First report of a '*Candidatus Phytoplasma trifolii*'-related strain (16SrVI-A) associated with white leaf disease in *Spirea japonica*

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Abstract

In August of 2018 phytoplasmas naturally infecting *Spirea japonica* were found in Şanlıurfa province, Turkey and they were identified by nested polymerase chain reaction (PCR), restriction fragment length polymorphism analysis (RFLP) and phylogeny based on sequence analysis. Plants exhibited typical white leaf disease symptoms with reduced leaf size. The phytoplasmas were found to be closely related to '*Candidatus Phytoplasma trifolii*' (16SrVI-A). This paper has reported that *Spirea japonica* is a new host for '*Ca. Phytoplasma trifolii*' which is associated with the white leaf disease in *S. japonica*.

Keywords *Spirea japonica* · 16SrDNA · '*Candidatus Phytoplasma trifolii*' · 16SrVI-A subgroup · White leaf disease

Main text

Spirea japonica is a perennial tree and extensively cultivated in China and Japan. It grows for landscape purposes in various regions of Turkey. In August of 2018, spirea plants exhibiting white leaf disease symptoms with reduced leaf size (Fig. 1) were observed in Şanlıurfa province, Turkey. White leaf disease was previously associated with phytoplasmas in some plant species (Çağlar et al. 2019; Pérez-López and Dumonceaux 2017; Ratchaseema et al. 2021; Song et al. 2016). Phytoplasma-infected *Spirea* spp. were previously recorded as showing severe stunting, little leaves, and witches' broom symptoms (Bellardi et al. 2018).

DNA was extracted by a spin column-based method (DNeasy plant mini kit, Qiagen) from leaf midribs and stems of five symptomatic and five asymptomatic plants to determine if the symptoms were associated with the phytoplasmas. Universal primers were used for the identification based on PCR of the 16S rDNA gene. The 16S rDNA sequences of the phytoplasmas were firstly amplified by direct PCR using the universal primers R16F1/R0 (Lee et al. 1994) amplifying 1.4 kb products. The PCR reaction mixture consisted of 1 µl template DNA, 1 µl of a 10-µM primers, 1 µl dNTP mix (10 mM of each), 5 µl

10X DreamTaq Buffer, 1.25 U DreamTaq DNA Polymerase (Thermo Scientific) and the final volume was adjusted to 50 µl with nuclease-free water. PCR conditions were 94°C for two min (1 cycle) and 94°C for 30 s, 58°C for 30 s (annealing temperature for nested PCR was 55°C) and 72°C for 1 min (35 cycles) followed by a final extension step at 72°C for 5 min. A 1:50-dilution of the direct PCR products was then used as target DNA for nested-PCR using the primer pair R16F2n/R2 (Gundersen and Lee 1996) amplifying ~ 1.2 kb products. With this procedure, amplicons of the expected length fragment (~ 1.2 kb in size) were obtained from all the symptomatic samples and the positive control but not from the negative control and asymptomatic plants. Nested-PCR amplicons obtained from the diseased plants were subjected to RFLP analysis by using *AluI*, *HhaI*, *HpaII*, *MseI* and *RsaI* enzymes according to manufacturer instructions (ThermoFisher Scientific). The digestion products were then electrophoresed in 2.0% agarose gel and visualized with a UV transilluminator. Nested-PCR amplicons gave the same restriction patterns. Thereafter, all nested-PCR amplicons were sequenced in both directions.

The 16S rDNA partial sequences showed 100% identity with each other. Two representative sequences data were submitted to GenBank and are accessible through the following accession numbers; MZ348974-MZ348975. To classify the sequences, the phylogenetic tree based on nested-PCR amplicons was constructed by MEGA7 (Fig. 2). In silico digestion analysis and determination of subgroups were also performed with the online tool *iPhyClassifier*

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(Zhao et al. 2009). According to *iPhyClassifier*, 16S rDNA sequences of the isolates shared 99.80% similarity with that of the '*Candidatus Phytoplasma trifolii*' reference strain (GenBank: AY390261). Wet and virtual RFLP patterns supported each other and the similarity coefficient was 1.00 with the reference pattern of 16SrVI-A (AY390261).

'*Candidatus Phytoplasma trifolii*' have been previously reported in pears (Usta et al. 2021), pepper (Yilmaz et al. 2019), and cabbage (Serçe and Yilmaz 2019) in Turkey. *Spirea* spp. infected with diverse phytoplasma subgroups causing stunting including 16SrIII-E from the USA (Griffiths et al. 1994), 16SrI-B from China (Gao et al. 2007), 16SrV-B from China (Li et al. 2010) have been reported. To the best of our knowledge, this is the first report about

Fig. 2 Phylogenetic tree constructed by neighbor-joining method inferred from the '*Candidatus Phytoplasma*' species nucleotide sequences of the 16S rDNA gene. The tree shows the relationships among the phytoplasma strains obtained from *Spirea japonica* plants from Turkey (red squares) and compared with some of '*Candidatus Phytoplasma*' species available in GenBank. The tree was constructed using MEGA 7 software. GenBank accession numbers and ribosomal subgroups are reported on the right of the strain names. *Achelasma laidlawii* was employed as outgroup to root the tree

a '*Candidatus Phytoplasma trifolii*'-related strain (16SrVI-A subgroup) associated with spirea white leaf disease on *Spirea japonica* in the world. These new findings are of practical significance to the region in terms of the pathogen/disease epidemiology.

Fig. 1 White leaf disease symptoms in infected *Spirea japonica* plants (yellow and red arrows show diseased and healthy plants, respectively)

Author contribution EŞ was responsible for designing the Nested-PCR and characterization analyses. HA and HA helped the Nested-PCR experiments. MEG and EŞ conducted the field observations and collected samples. MD conducted DNA isolation and phylogenetic studies. EŞ and MD wrote the manuscript and analysed the data. All authors have read and approved the manuscript.

Data availability statement The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Conflict of interest All the authors declare that there is no conflict of interest in this study.

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