in 1000 $m^2$ field within a 1 $m^2$ by moving on a diagonal transect across each field. The percentage of eggplant phyllody disease incidence was calculated by number of plants with symptoms out of the total number of plants present within a 1 $m^2$ multiplied by 100. From each field, five eggplant phyllody affected plants were potted, transferred to greenhouse for disease transmission and molecular analyses.

After phytoplasma identification from potted eggplants, one representative of each phytoplasma subgroup identified was dodder transmitted from two infected eggplants to 10 seed-grown 3-month-old periwinkle plants (Salehi, Esmailzadeh Hosseini, Salehi, & Bertaccini, 2016e) under insect-proof conditions. For graft transmission, small axillary shoots from a symptomatic eggplant (representative of an identified subgroup) were used as scions and side grafted on five 12-week-old seed grown eggplant plants. Each rootstock received two scions. Grafted areas were wrapped with parafilm and plants were covered with plastic bags for a week to maintain humidity. Healthy seed grown eggplant and periwinkle plants (five plants per each trial) were left as healthy controls. Presence of phytoplasmas in dodder and graft inoculated plants was confirmed by nested PCR assay.

Total DNA was extracted from 0.2 g of midrib tissue of eggplant phyllody infected, and dodder and graft inoculated plants using the procedure described by Zhang et al. (1998). Total DNA extracted from symptomless seed-grown eggplant was used as negative control. Positive control was a symptomatic periwinkle plant infected with Fars alfalfa witches' broom phytoplasmas (16SrII-C subgroup) (Salehi et al., 2011). Total DNA samples were tested for phytoplasma presence using primer pair P1/P7 (Deng & Hiruki, 1991; Schneider et al., 1995) followed by R16F2n/R16R2 (Gundersen & Lee, 1996). The molecular weight of the PCR products was estimated by comparison with 100 bp DNA ladder (Fermentas, Vilnius, Lithuania).

The R16F2n/R16R2 primed PCR products of 54 samples from the surveyed areas (one sample per area for which three sequences were screened) were ligated in pTZ57R/T vector and cloned into *Escherichia coli* DH5a cells using InsT / A clone$^{M}$ PCR Product Cloning Kit (Fermentas, Vilnius, Lithuania) according to manufacturer instructions. The presence of the correct size insert was confirmed by restriction endonuclease analysis using *Eco*R1 and *Pst*1 enzymes. Three plasmid DNAs from recombinant colonies were purified using GF-1 PCR Clean-Up Kit (Vivantis, Malysia, HQ) and sequenced. Sequencing was performed by Macrogenon both strands by using M13F/M13R primers (BioNeer, DNA sequencing service, South Korea). The phytoplasma 16Sr DNA partial sequences obtained (1250 bp) were used in Blastn analyses. Virtual RFLP was performed by *i*PhyClassifier (Zhao et al., 2009) to determine the ribosomal subgroup affiliation of the detected phytoplasmas. Partial 16S rDNA sequences of eggplant phyllody phytoplasma strains from Fars [Khafr, Fassa (Nowbandegan, Zahedshahr), Firooz Abad (Jaydasht), Sarvesta, Darab], Yazd (Abarkooh), Zanjan (Zanjan), Kerman (Sirjan), Khorasan Razavi (Mashhad) Bushehr [Bushehr (Bushehr1), Kangan, Dashtestan (Borazjan1, Borazjan2, Borazjan3, Bondarooz)] and Hormozgan (Roodan, Bandar Abbas) obtained from the present study were aligned and phylogenetic trees and sequence homologies were generated using MEGA 6 software (Tamura et al., 2013). *Acholeplasma laidlawii* was used as out-group to root the trees. Bootstrapping was performed 1000 times to estimate the stability and support for the tree branches.

The occurrence of eggplant phyllody was observed in all surveyed areas. The main disease symptoms were little leaf, internode shortening, flower virescence, phyllody, big bud, proliferation and sterility, witches' broom and stunting (Fig. 2). The highest disease percentage observed was 11% in Zahedshahr.

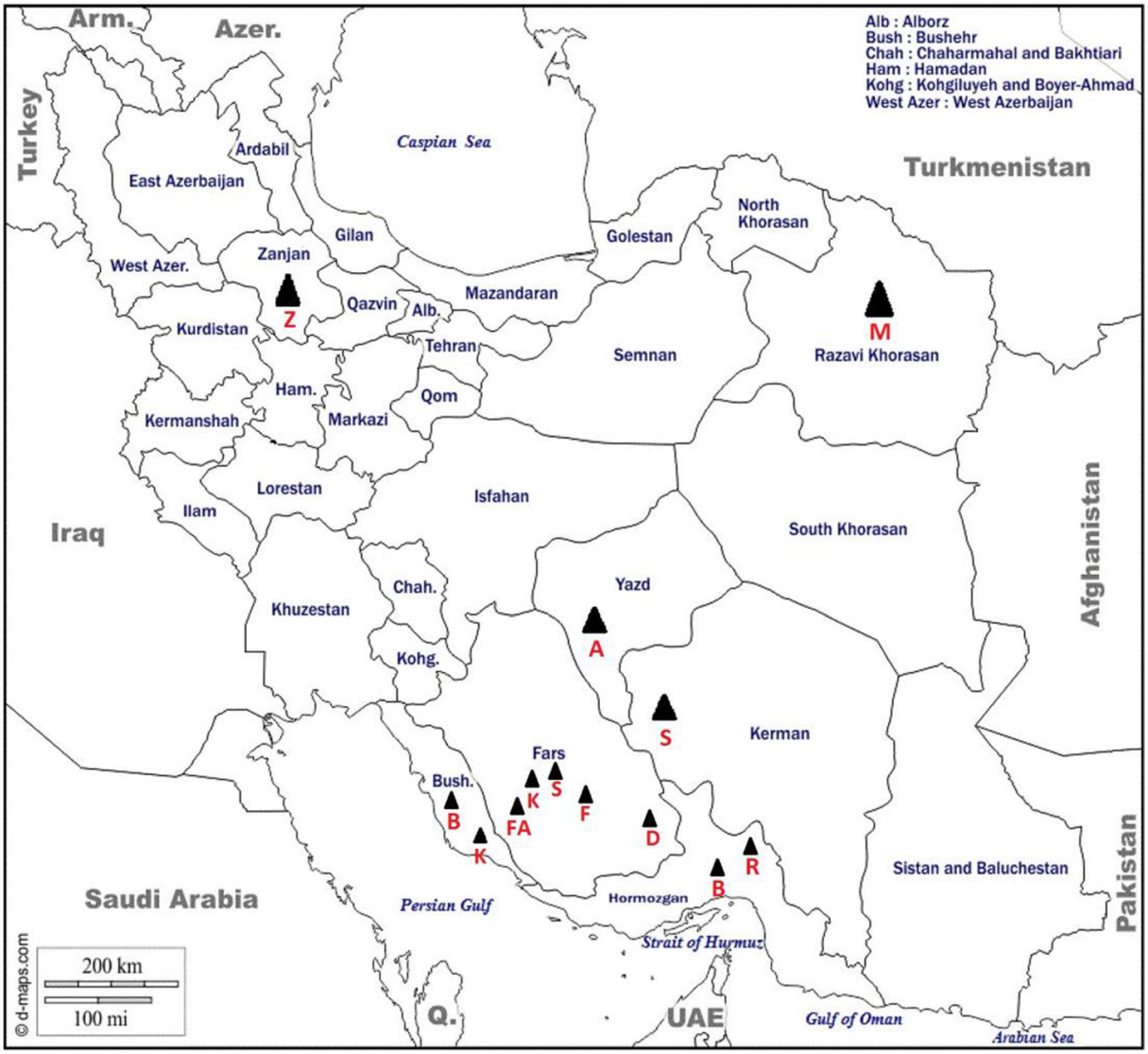


**Fig. 1** Map of Iran showing the sampling locations of the eggplant phyllody. In Fars area, K: Khafr, F: Fassa, FA: Firooz Abad, S: Sarvestan, D: Darab; in Yazd area, A: Abarkooh, in Zanjan, Z: Zanjan; in Kerman area, S: Sirjan, in Khorasan Razavi area, M: Mashhad, in Bushehr area, B: Bushehr, K: Kangan, D: Dashtestan and in Hormozgan area, R: Roodan, B: Bandar Abbas

The disease latency period varied between 6 weeks, in eggplants graft inoculated with 16SrII-A and -V subgroup strains, to 11 weeks in periwinkle plants dodder inoculated with 16SrIX-C strains. After dodder and graft inoculation of eggplant and periwinkle plants, at early stages of infection, there was no significant difference in symptoms among the diverse phytoplasma subgroups, except for 16SrVI-A strain, and the main symptoms were virescence, phyllody and moderate yellowing. At the late stage of infection, phytoplasma subgroups were differentiable from each other for the specific presence of virescence and phyllody (16SrII-A and –V), severe little leaf, internode shortening and stunting (16SrIX-C and –I), plant wilt and death (16SrVI-A), witches' broom and rosettes (16SrXII-A). However, in both eggplant and periwinkle plants 16SrII-A and –V were not differentiable on symptoms from each other, but resulted differentiable from those associated with the presence of phytoplasmas classified in the other ribosomal subgroups (Table 1 and Figs. 3 and 4).

DNA fragments of approximately 1800 and 1250 bp were amplified in direct and nested PCR, respectively

**Fig. 2** Symptoms of eggplant virescence and phyllody in plants from Zanjan (left) and Fasa (right)

from all the symptomatic eggplant plants, but no amplification was obtained from the asymptomatic plants. BLASTn search showed that eggplant phyllody strains were differentiable according to the diverse localities (Table 1) and the phylogenetic tree confirmed that the Iranian eggplant phyllody strains cluster with phytoplasmas enclosed in the diverse ribosomal groups listed above (Fig. 5). Since the sequences from eggplant phyllody phytoplasma samples collected in each province were identical to each other, only one representative of each province was submitted to GenBank (Table 1). Results of virtual RFLP analyses of eggplant phyllody

**Table 1** Ribosomal group/subgroup affiliation of eggplant phyllody disease associated phytoplasmas in Iran and their distribution

| Ribosomal group | Ribosomal subgroup* | Location | GenBank accession numbers |
|---|---|---|---|
| 16SrII | 16SrII-D | Khafr | MT248286 |
| | | Bandar Abbas | MT240535 |
| | 16SrII-D (97%) | Bondarooz | MT248287 |
| | 16SrII-D (97%) | Borazjan3 | MT248288 |
| | 16SrII-V | Syrjan | MT248285 |
| 16SrVI | 16SrVI-A | Abarkooh | MG760570 |
| | | Zanjan | MT240537 |
| | 16SrVI-A (90%) | Mashhad | MT240536 |
| 16SrIX | 16SrIX-C | Borazjan1 | MT248280 |
| | | Roodan | MT248278 |
| | | Darab | MT248276 |
| | | Zahedshahr | MT248275 |
| | 16SrIX-C (91%) | Borazjan2 | MT248284 |
| | 16SrIX-C (97%) | Sarvestan | MT248282 |
| | 16SrIX-C (97%) | Jaydasht | MT248281 |
| | 16SrIX-C (97%) | Nowbandegan | MT248283 |
| | 16SrIX-I | Bushehr1 | MT248274 |
| 16SrXII | 16SrXII-A | Kangan | MT248273 |

*, identity percentage is reported only for the strains in which it was lower than 100% to the sequence of the representative strain of each subgroup

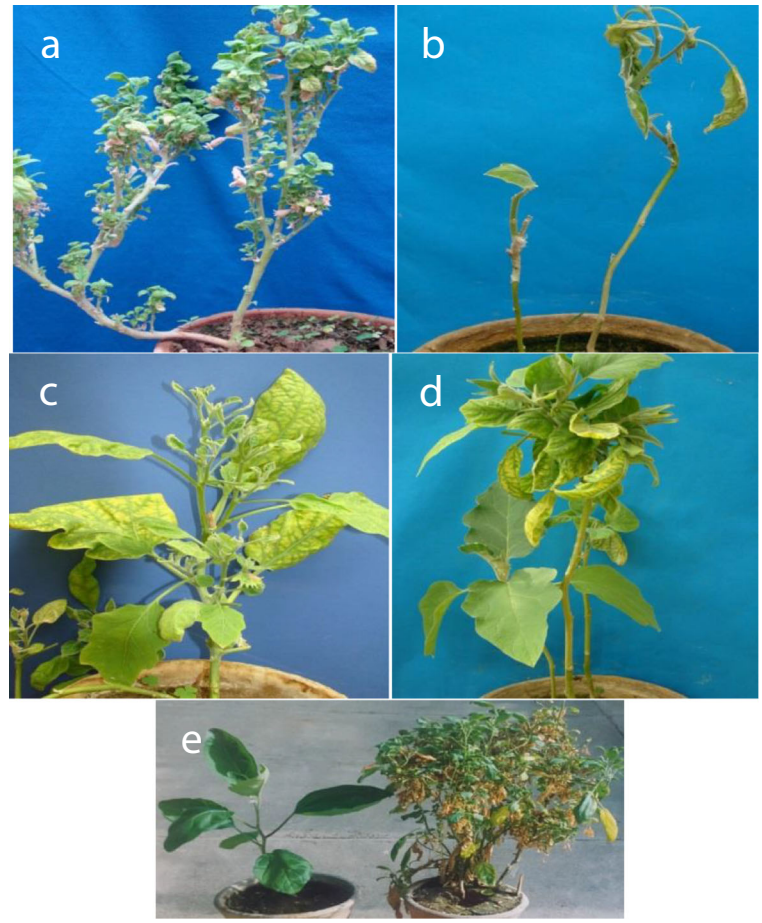


**Fig. 3** Eggplants 3-month-old graft inoculated with different eggplant phyllody phytoplasma strains four months after the grafting; a: 16SrIX-I; b: 16SrVI-A; c: 16SrIX-C; d: 16SrII-A and 16SrII-V; e: 16SrXII-A phytoplasmas. Left in d and e are healthy seed grown eggplants

strains showed the presence of six phytoplasma subgroups including 16SrII-V and -D, 16SrIX-C and -I, 16SrVI-A and 16SrXII-A, and of some that are variants of 16SrII-D, 16SrVI-A, 16SrIX-C (Table 1 and Fig. 6).

In the present study 16SrII-V and -D phytoplasma subgroups were detected; the 16SrII group encloses 22 subgroups (from 16SrII-A to 16SrII-V) and two '*Candidatus* Phytoplasma' species ('*Ca.* P. aurantifolia' and '*Ca*. P. australasia'). The 16SrII-V subgroup is here reported for the first time in Iran in Syrjan (Kerman province) after its description in *Praxelis clematidea* phyllody disease in tropical and subtropical regions of China (Yang et al., 2017).

In Iran the presence of destructive phytoplasma diseases adjacent to eggplant fields may indicate a role in the epidemiology of eggplant phyllody for alfalfa witches' broom (Esmailzadeh Hosseini, Khodakaramian, Salehi, Fani, Bolok Yazdi, et al., 2015a, b, c, Esmailzadeh Hosseini, Khodakaramian, et al., 2016a, b, c; Salehi et al. 2011), tomato witches' broom (Salehi et al., 2014), parsley phyllody (Salehi, Esmailzadeh Hosseini, Salehi, & Bertaccini, 2016f), squash phyllody (Salehi et al., 2015), garden beet witches' broom (Mirzaie et al., 2007), sesame phyllody (Salehi, Esmailzadeh Hosseini, Salehi, & Bertaccini, 2016d), carrot witches' broom (Salehi, Esmailzadeh Hosseini, Salehi, & Bertaccini, 2016e), pot marigold phyllody (Esmailzadeh Hosseini, Salehi, et al., 2016) and pomegranate little leaf (Salehi, Esmailzadeh Hosseini, Rasoulpour, et al., 2016a). Moreover, the 16SrVI-A phytoplasma strains identified in alfalfa witches' broom, *Sophora alopecuroides* yellowing (Esmaeilzadeh-Hosseini et al., 2020; Esmailzadeh Hosseini, Khodakaramian, et al., 2016b), cabbage yellows (Salehi et al., 2007), witches' broom and yellowing in jujube plants (Babaei et al., 2020) and tomato big bud (Davoodi et al., 2019; Salehi, Salehi, & Masoumi, 2016b) were adjacent to eggplant fields in Abarkooh,


**Fig. 4** Periwinkle plants 2-month-old dodder inoculated with different eggplant phyllody phytoplasma strains, four months after the beginning of the transmission trials. a: 16SrIX-C and 16SrIX-I phytoplasmas; b: 16SrVI-A phytoplasma; c: 16SrII-A and 16SrII-V phytoplasmas; d: 16SrXII-A phytoplasma; e: healthy seed grown periwinkle plant

Zanjan and Mashhad where the occurrence of eggplant phyllody was here reported. The phytoplasmas in the 16SrIX–C subgroup also were important in eggplant growing areas and were previously detected in Iran associated with sesame phyllody (Salehi, Esmailzadeh Hosseini, Salehi, & Bertaccini, 2016e), almond witches' broom (Salehi et al., 2006) and grapevine yellows (Salehi, Salehi, Taghavi, & Izadpanah, 2016c) diseases. The 16SrXII-A phytoplasma strains were associated with alfalfa witches' broom, *S. alopecuroides* yellowing (Esmaeilzadeh-Hosseini et al., 2020, Esmailzadeh Hosseini, Khodakaramian, et al., 2016b), *Vitis vinifera* yellows (Salehi, Salehi, Taghavi, & Izadpanah, 2016c) and decline (Ghayeb Zamharir et al., 2017), field bindweed witches' broom (Salehi et al., 2020), tomato witches' broom (Salehi & Esmailzadeh Hosseini, 2016).

Two phytoplasma insect vectors, *Circulifer haematoceps* and *Orosious albicinctus* are found inside the eggplant fields and on many weeds, trees and shrubs in eggplant marginal fields. *C. haematoceps* was reported as vector of eggplant big bud phytoplasma in Iran (Salehi & Izadpanah, 1995). These two insect species in Iran are vectoring several of the phytoplasmas identified in eggplant (Esmailzadeh Hosseini et al., 2007, 2011, 2017; Mirzaie et al., 2007; Salehi et al., 2015; Salehi, Esmailzadeh Hosseini, Salehi, & Bertaccini, 2016d).

The presence of phytoplasmas associated with eggplant phyllody in other crops and of *C. haematoceps* and *O. albicinctus* vectors of different phytoplasma subgroups in Iran provide indication that the eggplant fields may play an important role in the epidemiology of other diseases associated with these phytoplasmas. Collectively, based on the results of the present study and considering the reported presence of phytoplasmas belonging to the same ribosomal subgroups in other crops, eggplant fields contribute to the maintenance and

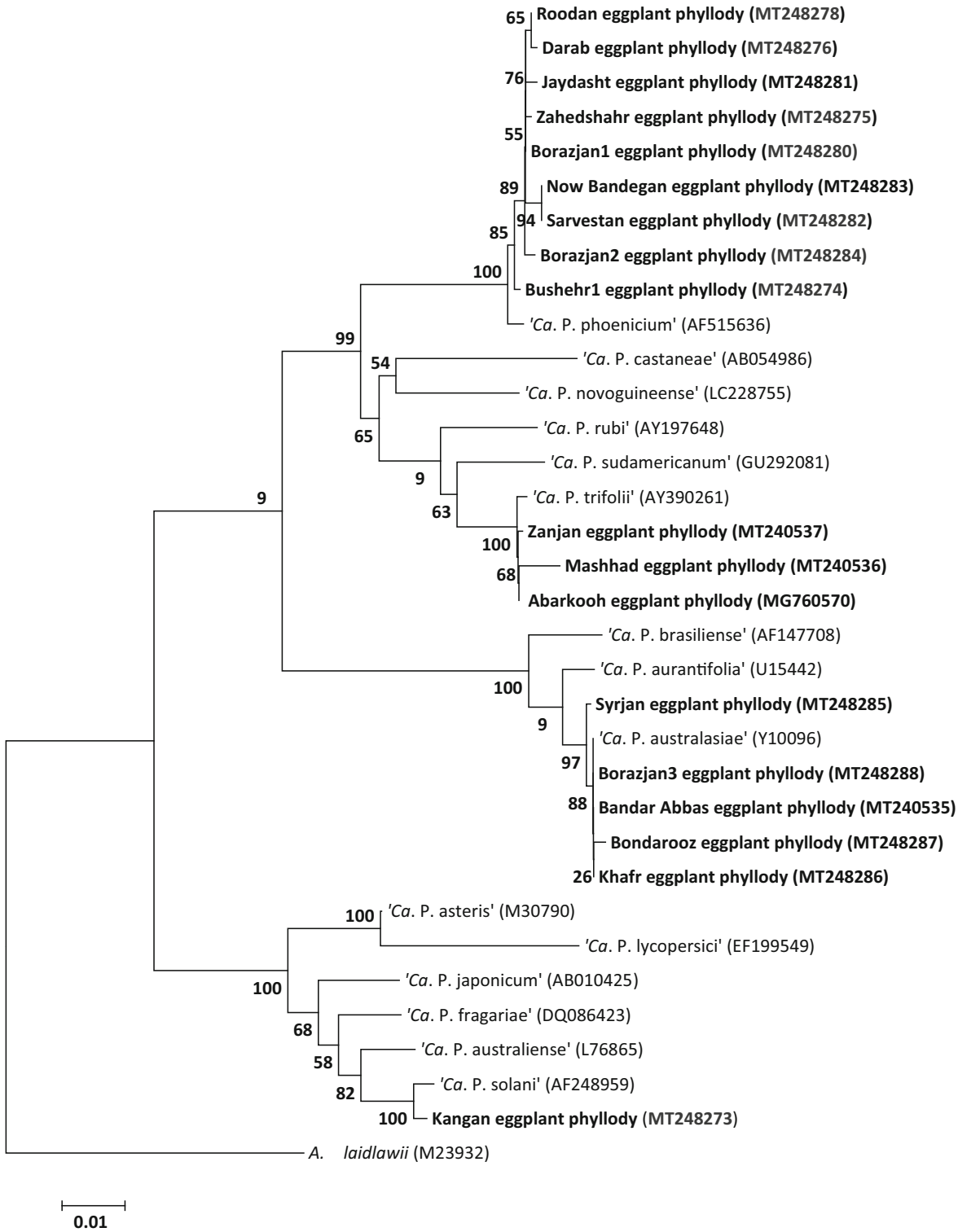


**Fig. 5** Phylogenetic tree constructed by the Neighbor-Joining method using partial 16S rRNA gene sequences (1250 bp) and *Acholeplasma laidlaw*ii as the outgroup; 'Ca. P.': '*Candidatus* Phytoplasma'; numbers at the nodes are bootstrap (confidence) values based on 1000 repetitions; GenBank accession numbers for sequences are given in parentheses following the phytoplasma names, Iranian eggplant phytoplasmas are in bold

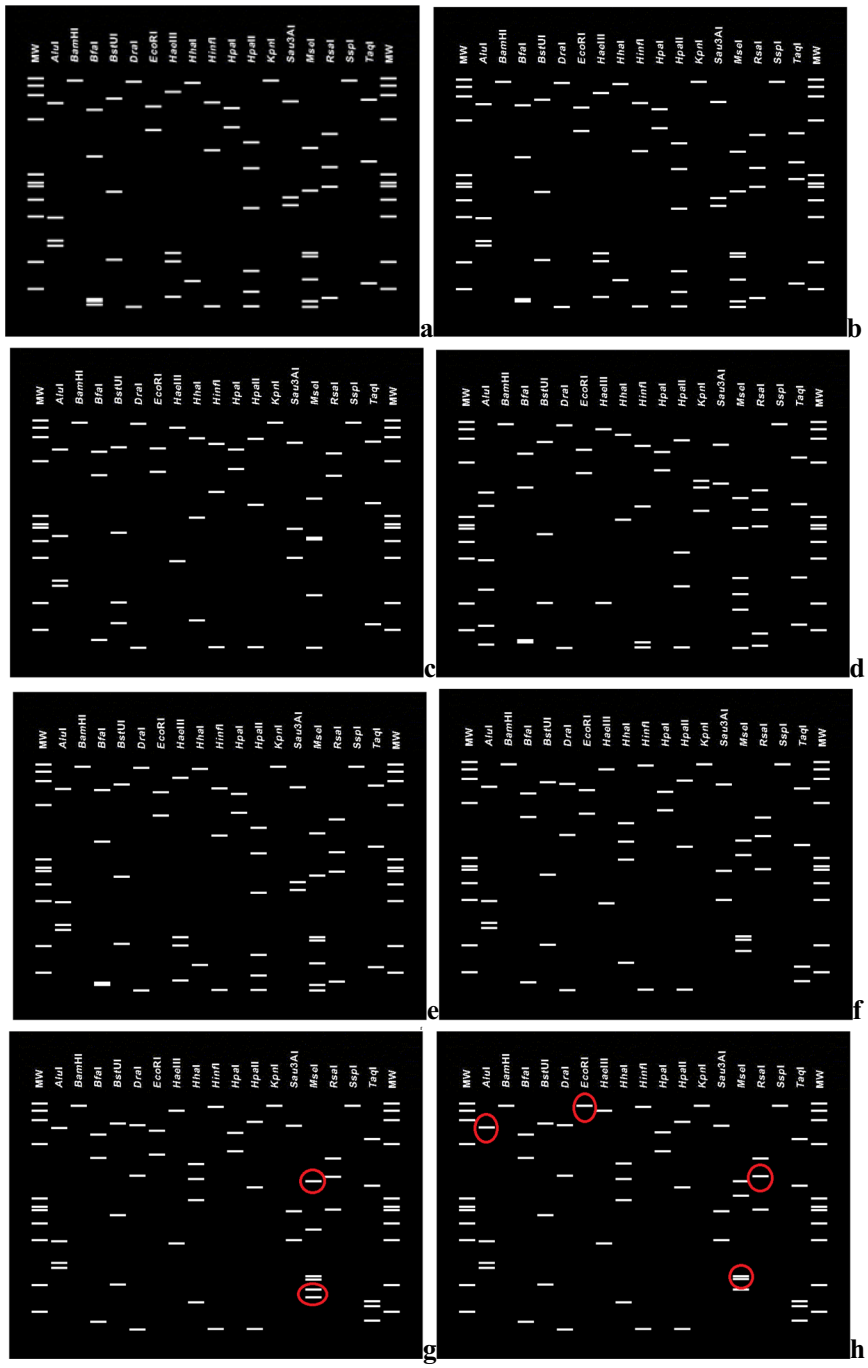


**Fig. 6** Virtual RFLP pictures generated with *i*PhyClassifier from in silico digestion of the R16F2n/R16R2 fragments of the diverse eggplant phytoplasma strains from Iran. In a) 16SrII-D strains Khafr (GenBank accession number, AC: MT248286), Bandar Abbas (AC: MT240535) and Bondarooz (AC: MT248287), Borazjan3 (AC: MT248288). In b) 16SrII-V (AC: MT248285) profiles. In c) strains 16SrVI-A, Yazd (AC: MG760570), Zanjan (AC: MT240537) and Mashhad (AC: MT240536). In d) strain 16SrXII-A, Kangan (AC: MT248273). In e) strains 16SrIX-C Borazjan1 (AC: MT248280); Roodan (AC: MT248278), Darab (AC: MT248276); Zahedshahr (AC: MT248275), and Borazjan2 (AC: MT248284). In f) strain 16SrIX-I, Bushehr1 (AC: MT248274). In g) strains related to 16SrIX-C (97%), Sarvestan (AC: MT248282); Nowbandegan (AC: MT248283). In h) strain related to 16SrIX-C (97%), Jaydasht (AC: MT248281). In g) and h) the red circles indicate enzymes differentiation from the 16SrIX-C profiles of the strains

spreading of diseases associated with these phytoplasmas in Iran.

### Declarations

**Conflict of interest** All authors affirm that 1) there exist no actual or potential conflict of interests to disclose, 2) the manuscript is original and has not been published previously (partly or in full), and is not under review for publication elsewhere, 3) all the necessary local, national and international standards, regulations and conventions, including normal scientific ethical practices, have been duly followed and respected. Additionally, all authors have endorsed the final version of the manuscript before submission.

**Research involving human participants and/or animals** The authors certify that no special permits were required for the field-work investigations. Investigations did not involve any species endangered or protected in Iran.

**Informed consent** All the authors declare that the principles of ethical and professional conduct were duly followed during the execution of this research. The research was funded by Agricultural Research, Education and Extension Organization (AREEO), Iran.

## References

Al-Subhi, A. M., Al-Saady, N. A., Khan, A. J., & Deadman, M. L. (2011). First report of a group 16SrII phytoplasma associated with witches' broom of eggplant in Oman. *Plant Disease, 95*, 360.

Amaral-Mello, A. P. O., Eckstein, B., Flôres, D., Kreyci, P. F., & Bedendo, I. P. (2011). Identification by computer- simulated RFLP of phytoplasmas associated with eggplant giant calyx representative of two subgroups, a lineage of 16SrIII-J and the new subgroup 16SrIII-U. *International Journal of Systematic and Evolutionary Microbiology, 61*, 1454–1461.

Azadvar, M., & Baranwal, V. K. (2012). Multilocus sequence analysis of phytoplasma associated with brinjal little leaf disease and its detection in *Hishimonas phycitis* in India. *Phytopathogenic Mollicutes, 2*, 15–21.

Babaei, G., Esmaeilzadeh-Hosseini, S. A., Zandian, M., & Nikbakht, V. (2020). Identification of phytoplasma strains associated with witches' broom and yellowing in *Ziziphus jujube* nurseries in Iran. *Phytopathologia Mediterranea, 59*(1), 55–61.

Barros, T. S. L., Kitajima, E. W., & Resende, R. O. (1998). Diversidade de isolados brasileiros de fitoplasmas atraves da analise do 16S rDNA. *Fitopatologia Brasilera, 23*, 459–465.

Bertaccini, A., & Duduk, B. (2009). Phytoplasma and phytoplasma diseases: a review of recent research. *Phytopathologia Mediterranea, 48*, 355–378.

Bertaccini, A., Duduk, B., Paltrinieri, S., & Contaldo, N. (2014). Phytoplasmas and phytoplasma diseases: a severe threat to agriculture. *American Journal of Plant Sciences, 5*, 1763–1788.

Davoodi, A., Panjekeh, N., Moslemkhani, K., & Taheri, A. (2019). Detection and molecular characterization of tomato big bud disease in Qazvin province. *Journal of Crop Protection, 8*(4), 379–388.

Deng, S. J., & Hiruki, C. (1991). Amplification of 16S ribosomal RNA genes from culturable and non culturable Mollicutes. *Journal of Microbiological Methods, 14*, 53–61.

Ember, I., Munyaneza, J. E., Crosslin, J. M., & Kolber, M. (2011). Survey and molecular detection of phytoplasmas associated with potato in Romania and southern Russia. *European Journal of Plant Pathology, 130*, 367–377.

Esmailzadeh Hosseini, S. A., Mirzaie, A., Jafari-Nodooshan, A., & Rahimian, H. (2007). The first report of transmission of a phytoplasma associated with sesame phyllody by *Orosius albicinctus* in Iran. *Australasian Plant Disease Notes, 2*(1), 33–34.

Esmailzadeh Hosseini, S. A., Salehi, M., & Mirzaie, A. (2011). Alternate hosts of alfalfa witches' broom phytoplasma and winter hosts of its vector *Orosius albicinctus* in Yazd-Iran. *Bulletin of Insectology*, *64*(Supplement), 247-248.

Esmailzadeh Hosseini, S. A., Khodakaramian, G., Salehi, M., Fani, S. R., Bolok Yazdi, H. R., Raoufi, D., Jadidi, O., & Bertaccini, A. (2015a). Status of alfalfa witches' broom phytoplasma disease in Iran. *Phytopathogenic Mollicutes, 5*(1-Supplement), 65–66.

Esmailzadeh Hosseini, S. A., Khodakaramian, G., Salehi, M., Fani, S. R., Mirchenari, S. M., Salehi, E., & Bertaccini, A. (2015b). Incidence, distribution and economic importance of alfalfa witches' broom disease in Sistan-Baluchestan (Iran) and characterization of associated phytoplasma. *Phytopathogenic Mollicutes, 5*(2), 84–90.

Esmailzadeh Hosseini, S. A., Salehi, M., Khodakaramian, G., Mirchenari, S. M., & Bertaccini, A. (2015c). An up-to-date status of alfalfa witches' broom disease in Iran. *Phytopathogenic Mollicutes, 5*(1), 9–18.

Esmailzadeh Hosseini, S. A., Khodakaramian, G., Salehi, M., & Bertaccini, A. (2016a). Characterization of 16SrII group phytoplasmas associated with alfalfa (*Medicago sativa*) witches' broom disease in diverse areas of Iran. *Journal of Crop Protection, 5*(4), 581–590.

Esmailzadeh Hosseini, S. A., Khodakaramian, G., Salehi, M., & Bertaccini, A. (2016b). First report of 16SrVI-A and 16SrXII-A phytoplasmas associated with alfalfa witches' broom diseases in Iran. *Journal of Plant Pathology, 98*(2), 369.

Esmailzadeh Hosseini, S. A., Khodakaramian, G., Salehi, M., & Bertaccini, A. (2016c). Molecular identification and phylogenetic analysis of phytoplasmas associated with alfalfa witches' broom diseases in the western areas of Iran. *Phytopathogenic Mollicutes, 6*(1), 16–22.

Esmailzadeh Hosseini, S. A., Salehi, M., Mirchenari, S. M., & Bertaccini, A. (2016). First report of a 16SrII-D phytoplasma associated with *Calendula officinalis* phyllody in Iran. *New Disease Reporter, 34*, 22.

Esmailzadeh Hosseini, S. A., Khodakaramian, G., Salehi, M., & Bertaccini, A. (2017). Biological, serological and molecular characteristics of two 16SrII-C related phytoplasma strains associated with alfalfa witches' broom disease in Yazd and

Fars provinces, Iran. *Iranian Journal of Plant Pathology, 53*(2), 165–174.

Esmaeilzadeh-Hosseini, S. A., Satta, E., Babaei, G., Salehi, M., & Bertaccini, A. (2020). Occurrence of '*Candidatus* Phytoplasma omanense'-related strains and other phytoplasmas in *Sophora alopecuroides* plants showing dwarfing and yellowing. *Australasian Plant Pathology, 49*, 403–411.

Faostat (2018) Food and agriculture organization corporate statistical database. [online] URL: http://faostat3.fao.org

Ghayeb Zamharir, M., Paltrinieri, S., Hajivand, S., Taheri, M., & Bertaccini, A. (2017). Molecular identification of diverse '*Candidatus* Phytoplasma' species associated with grapevine decline in Iran. *Journal of Phytopathology, 165*, 407–413.

Gundersen, D. E., & Lee, I.-M. (1996). Ultrasensitive detection of phytoplasmas by nested-PCR assays using two universal primer pairs. *Phytopathologia Mediterranae, 35*, 144–151.

Kelly, P. L, Arocha, Y., Dider, S. Z (2009). First report of a 16SrI, '*Candidatus* Phytoplasma asteris' isolate affecting eggplant and *Mikania* sp. in Bangladesh. *New Disease Reports, 18*, 52.

Kumar, J., Gunapati, S., Singh, S. P., Lalit, A., Sharma, N. C., & Tuli, R. (2012). First report of a '*Candidatus* Phytoplasma asteris' (16SrI group) associated with little leaf disease of *Solanum melongena* in India. *New Disease Reports, 26*, 21–21.

Kumari, S., Nagendran, K., Rai, A. B., Singh, B., Rao, G. P., & Bertaccini, A. (2019). Global status of phytoplasma diseases in vegetable crops. *Frontiers in Microbiology, 10*, 1349.

Lee, I.-M., Gundersen-Rindal, D. E., Davis, R. E., & Bartoszyk, I. M. (1998). Revised classification scheme of phytoplasmas based on RFLP analyses of 16S rRNA and ribosomal protein gene sequences. *International Journal of Systematic Bacterioloy, 48*, 1153–1169.

Lee, I-M., Davis, R. E., & Gundersen-Rindal, D. E. (2000). Phytoplasma: phytopathogenic mollicutes. *Annual Revue of Microbiology, 54*, 221–255.

Mirzaie, A., Esmailzadeh Hosseini, S. A., Jafari-Nodooshan, A., & Rahimianm, H. (2007). Molecular characterization and potential insect vector of a phytoplasma associated with garden beet witches' broom in Yazd, Iran. *Journal of Phytopathology, 155*(4), 198–203.

Okuda, S., Prince, J. P., Davis, R. E., Dally, E. L., Lee, I-M., Mogen, B., & Kato, S. (1997). Two groups of phytoplasma from Japan distinguished on the basis of amplification and restriction analysis of 16S rDNA. *Plant Disease, 81*, 301–305.

Omar, A. F., & Foissac, X. (2012). Occurrence and incidence of phytoplasmas of the 16SrII-D subgroup on solanaceous and cucurbit crops in Egypt. *European Journal of Plant Pathology, 133*, 353–360.

Salehi, M., & Izadpanah, K. (1995). *Big bud of tomato and eggplant in Fars*. Proceedings of the 12th Iranian Plant Protection Congress 2-7 September, 127.

Salehi, E., Salehi, M., Taghavi, S. M., & Izadpanah, K. (2014). 16SrII-D phytoplasma strain associated with tomato witches' broom in Bushehr province. *Iran Journal of Crop Protection, 3*(3), 377–388.

Salehi, M., Izadpanah, K., & Heydarnejad, J. (2006). Characterization of a new almond witches' broom phytoplasma in Iran. *Journal of Phytopathology, 154*(7–8), 386–391.

Salehi, M., Izadpanah, K., & Siampour, M. (2007). Characterization of a phytoplasma associated with cabbage yellows in Iran. *Plant Disease, 91*(5), 625–630.

Salehi, M., Izadpanah, K., Siampour, M., & Esmailzadeh Hosseini, S. A. (2011). Polyclonal antibodies for the detection and identification of Fars alfalfa witches' broom phytoplasma. *Bulletin of Insectology*, *64*(Supplement): 59-60.

Salehi, M., Siampour, M., Esmailzadeh Hosseini, S. A., & Bertaccini, A. (2015). Characterization and vector identification of phytoplasmas associated with cucumber and squash phyllody in Iran. *Bulletin of Insectology, 68*, 311–319.

Salehi, M., & Esmailzadeh Hosseini, S. A. (2016). The first report of a 16SSrII-A phytoplasma associated with tomato big bud disease in Iran. *Journal of Plant Pathology, 98*(3), 692.

Salehi, M., Esmailzadeh Hosseini, S. A., Rasoulpour, R., Salehi, E., & Bertaccini, A. (2016a). Identification of a phytoplasma associated with pomegranate little leaf disease in Iran. *Crop Protection, 87*, 50–54.

Salehi, M., Esmailzadeh Hosseini, S. A., Salehi, E., & Bertaccini, A. (2016d). Genetic diversity and vector transmission of phytoplasmas associated with sesame phyllody in Iran. *Folia Microbiologica, 62*(2), 99–109.

Salehi, M., Esmailzadeh Hosseini, S. A., Salehi, E., & Bertaccini, A. (2016e). Molecular and biological characterization of a 16SrII phytoplasma associated with carrot witches' broom in Iran. *Journal of Plant Pathology, 98*(1), 83–90.

Salehi, M., Esmailzadeh Hosseini, S. A., Salehi, E., & Bertaccini, A. (2016f). Occurrence and characterization of a 16SrII-D subgroup phytoplasma associated with parsley witches' broom disease in Iran. *Journal of Phytopathology, 164*(11–12), 996–1002.

Salehi, E., Salehi, M., & Masoumi, M. (2016b). Biological and molecular characterization of the phytoplasma associated with tomato big bud disease in Zanjan province, Iran. *Iranian Journal of Plant Pathology, 52*(3), 415–427.

Salehi, E., Salehi, M., Taghavi, S., & Izadpanah, K. (2016c). First report of a 16SrIX group (pigeon pea witches' broom) phytoplasma associated with grapevine yellows in Iran. *Journal of Plant Pathology, 98*(2), 376.

Salehi, M., Esmaeilzadeh-Hosseini, S. A., Salehi, E., Quaglino, F., & Bianco, P. A. (2020). Peach witches` broom, an emerging disease associated with `*Candidatus* Phytoplasma phoenicium` and `*Candidatus* Phytoplasma aurantifolia` in Iran. *Crop Protection, 127*, 104946.

Satta, E., Paltrinieri, S., & Bertaccini, A. (2019). Phytoplasma transmission by seed. In Bertaccini A., Weintraub P.G., Rao G.P. & Mori N. (Eds.). *Phytoplasmas: Plant Pathogenic Bacteria-II Transmission and Management of Phytoplasma-Associated Diseases.*(pp. 131–147). Springer.

Schneider, B., Seemüller, E., Smart, C. D., & Kirkpatrick, B. C. (1995). Phylogenetic classification of plant pathogenic mycoplasma-like organisms or phytoplasmas. In S. Razin & J. G. Tully (Eds.), *Molecular and Diagnostic Procedures in Mycoplasmology* (Vol. 1, pp. 369–380). Academic Press.

Sertkaya, G., Martini, M., Musetti, R., & Osler, R. (2007). Detection and molecular characterization of phytoplasmas infecting sesame and solanaceous crops in Turkey. *Bulletin of Insectology, 60*, 141–142.

Siddique, A. B. M., Agrawal, G. K., Alam, N., & Krishina Reddy, M. (2001). Electron microscopy and molecular characterization of phytoplasmas associated with little leaf disease of

brinjal (*Solanum melongena* L.) and periwinkle (*Catharanthus roseus*) in Bangladesh. *Journal of Phytopathology, 149*, 237–244.

Tamura, K., Stecher, G., Peterson, D., Filipski, A., & Kumar, S. (2013). MEGA 6: molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution, 30*, 2725–2729.

Tohidi, Z., Salehi, M., Ghasemi, S., Khanchezar, A., & Shahamiri, M. (2015). Association of a 16SrIX-C phytoplasma with eggplant phyllody in Iran. *Journal of Crop Protection, 4*, 247–256.

Yadav, V., Mahadevakumar, S., Tejaswini, G. S., Shilpa, N., Amruthavalli, C., & Janardhana, G. R. (2016). First report of 16SrII-D phytoplasma associated with eggplant big bud (*Solanum melongena* L.) in India. *Plant Disease, 100*, 517.

Yang, Y., Jiang, L., Tian, Q., Lu, Y., Zhang, X., & Zhao, W. (2017). Detection and identification of a novel subgroup 16SrII-V phytoplasma associated with *Praxelis clematidea* phyllody disease. *International Journal of Systematic and Evolutionary Microbiology, 67*, 5290–5295.

Zhang, Y. P., Uyemoto, J. K., & Kirkpatrick, B. C. (1998). A small-scale procedure for extracting nucleic acids from woody plants infected with various phytoplasmas for PCR assay. *Journal of Virological Methods, 71*, 45–50.

Zhao, Y., Wei, W., Lee, I-M., Shao, J., Suo, X., & Davis, R. E. (2009). 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, 59*, 2582–2593.