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Occurrence and identification of a '*Candidatus* Phytoplasma asteris' (subgroup 16SrI-F) strain infecting *Lolium rigidum* in Iran

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Summary. From 2016, witches' broom and stunting symptoms were observed in *Lolium rigidum* grown in some fruit tree nurseries in Faragheh (Abarkouh, Yazd province, Iran). Total DNAs were extracted from symptomatic and asymptomatic plants and assessed for phytoplasma presence using direct and nested PCR to detect the 16S ribosomal RNA gene. From all symptomatic *L. rigidum* plant samples, expected length PCR amplicons were obtained. RFLP analysis with informative restriction enzymes showed identical profiles in all the samples resulted positive, that were also consistent with those of one of the subgroups of the aster yellows phytoplasmas (16SrI). The 16S rRNA gene sequence of Faragheh *L. rigidum* bushy stunt strain was 100% identical to some '*Candidatus* Phytoplasma asteris' related strains, and 99.12% similar to the reference '*Ca.* P. asteris' strain. The virtual RFLP pattern was identical (similarity coefficient 1.00) to the pattern of phytoplasmas in subgroup 16SrI-F. This is the first report of occurrence and molecular identification of this phytoplasma strain in *L. rigidum* and indicates a potential phytoplasma reservoir for trees in fruit tree nurseries where insect vectors may be present. This phytoplasma strain has been reported in symptomatic stone fruits in Spain and in potato in Ecuador. Further research on the epidemiology of witches' broom and stunting in *L. rigidum* is required to develop elimination the phytoplasma from areas surrounding agricultural crops and avoid the risks of epidemics.

Keywords. Annual ryegrass, aster yellows, Yazd province, epidemiology.

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INTRODUCTION

Lolium rigidum (*Poaceae*) is an annual ryegrass, that is an important weed but is also planted to a limited extent in some local areas in Iran as fodder for livestock feed. This plant species is native to the Mediterranean region and grows naturally in Europe, Africa, Asia and the Indian sub-continent. It is considered invasive in some regions, such as Australia, where it

was introduced as a forage crop in approx. 1880, but it has since become an economically damaging weed. *Lolium rigidum* is mainly grown as a forage crop, but it may be a host for the human and animal pathogens *Clavibacter* spp. and *Claviceps purpurea* (McKay and Riley, 1993; Wegulo and Carlson, 2011).

Phytoplasmas are plant pathogenic bacteria without cell walls, that are associated with many destructive plant diseases, with a variety of symptoms (Bertaccini *et al*., 2014; Bertaccini, 2022). These pathogens are transmitted by phloem feeding insects (mainly leafhoppers and psyllids) and are identified based on 16S rRNA gene sequences due to difficulty growing these organisms in axenic culture (Contaldo *et al*., 2019). Identified phytoplasma strains are grouped in more than 50 '*Candidatus* Phytoplasma' species that are designated based on 16S rRNA gene sequencing (IRPCM, 2004; Bertaccini *et al*., 2022). Moreover, an RFLP-based system distinguishes phytoplasmas into ribosomal groups and subgroups (Lee *et al*., 1998) discriminating strains with high similarities in the 16S rRNA gene.

Presence of phytoplasmas in *Lolium* species in fields has not been previously reported. Symptoms resembling those associated with the phytoplasma presence were observed in plants growing in stone fruit tree nurseries in Faragheh, Abarkouh, Yazd province, Iran. The present study aimed to determine presence and identity of phytoplasmas associated with witches' broom and stunting symptoms in *L. rigidum*, as first step to devise appropriate disease management and maintain nursery plants free from these pathogens.

MATERIALS AND METHODS

Plant sampling and disease incidence

From 2016, witches' broom and stunting symptoms were repeatedly observed in scattered *L. rigidum* plants grown in stone fruit tree nurseries (mainly apricot, plum and peach) in Faragheh, Iran. A survey was carried out from 2016 to 2018 in 10 fruit tree nurseries. These nurseries were of approx. 500 to 1,000 m^2 each. Disease incidence in each nursery was determined by sampling within 1 m^2, and the disease percentage was calculated as the total number of *L. rigidum* plants with symptoms divided by the total number of *L. rigidum* plants growing in each quadrat. Twelve symptomatic and four asymptomatic *L. rigidum* samples were collected and subjected to molecular studies to determine phytoplasma presence and identity.

Molecular detection of phytoplasma presence

Total DNA was extracted from 0.2 g of midrib tissue of fresh leaves from the sampled *L. rigidum* plants showing witches' broom and stunting and from the asymptomatic plants, using the procedure of Healey *et al.* (2014). Total DNA extracted from a witches' broom-symptomatic *Medicago sativa* plant infected by a 16SrII-C phytoplasma strain was used as positive control (Salehi *et al*., 2011). The quality and quantity of extracted total DNA was estimated by spectrophotometer and agarose gel electrophoresis (Green and Sambrook, 2012), and 100 ng of nucleic acids were used for each sample as PCR template. One µL of the P1/P7 PCR product (Deng and Hiruki, 1991; Schneider *et al*., 1995) diluted 1:30 with sterile deionized water, was amplified in nested PCR with R16mF2/R16mR2 and R16F2n/R2 primer pairs (Gundersen and Lee, 1996). The PCR reactions were carried out in 50 µL mixtures, as described by Esmaeilzadeh-Hosseini *et al*. (2020). Five µL of each reaction mixture were electrophoresed in a 1% (w/v) agarose gel in TBE buffer, and were visualized after ethidium bromide staining, using a UV imaging system (Isogene Life Science, Netherlands). The sizes of the PCR products were estimated by comparison with a 100 bp DNA ladder (Biobasic, Canada). The R16F2n/R2 amplified products from *L. rigidum* witches' broom were digested separately with *Mse*I, *Hha*I, *Alu*I, *Hae*III, *Rsa*I, *Hpa*II, *Taq*I and *Kpn*I restriction enzymes, according to the manufacturer's instructions (Thermo Fisher Scientific, USA). These enzymes were selected to compare the main differential profiles reported for aster yellows phytoplasmas (16SrI). The restriction products were separated by 8% polyacrylamide gel electrophoresis, then stained by ethidium bromide and visualized using the UV imaging system (above) for comparison with the reported 16S rDNA pattern profiles of previously described phytoplasma strains (Lee *et al*., 1998).

Sequencing and phylogenetic analyses

Only samples from two of the surveyed nurseries were positive for phytoplasmas for all the 12 symptomatic samples collected. These positive samples showed identical RFLP profiles. The R16mF2/R16mR2 primed PCR products of the nested PCR (1.4 kb) from four randomly selected *L. rigidum* witches' broom and stunting samples were directly sequenced from both ends (Macrogen, South Korea), using the same primers as for the nested amplification. The assembled sequences (DNA BASER assembler program) were compared with sequences deposited in the GenBank database using BLAST analyses at the National Center for Biotechnology Information (NCBI) and were

aligned with the BioEdit 7.2 tool. The comparison with the reference strain of '*Ca*. P. asteris (GenBank accession number M30790) showed 99.12% similarity, identifying the strains as '*Ca*. P. asteris'. A number of other phytoplasma strains showed identity percentages greater than this to the *L. rigidum* witches' broom phytoplasmas and those with 100% similarity were the strains AVUT (GenBank accession number LB388958), AAY (apricot aster yellows operon A, GenBank accession number AB639057), and *Psammotettix* sp. B4 (GenBank accession number MZ458767). The 1,246 bp of 16S rDNA sequences of '*Ca*. Phytoplasma' (Bertaccini *et al.,* 2022) and those of selected '*Ca*. P. asteris' strains, including the *L. rigidum* witches' broom and stunting phytoplasma Faragheh strain of the present study, were aligned using MEGA7 software (Kumar *et al*., 2016). A phylogenetic tree was constructed using the neighbor-joining method (Saitou and Nei, 1987) in MEGA7, with *Acholeplasma laidlawii* as an outgroup to root the tree. Bootstrapping was carried out 1,000 times to estimate stability and support for relationship branches (Felsenstein, 1985). The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura *et al*., 2004). Virtual RFLP analysis using online program *i*PhyClassifier (Zhao *et al*., 2009) was used on the obtained sequences to determine the ribosomal subgroup affiliations of the strains, digesting them *in silico* with the 17 restriction enzymes available in the program.

RESULTS

Figure 1 shows disease symptoms observed in two of the ten nurseries inspected. All the plants of *L. rigidum* showing witches' broom and stunting symptoms were positive for phytoplasmas, while the asymptomatic samples were negative for phytoplasma.

The overall infection percentage of the disease in the two nursery fields was about 4% and was constant in numbers and locations in both the years of the survey. From all symptomatic *L. rigidum* samples, fragments of about 1.8, 1.4 and 1.2 kb were obtained, while from the symptomless plants no amplifications were obtained.

Restriction fragment length polymorphism (RFLP) analysis of the R16F2n/R2 amplicons using informative selected restriction enzymes produced patterns identical to each other and indistinguishable from those of 16SrI group (aster yellows) (Lee *et al.,* 1998) (Figure 2).

The 1.2 kb DNA fragments of the R16F2n/R2 amplicons sequenced from Faragheh *L. rigidum* witches' broom and stunting phytoplasmas were 100% identi-

Figure 1. *Lolium rigidum* witches' broom and stunting symptoms observed in two of the surveyed stone fruit nurseries in Faragheh (Iran).

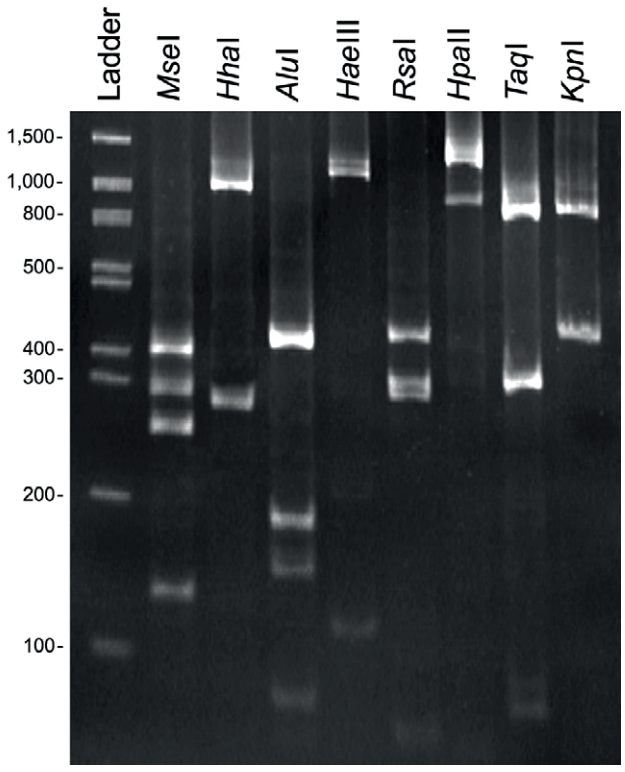

Figure 2. Polyacrylamide gel showing RFLP profiles of 16S rRNA gene fragment a amplified by nested PCR using P1/P7 followed by R16F2n/R2 primer pairs from the *Lolium rigidum* witches' broom and stunting phytoplasma. PCR products were digested by the restriction enzymes listed at the top. Ladder is the 100 bp DNA (Biobasic, Canada).

cal to each other, so one sequence was submitted to the GenBank database under accession number OR365262. The virtual RFLP pattern derived from the R16F2n/R2 fragment of this sequence was identical (similarity coefficient 1.00) to the pattern of the 16SrI-F reference strain in the *i*PhyClassifier (GenBank accession number AY265211) (Figures 3 and 4).

The phylogenetic analysis confirmed that the *L. rigidum* witches' broom and stunting phytoplasma clustered within the '*Ca*. P. asteris' strains enclosed in the subgroup 16SrI-F including phytoplasma strains identified in the putative insect vector *Psammotettix* sp., captured on apricot trees in Turkey (Figure 5).

DISCUSSION

Identification of aster yellows phytoplasmas ('*Ca*. P. asteris', subgroup 16SrI-F) in *L. rigidum* is among the few reports of phytoplasmas in a monocot *Lolium* species. The phytoplasma was detected in nurseries where mainly stone fruit trees are grown. *Lolium rigidum* weeds in apricot seedling nurseries and in general in stone fruit nurseries, cause problems also for weed management. This weed competes with nursery seedlings for water and soil resources, and absorbs many of the fertilizer nutrients required to rapidly produce young fruit tree plants for orchard eastablishment. This competition weakens the fruit tree seedlings and increases their susceptibility to pests and pathogens. Plant pathogens also survive on weeds that act as alternative hosts that are important in disease epidemiology, and can lead to outbreaks of diseases in nurseries and orchards. In the present study cases, *L. rigidum* hosted 16SrI-F phytoplasmas, and could be a source/reservoir for infection by this pathogen.

The only previous reports of phytoplasmas in *Lolium* are in *L. multiflorum* where a phytoplasma 16SrI-L was detected in Lithuania associated with yellowing of leaves and spikes, and general stunting symptoms (Urbanaviciene *et al.,* 2005), and of a 16SrI phytoplasmas in *L. perenne* with similar symptoms in the Russian Federation (Bogoutdinov *et al*., 2021). The symptoms observed in the present study were different from those reported in other agriculturally important monocotledonous plants. These include rice orange leaf and maize leaf reddening (Duduk and Bertaccini, 2006; Jonson *et al*., 2020).

From surveys carried out during the last two decades, the main phytoplasmas identified in Iran were within 16SrI (aster yellows), 16SrII (peanut witches' broom), 16SrIII (X-disease), 16SrVI (clover proliferation), 16SrVII (ash yellows), 16SrIX (pigeon pea witches' broom), 16SrX (apple proliferation), 16SrXI (rice yellow dwarf), 16SrXII ("stolbur"), 16SrXIV (Bermudagrass white leaf), 16SXXIX (*Cassia* witches' broom), and 16SrXXX (salt cedar witches' broom) groups (Esmaeilzadeh Hosseini *et al*., 2023a; 2023b). Aster yellows phytoplasma ('*Ca*. P. asteris', 16SrI) (Lee *et al*., 2004) is the third most widespread phytoplasma identified in Iran, reported from 43 plant species in 19 families. Among the three '*Ca*. Phytoplasma' species described in the 16SrI ribosomal group (*i.e.,* '*Ca*. P. asteris', '*Ca*. P. lycopersici' and '*Ca*. P. tritici') (Bertaccini *et al*., 2022), '*Ca*. P. asteris' and '*Ca*. P. tritici' strains were both detected and identified in this Country. Moreover, for these phytoplasmas four subgroups were reported 16SrI-B, 16SrI-F, 16SrI-R and 16SrI-S (Esmailzadeh Hosseini *et al*., 2023a; 2023b; Salehi *et al*., 2025); however the identified strains are mainly included in subgroup 16SrI-B and have been associated with many diseases including yellowing of *Allium cepa*, phyllody of *Eruca sativa*, little leaf of *Eucalyptus camaldulensis*, phyllody of *Lactuca*

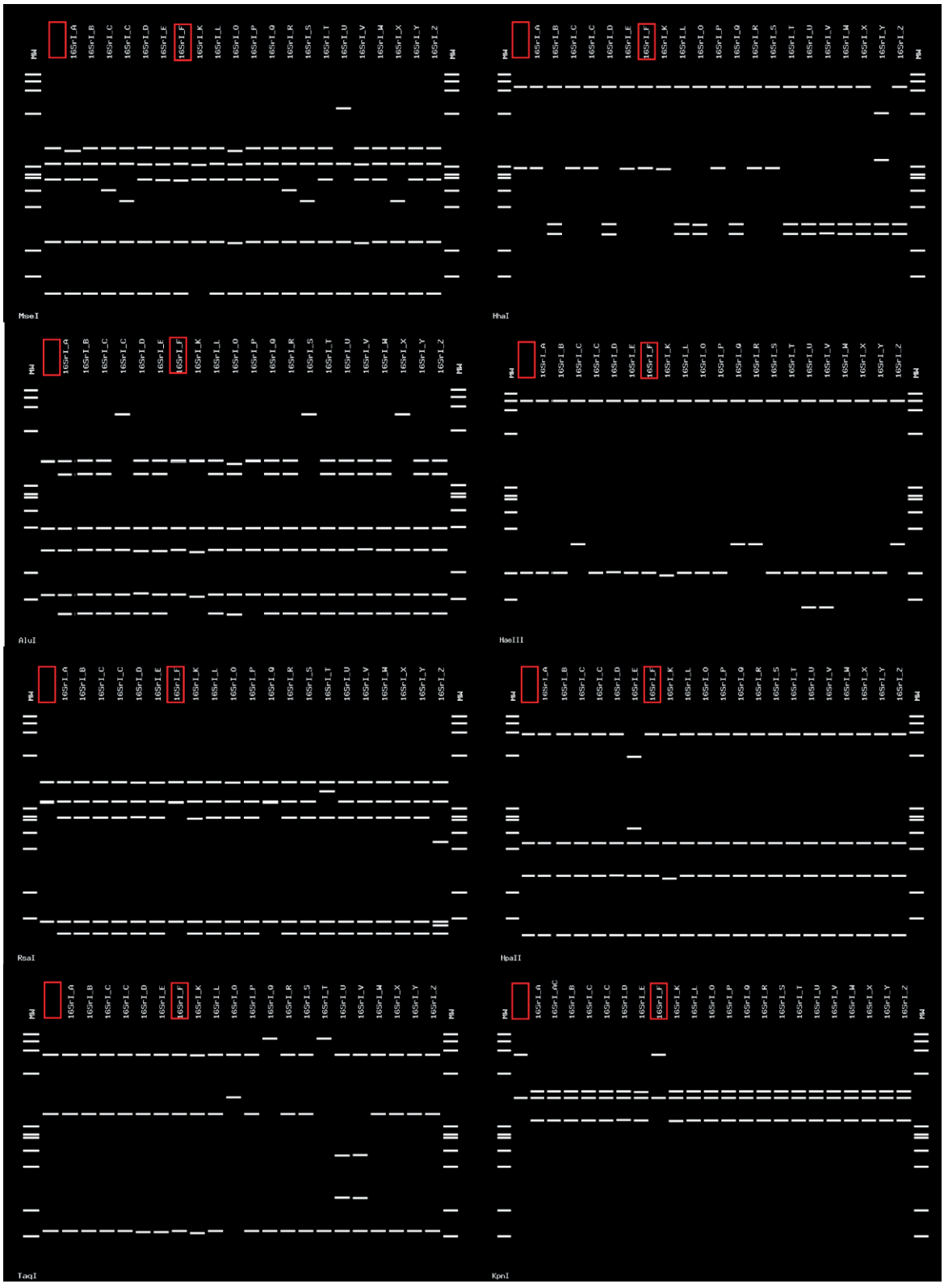

Figure 3. Virtual RFLP patterns generated with the *i*PhyClassifier from *in silico* digestion of the R16F2n/R2 fragment of the *L. rigidum* witches' broom and stunting (GenBank accession number OR365262; lane with no label) with the enzymes used in RFLP on amplicons in Figure 2 confirming that the strain studied is in subgroup 16SrI-F.

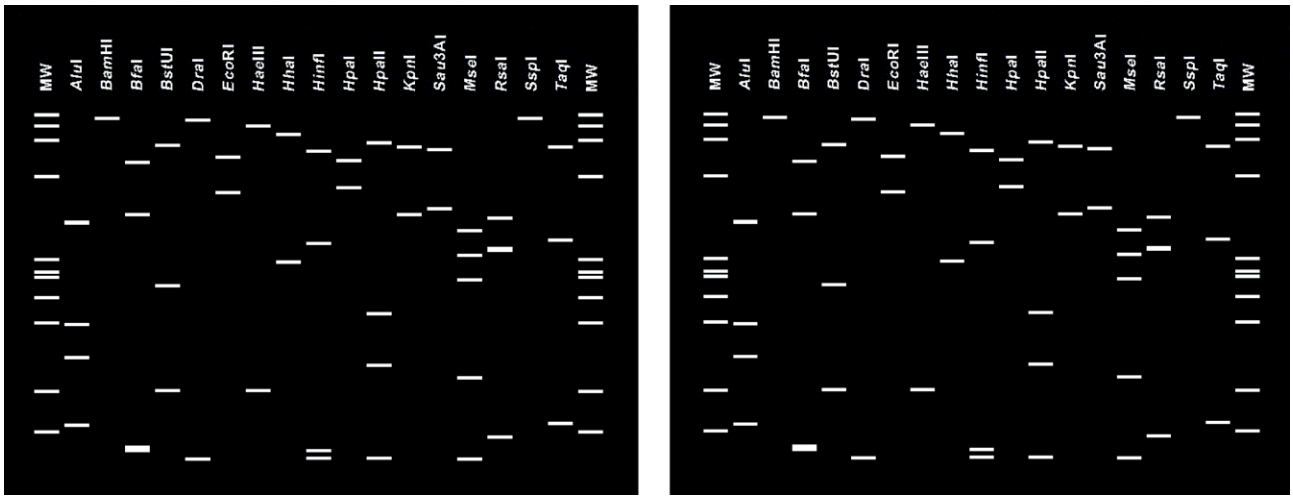

Figure 4. Collective RFLP profile of the Faragheh *L. rigidum* witches' broom and stunting phytoplasma strain on the left (GenBank accession number OR365262) was identical to the pattern for the 16SrI-F reference strain on the right (GenBank accession number AY265211).

sativa, witches' broom of *Morus alba*, *Prunus armeniaca* decline, yellowing of *Rosa canina*, purple top of *Solanum tuberosum*, phyllody of *Sonchus oleraceus*, *Tamarix aphylla* witches' broom, witches' broom of *Tragopogon dubius*, *Vitis vinifera* yellows, and witches' broom and yellowing of *Ziziphus jujuba*. Phytoplasmas in subgroup 16SrI-R was detected and identified in *Aquilegia vulgaris* with phyllody, and subgroup 16SrI-S in *Calendula officinalis* with phyllody (Esmaeilzadeh Hosseini *et al*., 2023a, 2023b). Phytoplasmas belonging to subgroup 16SrI-F were reported in periwinkle plants showing little leaf symptoms in Fars province, Iran (Salehi *et al*., 2025). This phytoplasma has now been identified in *L. rigidum* in Iranian nurseries producing fruit tree propagation materials (apricots). Considering that the same phytoplasma was detected in apricot in Spain, in the putative leafhopper vectors *Psammotettix* sp. in Germany (Bertaccini, 2023) and Turkey (Randa-Zelyut *et al*., 2022), and in *Euscelis incisus* in Serbia (Jakovljevic *et al*., 2020), the present study results have relevance for possible epidemic spread in agricultural situations, since the potential insect vectors are polyphagous. The feeding preferences of these insect vectors and their ability to survive on *L. rigidum* requires further verification. Castillo-Carrillo *et al*. (2018) identified 16SrI-F phytoplasmas in potato plants with purple top symptoms in Ecuador. It is therefore likely that transmission of this phytoplasma from and to nursery trees from herbaceous host species cannot be excluded, although separate surveys in these nurseries have not identified this phytoplasma in trees in Iran. Although stone fruit plants of the investigated nurseries did not have phytoplasma symptoms, there is a risk that this phytoplasma could be transmitted to nurseries.

On the other hand, the presence of the phytoplasma could affect *L. rigidum* when cultivated as forage crop (Oshib Nataj *et al*., 2012), and affect other economically important monocots such as wheat, maize, or barley which are extensively grown in Iran. Since *L. rigidum* is a weed with widespread distribution in this country, its role as phytoplasma reservoir plant should be considered, because a similar role has been demonstrated for phytoplasma-infected alfalfa (Esmailzadeh Hosseini *et al*., 2016) in some areas of Iran. Seeds can be sources of phytoplasma infections in herbaceous hosts including alfalfa, corn, tomato, pea, carrot and eggplant (Calari *et al*., 2011; Zwolinska *et al*., 2012; Satta *et al*., 2020; Mateeti *et al*., 2022, 2023; Gungoosingh Bunwaree *et al*., 2023; Darabakula *et al*., 2024; Bertaccini *et al*., 2025). This makes the detection of 16SrI-F phytoplasmas in *L. rigidum* relevant for management of healthy stone fruit propagation material in Iranian nurseries and in other environments where these crops are grown or where *L. rigidum* is an infesting weed. Large use of herbicides for *L. rigidum* management has resulted in widespread herbicide resistance (Heap, 2019). The majority of *L. rigidum* fields in southern Australian cropping regions are herbicide resistant, based on periodic random surveys (Owen *et al*., 2014), posing threats to elimination of this weed from crops. In some regions environmental temperatures also affect herbicide efficacy. Herbicide resistant *L. rigidum* populations and climate change may provide perennial sources of phytoplasma inoculum, when insect vectors are present. Further research to clarify the epidemiology of diseases associated with 16SrI-F phytoplasmas under Iranian conditions will assist containment of possible disease outbreaks.

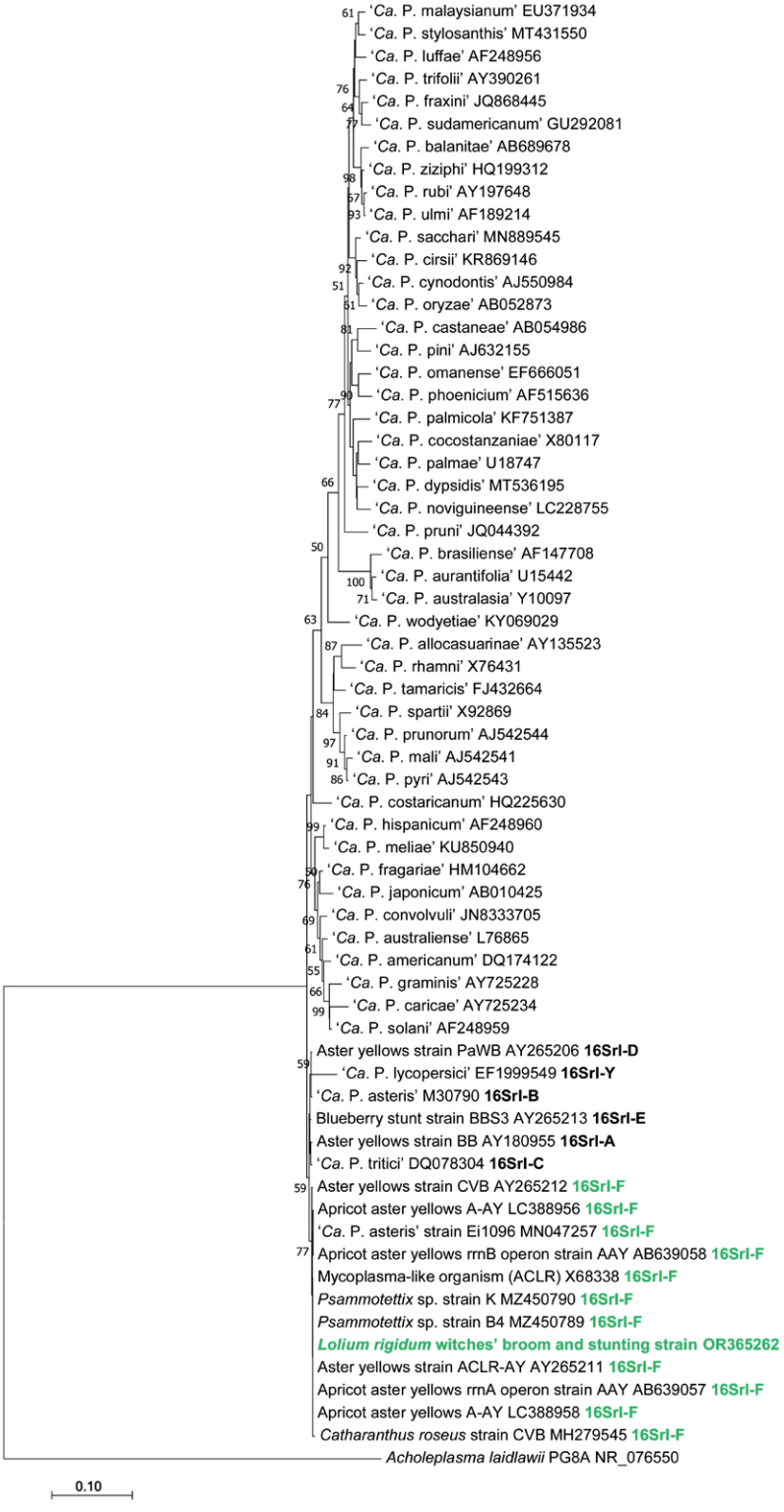

Figure 5. Phylogenetic tree (constructed in MEGA 7) of 16S rRNA gene sequences from 64 phytoplasma strains, and *Acholeplasma laidlawii* as the outgroup. The *Lolium rigidum* witches' broom and stunting phytoplasma and the ribosomal subgroup of related strains, are highlighted in bold green font. Other ribosomal subgroups in group 16SrI are in bold. Proportions (>50%) of replicate trees in which the associated taxa clustered together in the bootstrap test are shown next to relevant branches, the GenBank accession numbers are indicated to the right of phytoplasma names.

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LITERATURE CITED

Bertaccini A., 2022. Plants and phytoplasmas: when bacteria modify plants. *Plants* 11: 1425. https://doi.org/10.3390/plants11111425

Bertaccini A., 2023. Phytoplasma collection. https://www.ipwgnet.org/collection

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. https://doi.org/10.4236/ajps.2014.512191

Bertaccini A., Arocha-Rosete Y., Contaldo N., Duduk B., Fiore N.,... Zamorano A., 2022. Revision of the '*Candidatus* Phytoplasma' species description guidelines. *International Journal of Systematic & Evolutionary Microbiology* 72: 005353. https://doi.org/10.1099/ijsem.0.005353

Bertaccini A., Gandra R.R., Mateeti S., Pacini F., 2025. Phytoplasma transmission by seeds in alfalfa: a risk for agricultural crops and environment. *Seeds* 4: 39. https://doi.org/10.3390/seeds4030039

Bogoutdinov D., Girsova N., Kastalyeva T., 2021. Danger of phytoplasma diseases for fodder crop cultivation. *Economic and Phytosanitary Rationale for the Introduction of Feed Plants IOP Conference Series: Earth Environment Sciences* 663: 012033. doi 10.1088/1755-1315/663/1/012033

Calari A., Paltrinieri S., Contaldo N., Sakalieva D, Mori N., ... Bertaccini A., 2011. Molecular evidence of phytoplasmas in winter oilseed rape, tomato and corn seedlings. *Bulletin of Insectology* 64(Supplement): S157–S158.

Castillo-Carrillo C., Paltrinieri S., Buitrón Bustamante J., Bertaccini A., 2018. Detection and molecular characterization of a 16SrI-F phytoplasma in potato showing purple top disease in Ecuador. *Australasian Plant Pathology* 47: 311–315. https://doi.org/10.1007/s13313-018-0557-9

Contaldo N., D'Amico G., Paltrinieri S., Diallo H.A., Bertaccini A., Arocha Rosete Y., 2019. Molecular and biological characterization of phytoplasmas from coconut palms affected by the lethal yellowing disease in Africa. *Microbiological Research* 223–225: 51–57. https://doi.org/10.1016/j.micres.2019.03.011

Darabakula M., Mateeti S.T., Pacini F., Bertaccini A. Contaldo N., 2024. Eggplant little leaf-associated phytoplasma detection in seedlings under insect-proof conditions. *International Journal of Plant Biology* 15: 217–229. https://doi.org/10.3390/ijpb15020018

Deng S., Hiruki C., 1991. Amplification of 16S rRNA genes from culturable and non-culturable mollicutes. *Journal of Microbiological Methods* 14: 53–61. https://doi.org/10.1016/0167-7012(91)90007-D

Duduk B., Bertaccini A., 2006. Corn with symptoms of reddening: new host of "stolbur" phytoplasma. *Plant Disease* 90: 1313–1319. https://doi.org/10.1094/PD-90-1313

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

Esmaeilzadeh-Hosseini S.A., Babaei G., Davoodi S., Bertaccini A., 2020. Identification and impact of phytoplasmas associated with greenhouse cucumber phyllody in Iran. *Advances in Horticultural Science* 34(4): 413418. https://doi.org/10.5958/2249-4677.2016.00003.7

Esmailzadeh Hosseini S.A., Azadvar M., Babaei G., Salehi M., Bertaccini A., 2023a. Diversity, distribution and status of phytoplasma diseases in Iran. In: *Phytoplasma Diseases in Asian countries. Diversity, Distribution and Current Status* (Tiwari A.K., Caglayan K., Al-Sadi A., Azadvar M., Abeysinghe S., ed.). Academic Press, pp. 39-83

Esmailzadeh Hosseini S.A., Azadvar M., Babaei G., Salehi M., Bertaccini A., 2023b. Important phytoplasma ribosomal subgroups distributed in Iran. *Phytopathogenic Mollicutes* 13(1): 125–126. https://doi.org/10.5958/2249-4677.2023.00063.4

Felsenstein J., 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39: 783–791. https://doi.org/10.2307/2408678

Green M.R., Sambrook J., 2012. Molecular cloning: a laboratory manual. Fourth edition. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y, USA.

Gundersen D.E., Lee I-M., 1996. Ultrasensitive detection of phytoplasmas by nested-PCR assays using two universal primer sets. *Phytopathologia Mediterranea* 35: 144–151. https://www.jstor.org/stable/42685262

Gungoosingh Bunwaree A., Contaldo N., Bertaccini A., 2023. Seed transmission of phytoplasmas in tomato and chili varieties commonly grown in Mauritius. *Phytopathogenic Mollicutes* 13: 55–56. doi/10.5958/2249-4677.2023.00028.2

Healey A., Furtado A., Cooper T., Henry R.J., 2014. Protocol: a simple method for extracting next-generation

sequencing quality genomic DNA from recalcitrant plant species. *Plant Methods* 10: 21. https://doi.org/10.1186/1746-4811-10-21

Heap I., 2025. The International Herbicide-Resistant Weed Database. Online. February 5, 2025. Available www.weedscience.org

IRPCM., 2004. '*Candidatus* Phytoplasma', a taxon for the wall-less, non-helical prokaryotes that colonize plant phloem and insects. *International Journal of Systematic & Evolutionary Microbiology* 54: 1243–1255. https://doi.org/10.1099/ijs.0.02854-0

Jakovljević M., Jović J., Krstić O., Mitrović M., Marinković S., Cvrković T., 2020. Diversity of phytoplasmas identified in the polyphagous leafhopper *Euscelis incisus* (Cicadellidae, Deltocephalinae) in Serbia: pathogen inventory, epidemiological significance and vectoring potential. *European Journal of Plant Pathology* 156: 201–221. https://doi.org/10.1007/s10658-019-01878-w

Jonson G.B., Matres J.M., Ong S., Tanaka T., Choi I-R., Chiba S., 2020. Reemerging rice orange leaf phytoplasma with varying symptoms expressions and its transmission by a new leafhopper vector—*Nephotettix virescens* distant. *Pathogens* 9: 990. https://doi.org/10.3390/pathogens9120990

Kumar S., Stecher G., Tamura K., 2016. MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution* 33: 1870-1874. https://doi.org/10.1093/molbev/msw054

Lee I-M., Gundersen-Rindal D.E., Davis R.E., Bartoszyk I., 1998. Revised classification scheme of phytoplasmas based on RFLP analyses of 16S rRNA and ribosomal protein gene sequences. *International Journal of Systematic & Evolutionary Microbiology* 48: 1153–1169. https://doi.org/10.1099/00207713-48-4-1153

Lee I-M., Gundersen-Rindal D.E., Davis R.E., Bottner K.D., Marcone C., Seemüller E. 2004., '*Candidatus* Phytoplasma asteris', a novel phytoplasma taxon associated with aster yellows and related diseases. *International Journal of Systematic & Evolutionary Microbiology* 54: 1037–1048. https://doi.org/10.1099/ijs.0.02843-0

Mateeti S.T., Checchi G., Messina N.A., Feduzi G., Bertaccini A., Contaldo, N., 2022. Presence and seed transmission of phytoplasmas in tomato fields in Italy. *Phytopathogenic Mollicutes* 12: 1–6. doi.org/10.5958/2249-4677.2022.00001.9

Mateeti S.T., Darabakula M., Contaldo N., Pacini F., Bertaccini, A., 2023. Seed transmission of phytoplasmas infecting eggplants in India. *Phytopathogenic Mollicutes* 13: 57–58. doi.org/10.5958/2249-4677.2023.00029.4

McKay A.C., Riley I.T., 1993. Sampling ryegrass to assess the risk of annual ryegrass toxicity. *Australian Veterinary Journal* 70(7): 241–243. https://doi.org/10.1111/j.1751-0813. 1993.tb08038.x

Oshib Nataj M., Shekarchi H., Akbarzadeh M., Keshavarzi M., 2012. An autecological study of *Lolium rigidum* L. in Mazandaran Province. *Journal of Plant Biological Science*, 3(10): 37–46. https://doi.org/20.1001.1.20088264.1390.3.10.5.7

Owen M.J, Martinez N.J., Powles S.B., 2014. Widespread occurrence of multiple herbicide resistance in Western Australian annual ryegrass (*Lolium rigidum*) populations. *Australian Journal of Agricultural Research* 58: 711–718. https://https://doi.org/10.1071/AR06283

Randa-Zelyut F., Inak E., Demire Ozden E., Senal D., Ertunc F., 2022. Determination of potential insect vectors and subgroups of aster yellows phytoplasma in the carrot (*Daucus carota* L.) (Apiaceae) cultivation areas of Ankara and Konya Provinces, Türkiye. *Türkish Entomology Derg* 46(4): 385–398. https://doi.org/https://dx.doi.org/10.16970/entoted.1118787

Saitou N., Nei M., 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology & Evolution* 4: 406–425. https://doi.org/10.1093/oxfordjournals.molbev.a040454

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., Esmaeilzadeh-Hosseini S.A., Faghihi M.M., Salehi E., Bertaccini A., 2025. Identification of a '*Candidatus* Phytoplasma asteris' 16SrI-F strain infecting periwinkle in Iran. *Phytopathogenic Mollicutes*, 15(2): 175–180.

Satta E., Carminati G., Bertaccini A., 2020. Phytoplasma presence in carrot seedlings. *Australasian Plant Disease Notes* 15: 11. https://doi.org/10.1007/s13314-020-0377-y

Schneider B., Seemüller E., Smart C.D., Kirkpatrick B.C., 1995. Phylogenetic classification of plant pathogenic mycoplasma-like organisms or phytoplasmas. In: *Molecular and diagnostic procedures in Mycoplasmology* (Razin S. and Tully J.G., ed.). Academic Press. San Diego, CA (USA) pp. 369–380. https://doi.org/10.1016/B978-012583805-4/50040-6

Tamura K., Nei M., Kumar S., 2004. Prospects for inferring very large phylogenies by using the neighbor-joining method. *Proceedings of National Academy of Science (USA)* 101: 11030–11035. https://doi.org/10.1073/pnas.0404206101

Urbanaviciene L., Valiunas D., Jomantiene R., 2005. Molecular detection and identification of subgroup 16SrI-L phytoplasma in ryegrass (*Lolium multiflorum* Lam.). *Phytopathologia Polonica* 35: 121–124.

Wegulo S.N, Carlson M.P., 2011. Ergot of small grain cereals and grasses and its health effects on humans and livestock. University of Nebraska–Lincoln Extension.

Zhao Y., Wei W., Lee I-M., Shao J., 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 & Evolutionary Microbiology* 59: 2582–2593. https://doi.org/10.1099/ijs.0.010249-0

Zwolinska A., Krawczyk K., Pospieszny H., 2012. Molecular characterization of "stolbur" phytoplasma associated with pea plants in Poland. *Journal of Phytopathology* 160: 317–323. https://doi.org/10.1111/j.1439-0434.2012.01903.x