First report of a '*Candidatus Phytoplasma phoenicium*'-related strain (16SrIX-C) associated with phyllody disease of *Reseda lutea*

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Abstract

During a survey in 2019, *Reseda lutea* plants showing phyllody symptoms were observed in Fars province, Iran. Presence of phytoplasma in the symptomatic plants was confirmed by PCR amplifications of *16S rRNA* and *rp* genes. Sequence analysis revealed that the phytoplasma associated with the phyllody affected plants belongs to subgroup 16SrIX-C of '*Candidatus Phytoplasma phoenicium*' species. This is the first report of a 16SrIX-C phytoplasma strain associated with phyllody symptoms of *Reseda lutea* in Iran and probably in the world.

Keywords PCR · *Rp* gene · Virtual RFLP · Yellow mignonette · *16S rRNA* gene

Reseda lutea (Family: Resedaceae) known as yellow mignonette or wild mignonette is a perennial plant that grows widely in arid and semi-arid rangeland in Iran (Moghaddam 1977). *R. lutea* has been used as a medicinal plant and in the carpet and rug industry as a source of natural dye (Abdallah and De Witt 1967). In Iran, it is mainly used in animal husbandry as a grazing plant (Moghaddam 1977). *Reseda lutea* has been reported as a harmful weed in field crops (Bailey and Wicks 1995) and a potential host of *Watermelon mosaic virus* (Amiri and Ebrahim-Nesbat 1977) in Iran.

During a survey in April 2019, *R. lutea* phyllody (RLPh) disease was observed in Shiraz, Fasa, Darab and Firuzabad areas (Fars province, Iran). The main disease symptoms were flower virescence, phyllody, proliferation, little leaf and shortened internodes (Fig. 1). Disease incidence rate in certain patches of *R. lutea* was up to 15%. Phyllody affected and symptomless *R. lutea* plants were sampled from Golestan town (Shiraz area). Total DNA was extracted from 0.3 g of flower tissue from six symptomatic and six symptomless plants using the small-scale procedure of Zhang et al. (1998) as modified by Abou-Jawdah et al. (2002). PCR amplifications of *16S rRNA* and *rp* genes was used to confirm the presence of phytoplasma in the symptomatic plants.

The amplification of ribosomal DNA was done by nested PCR using primers pairs P1/P7 (Deng and Hiruki 1991; Schneider et al. 1995) and R16F2n/R16R2 (Gundersen and Lee 1996). A 1:30 dilution of PCR products amplified by primer pair P1/P7 was used as a template for the second round of PCR with primer pair R16F2n/R16R2. Primer pair R16F2n/R16R2 amplifies about 1250 bp of the phytoplasma *16S rRNA* gene. Additionally, rpF1/rpR1 (Lim and Sears 1992) primer pair was used in direct PCR to amplify approximately 1250 bp of the *ribosomal protein (rp)* gene. PCR was performed in 50 µl reaction mixture containing 100 ng DNA, 0.4 µM of each primer, 0.2 mM of dNTP mix, 1.25 U Taq DNA polymerase (CinnaGen, Tehran, Iran) and 1X PCR buffer. The reactions were denatured at 95 °C for 120 s, followed by 35 cycles of 95 °C for 60 s, 55 °C (42 °C for *rp* amplification) for 60 s and 72 °C for 180 s, with a final extension of 72 °C for 10 min.

Amplicons of 1250 and 1256 bp, respectively, corresponding to R16F2n/R16R2 and rpF1/rpR1 primer pairs were yielded only in symptomatic plants. Amplicons were ligated onto pTZ57R/T vector and cloned into *Escherichia coli* DH5α cells using InsT/A Clone™ PCR Product Cloning Kit (Metabion, Germany) according to manufacturer's instructions. Presence of the correct insert was confirmed by restriction endonuclease analysis using *EcoRI* and *PstI* enzymes. Plasmid DNAs from recombinant colonies were purified using GF-1 PCR Clean-Up Kit (Vivantis, Malaysia, HQ) and the cloned R16F2n/R16R2 and rpF1/rpR1 fragments

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Fig. 1 Symptoms of *Reseda lutea* phyllody disease: a, a symptomless plant; b, flower phyllody, virescence and proliferation in a phyllody affected plant; c, comparison of phyllody affected (left) and healthy (right) branches of *Reseda lutea* plant

were sequenced on both strands by a commercial DNA sequencing service (Macrogen, South Korea). M13 forward and reverse primers were used as sequencing primers. Each of the R16F2n/R16R2 and rpF1/rpR1 primed sequences from six phyllody affected *R. lutea* showed 100% sequence identity and the consensus sequences were submitted to GenBank with the accession numbers MZ536389 and MZ542534, respectively. BLASTn searches using obtained sequences showed that RLPh phytoplasma strain had 100% sequence identity with *Knautia arvensis* strains (accession Nos. EF186823 and EF186801, respectively), representatives of 16SrIX-C subgroup phytoplasmas ('*Candidatus* Phytoplasma phoenicium'-related) (Lee et al. 2012). Phylogenetic trees

constructed with *16S rRNA* (Fig. 2) and *rp* (Fig. 3) genes sequences showed that RLPh phytoplasma is classified in the 16SrIX group, subgroup C.

The 16SrIX-C subgroup phytoplasma strains have been previously detected in almond (Salehi et al. 2006), eggplant (Tohidi et al. 2015), tomato (Jamshidi et al. 2014), hemp (Rasoulpour et al. 2017), grapevine (Salehi et al. 2016; Ghayeb Zamharir et al. 2017) and sesame (Salehi et al. 2017) in Iran. Considering that 16SrIX-C phytoplasma strains can be vectored by *Circulifer haematoceps* (Salehi et al. 2017), a highly polyphagous insect feeding on a variety of herbaceous plants (EFSA 2015), *R. lutea* may act as a reservoir plant host for transmission of these strains to above mentioned plant species.

Fig. 2 Phylogenetic tree based on partial 16S rDNA sequences constructed by the neighbor-joining method showing relationship among *Reseda lutea* phyllody phytoplasma (rectangular) and reference phytoplasmas from GenBank. *Acholeplasma laidlawii* was used as an outgroup to root the tree. Numbers at the nodes are bootstrap (confidence) values based on 1000 repetitions. The scale bars indicates 0.07 substitution per nucleotide position

Fig. 3 Phylogenetic tree based on partial sequences of *rp* gene constructed by the neighbor-joining method showing relationship between *Reseda lutea* phyllody phytoplasma (rectangular) and reference phytoplasmas from GenBank. *Acholeplasma laidlawii* was used as an outgroup to root the tree. Numbers at the nodes are bootstrap (confidence) values based on 1000 repetitions. The scale bars indicates 0.5 substitution per nucleotide position

To our knowledge, this is the first report of the 16SrIX-C subgroup phytoplasma associated with *R. lutea* in Iran and probably worldwide.

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Declarations

Conflict of Interest Statement The authors declare that there is no conflict of interest.

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