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First report of '*Candidatus* Phytoplasma asteris' (16SrI) associated with yellows disease of *Trachyspermum ammi* from India

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Abstract

Trachyspermum ammi is a traditional medicinal plant and is widely used for curing various diseases in humans. During a 2022 survey, yellowing symptoms associated with phytoplasma disease were observed in Bhopal. The disease was detected by direct and nested PCR using phytoplasma-specific primers in symptomatic plant leaf samples. The suspected ~ 1.2 kb amplified amplicons were sequenced and submitted in GenBank under accession number OQ216747. The phytoplasma isolate showed the highest 99.42% sequence identity and phylogenetic relationships with the 16 S ribosomal RNA gene of an isolate of aster yellows phytoplasma ('*Candidatus* Phytoplasma asteris'; 16SrI group). This is the first report of *'Ca.* P. asteris' (16SrI) associated with the yellows disease of *T. ammi.*

Keywords *Trachyspermum ammi* · Yellows disease · Sequence analysis · *'Candidatus* Phytoplasma asteris'

Trachyspermum ammi L. (family *Apiaceae*), commonly known as ajwain, is a highly valued traditional medicinal plant. Its seeds are used in small quantities for flavoring numerous foods, as preservatives, in medicine and for the manufacture of essential oil in perfumery. In the Indian ayurveda medicinal system, *T. ammi* (ajwain) leaves and seeds are administered for curing stomach disorders, relieving colic pains and asthma, also having antimicrobial, hepatoprotective, gastroprotective, diuretic, anti-inflammatory and other various pharmacological effects (Bairwa et al. 2012). *T. ammi* is a native of Egypt and is also cultivated in West Asian countries including India. In India, it is cultivated in Madhya Pradesh, Uttar Pradesh, Gujarat, Rajasthan, Maharashtra, Bihar and West Bengal for its medicinal values (Bairwa et al. 2012).

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Some fungal and bacterial diseases have been reported previously on *T. ammi* (Sattar and Alam 1993). Phytoplasma disease associated with chlorotic leaves, proliferation of shoots and witches' broom disease were reported previously on *T. ammi* from the Uttar Pradesh state of India (Samad et al. 2011).

Phytoplasma are intracellular obligate prokaryotes that lack cell walls, have small genomes and are mainly transmitted by leafhoppers. They are associated with typical yellowing, stunting of the whole plant, virescence, phyllody, the proliferation of axillary buds, witches' broom and die-back symptoms (Al-Saady and Khan 2006; Bertaccini 2007; Harrison et al. 2008). Phytoplasma is also associated with severe yield losses in a variety of plant species of horticultural, agricultural, and ornamental importance grown in India (Chaturvedi et al. 2010; Rao et al. 2011).

In April 2022, we observed the chlorotic and yellow leaf symptoms on some plants of *T. ammi* (Fig. 1) during the survey at the nursery of Bhopal and we suspected phytoplasma on this plant on the basis of symptomatology.

For the detection of phytoplasma, total DNA was extracted from approximately 100 mg of leaf tissues from the symptomatic and asymptomatic samples of ajwain employing a phytoplasma enrichment procedure (Ahrens and Seemüller 1992). The Polymerase Chain Reaction (PCR) was performed using the total DNA and P1/P6 universal primers specific to the 16 S rRNA gene of phytoplasma (Deng

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Fig. 1 Naturally infected *T. ammi* plant showing excessive chlorotic and yellows leaves symptoms (**a**) compared with the healthy plant (**b**) commercially grown in a nursery at Bhopal

and Hiruki 1991). Further, the nested PCR was carried out using 1:10 diluted first stage (P1/P6) products and R16F2n/R16R2 primers (Gundersen and Lee 1996). The resulting products of direct and nested PCR were electrophoresed on 1.0% agarose gel visualised under UV followed by staining with ethidium bromide using an 100 bp DNA ladder marker for comparison and assessing the size of amplicons. The ~ 1.5 kb and ~ 1.2 kb fragments, respectively, were obtained in both the symptomatic samples (2/2), and the band corresponded to a similar size obtained in the positive control (little leaf diseased of *Ziziphus oenophilia*). However, no such bands could be obtained from a healthy asymptomatic plant collected from the same location. The amplicons of ~ 1.2 kb obtained from nested PCR were purified from the gel using the Wizard® SV Gel and PCR Clean-Up System Kit (Promega Pvt., USA) and were sequenced in both the orientations by Sanger sequencing method (Barcode Biosciences Pvt. Ltd., Bengaluru, India) and consensus identical sequence data obtained was submitted in NCBI GenBank database under the accession number: OQ216747.

BLASTn analysis of sequence data of *T. ammi* (OQ216747) showed the highest 99.42% sequence identities with 16 S ribosomal RNA gene of '*Ca.* P. asteris' (16SrI) '*Empoasca sp.*' aster yellows phytoplasma (OQ058850, OQ058849); '*Cyamopsis tetragonoloba*' aster yellows phytoplasma (OQ0588848, OQ058847) from India; '*Tachycixius sp.*' phytoplasma (KF583778); '*Eumercurus sp.*' phytoplasma (KF583783); '*Pentastiridius sp.*' phytoplasma (KF583782); '*Hyalesthes obsoletus*' phytoplasma (KF583780) from Lebnon; *Cajanus cajan'* aster yellow phytoplasma (OQ417510); *Sesamum indicum'* aster yellows phytoplasma (OQ417508, OQ417505) from India.

Analysing the phylogenetic tree, the understudy phytoplasma isolates (OQ216747) shared close relationships with the isolates of '*Ca.* P. asteris' (16SrI group) and distinct relationships with other '*Ca.* P. species'. The tree was generated by the neighbor-joining method MEGA 11.0 software and the analysis was subjected to a bootstrap test with 1000 repeat values (Fig. 2).

Computer-simulated analysis of the 16 S rRNA of the phytoplasma isolate (OQ216747) of *T. ammi* analysed with 17 restriction endonucleases enzymes using *i*PhyClassifier online tool (https://plantpathology.ba.ars.usda.gov/cgi- bin/resource/iphyclassifier.cgi) with three representative phytoplasma isolates of '*Ca.* P. asteris' (AP006628, OQ058849, OQ0588850), showed a similar restriction pattern with the exception of the *Kpn*I restriction enzyme (Fig. 3).

On the basis of the highest BLASTn sequence identities, close phylogenetic relationships and virtual RFLP pattern, the phytoplasma isolate was identified as an isolate of '*Ca.* P. asteris, 16SrI group, of *T. ammi*.

The '*Ca.* P. asteris' (16SrI) is the most important phytoplasma in India affecting ornamentals, vegetables, fruit crops, tree species and pulses (Raj et al. 2011; Reddy et al. 2012). There are some reports of '*Ca.* P. asteris' (16SrI group) on the medicinal plant from India which was associated with yellows disease of *Piper nigrum* (Adkar-Purushothama et al. 2009); witches' broom disease of *Cannabis sativa (*Raj et al. 2008a); virescence and witches"-broom *Parthenium hysterophorus* (Raj et al. 2008b) and yellows disease of *Achyranthes aspera* (Raj et al. 2009) & *Gerbera jamesonii* (Gautam et al. 2015) from north India.

Only '*Ca.* P. trifolii' (Clover proliferation group; 16SrVI) associated with yellowing and little leaf disease of ajwain (*T. ammi*) have been reported earlier from India (Samad et

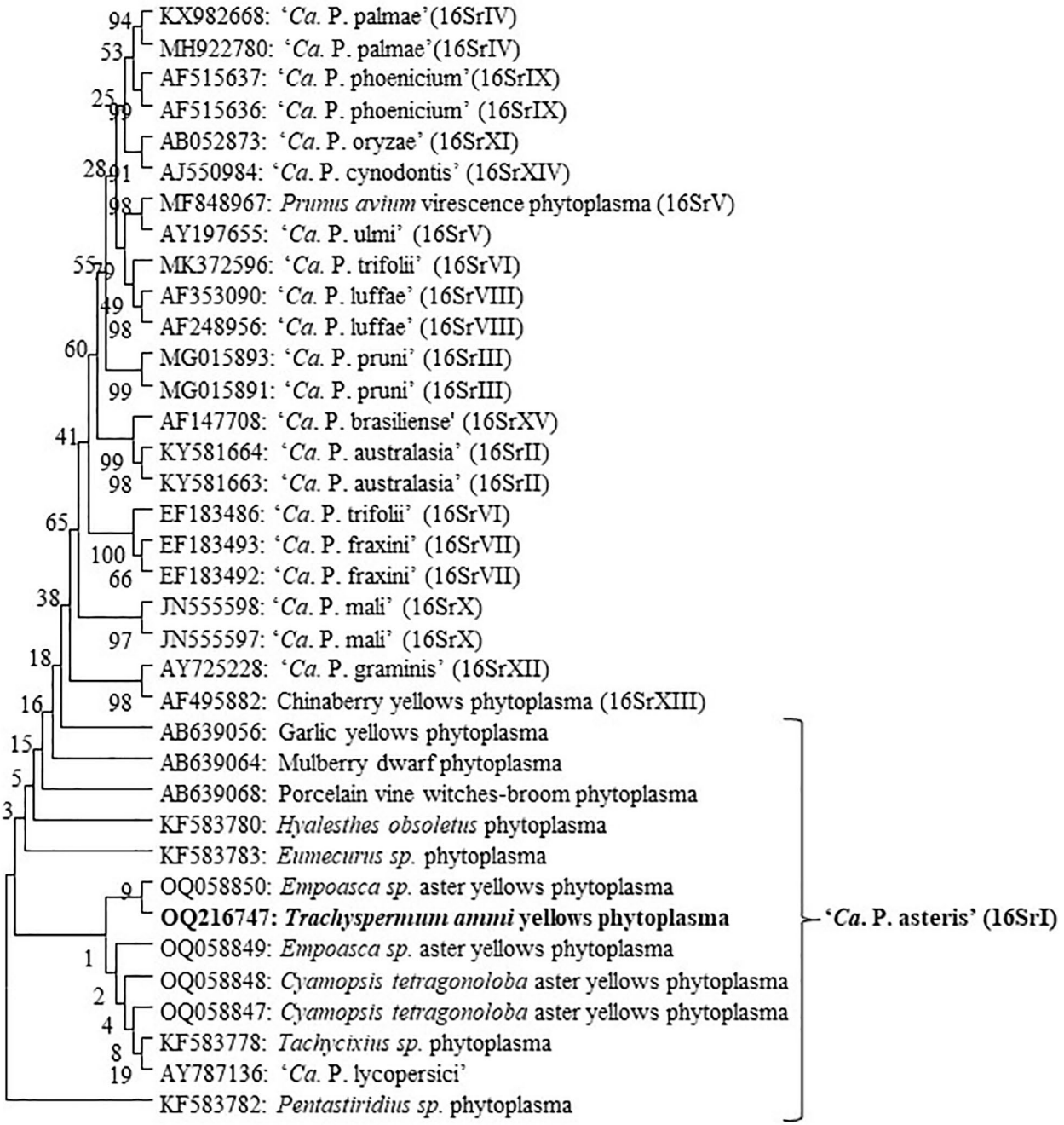

Fig. 2 The phylogenetic tree of the phytoplasma isolate (OQ216747) shared close relationships with the isolates of '*Ca.* P. asteris' (16SrI group) and distinct relationships with other '*Ca.* P. species'. The tree was generated by neighbor-joining method MEGA 11.0 software and analysis were subjected to a bootstrap test with 1000 repeat values

al. 2011). However, this is the first report of a '*Ca.* P. asteris' strain (16SrI group) associated with a yellows disease of *T. ammi*.

The present study also suggests that yellows disease ('*Ca*. P. asteris': 16SrI group) in *T. ammi* may be spread to other cultivated medicinal, ornamental, and vegetable plants in the near future as per previous studies (Rao et al. 2011), so more research is needed on host-pathogen interactions and disease management.

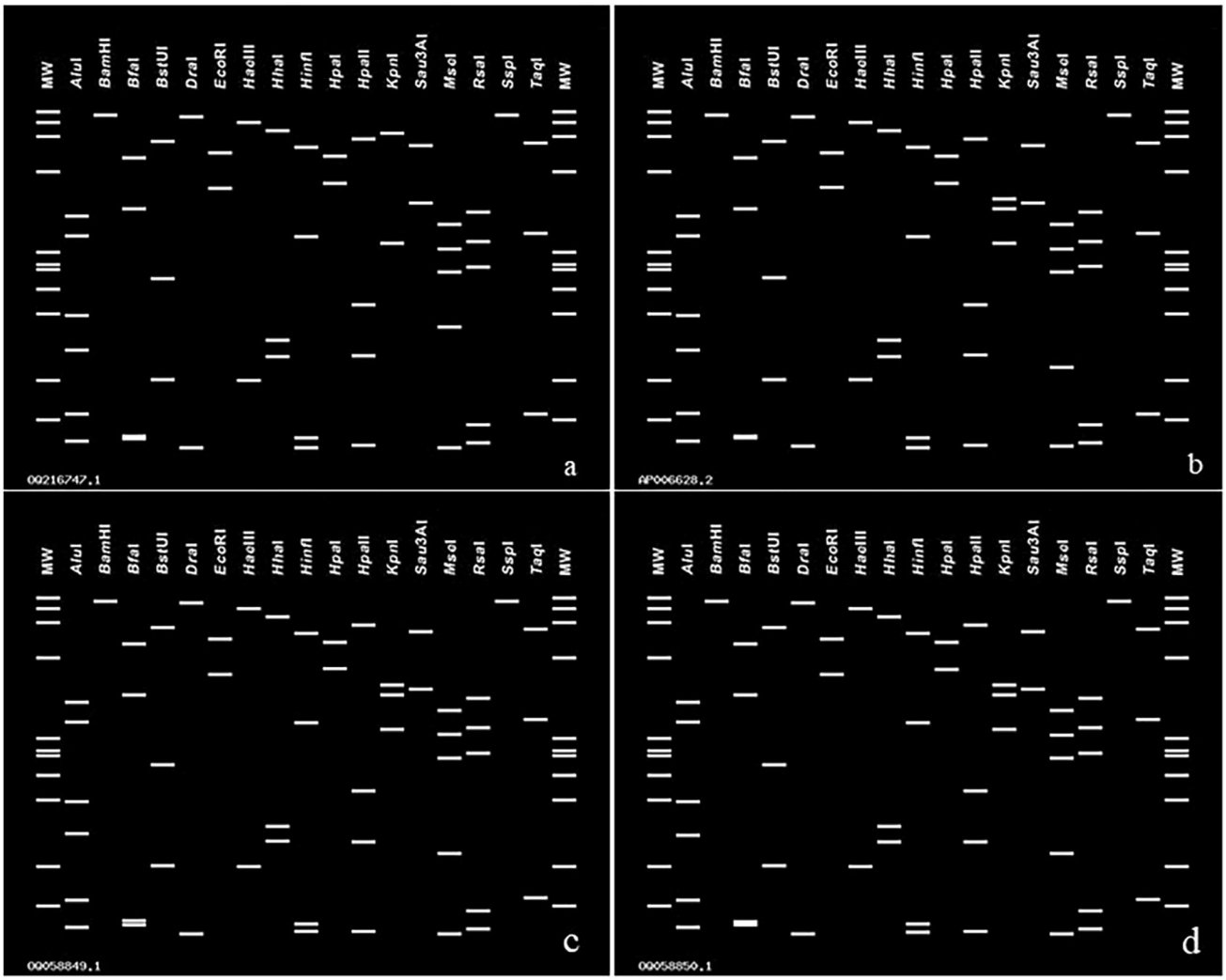

Fig. 3 Virtual RFLP pattern of 16 S ribosomal DNA sequence of the R16F2n/R16R2 fragments of *T. ammi* phytoplasma isolate (OQ216747) **a.** comparing with '*Ca.* P. asteris' reference strain (AP006628) **b** and '*Empoasca sp.*' aster yellows phytoplasma (OQ058849, OQ058850) '*Ca.* P. asteris' (16SrI group), **c & d** respectively, using the *i*PhyClassifier online tool via 17 restriction enzymes. MW: 1 kb DNA molecular weight marker

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Declarations

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

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