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RAPID COMMUNICATION

Association of '*Candidatus* Phytoplasma aurantifolia' with *Cosmos bipinnatus* phyllody disease in Iran

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Abstract

In 2017 growing season numerous examinations of *Cosmos bipinnatus* in Hormozgan province, Iran revealed the disease symptoms similar to those associated with phytoplasmas. Phytoplasmas were detected from all symptomatic plants by the specific polymerase chain reaction (PCR) utilizing phytoplasma universal primer pairs. Amplification, sequencing and BLAST analysis of 16S rDNA fragment (*ca.* 1.2 kb) demonstrated that *C. bipinnatus* plants were infected by a phytoplasma belonging to the 16SrII group. This is the first report of association of a '*Candidatus* Phytoplasma aurantifolia'-related strain with *C. bipinnatus* phyllody in Iran.

Key words: '*Candidatus* Phytoplasma aurantifolia', *Cosmos bipinnatus,* Mexican aster, phyllody, phytoplasma

Phytoplasmas, cell wall-less phytopathogenic bacteria, belonging to the Mollicutes class (Firrao *et al.* 2005) are associated with numerous devastating diseases in ornamental plants worldwide. Epidemics of these diseases have compelled withdrawal of many ornamental plant varieties from cultivation. Symptoms of general yellowing and stunting of plants, proliferation of shoots, phyllody, virescence and reduced size of flowers as well as reddening of leaves are commonly observed on ornamental plants and their occurrence often results in high economic losses (Chaturvedi *et al.* 2010).

Cosmos bipinnatus (Asteraceae, Coreopsidae) commonly called the garden cosmos or Mexican aster is a medium-size flowering herbaceous plant native to Mexico. The species and its varieties are popular as an ornamental plant in temperate climate gardens (Paniagua-Ibanez *et al.* 2015). *Cosmos bipinnatus* is an economically important ornamental plant species used in residential and commercial landscapes in Hormozgan province.

In February 2017, typical symptoms of phytoplasma diseases including phyllody, virescence, little leaf and stunting (Fig. 1) were observed in several *C. bipinnatus* plants in urban green space of Bandar Abbas, Hormozgan province, Iran.

Five samples of both symptomatic and two samples of asymptomatic *C. bipinnatus* were collected from urban green space of Bandar Abbas (N27°11'40"; E56°19'58"), Hormozgan province, Iran. To verify the possibility of a phytoplasma association with the symptoms, a total DNA was extracted from all symptomatic and asymptomatic plants using cetyltrimethylammonium bromide (CTAB) extraction procedure described by Doyle and Doyle (1990). The samples were examined for phytoplasma DNA by direct-PCR using the phytoplasma universal primer pair P1/P7 (Deng and Hiruki 1991; Schneider *et al.* 1995) and nested-PCR using primers P1/P7 (first round) followed by R16F2n/R16R2 (Gundersen and Lee 1996). A nested-PCR product of approximately 1.2 kb was directly sequenced. The PCR was performed in 20 µl of reaction mixture containing 10 µl PCR Master Mix (Amplicon), 1 µl of each primer (10 µM), 2 µl of template DNA and 6 µl sterile distilled water. The thermocycling program

Fig. 1. Symptoms of little leaf, virescence and phyllody in *Cosmos bipinnatus* (shown in red circle): A – plant with visible symptoms, B – healthy plant

consisted of an initial denaturation step at 95°C for 3 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 40 s and extension at 72°C for 90 s, with a final extension step at 72°C for 10 min. The P1/P7 primed PCR product was diluted at 1 : 10 ratio in sterile water and 2 µl was used in nested-PCR as a template. The nested-PCR cycles were the same as for the first round PCR**.**

Sequence used in phylogenetic analysis was checked and aligned using software DNAstar and ClustalX. Phylogenetic analysis was conducted by neighbor joining (NJ) methods using MEGA 6.0. Software (Tamura *et al*. 2013). The sequences of 16S rDNA of phytoplasma used in comprehensive phylogenetic analysis were downloaded from GenBank (the accession numbers are given in brackets in Figure 2).

Amplicons of *ca*. 1.8 and 1.25 kb were amplified from five symptomatic plants with direct and nested-PCR, respectively, but not from symptomless plants or sterile distilled water as negative controls. A positive nested-PCR product was directly sequenced, edited and deposited in GenBank under MF186858 accession number.

Blast analysis of the 1.25 kb of ribosomal RNA gene revealed that the phytoplasma associated with *C. bipinnatus* phyllody (CbP) shared 99% identity with strains of group 16SrII (peanut witches' broom group), including alfalfa witches' broom (KT6341420), '*Candidatus* Phytoplasma aurantifolia' (KX013260), tomato big bud phytoplasma (KP027532) and faba bean phyllody phytoplasma (KP869129). Phylogenetic analysis based on the 16S rRNA gene sequence of the phytoplasma associated with *C. bipinnatus* and other phytoplasmas exhibited that the *C. bipinnatus* phyllody phytoplasma is a member of the 16SrII phytoplasma clade (Fig. 2). This result was further confirmed by the analysis using *i*PhyClassifier online tool (Zhao *et al*. 2009), where it was determined that *C. bipinnatus* phytoplasma related to 16SrII group, subgroup D.

In Iran, group16SrII phytoplasmas have been identified in association with many diseases such as witches' broom disease of lime (WBDL), cabbage yellow, clover little leaf, alfalfa witches' broom, tomato witches' broom, sunflower phyllody, cucumber and squash phyllody, carrot witches' broom, parsley witches' broom, pomegranate little leaf, bell pepper big bud and elegant zinnia phyllody (Hemmati and Nikooei 2017). Association between phytoplasmas and *C. bipinnatus* has been previously reported by Wang and Hiruki (2001) and Chen *et al*. (2011) who showed the *C. bipinnatus*, infected by '*Candidatus* Phytoplamsa asteris'-related strains in Canada and Taiwan, respectively. In addition, Rojas-Martinez *et al*. (2003) revealed that the phytoplasma associated with cosmos disease in Mexico belongs to subgroup B from the aster yellows phytoplasma group 16SrI. Since symptoms affect the architecture and aesthetics of private and public gardens and parks, phytoplasmas infected ornamentals

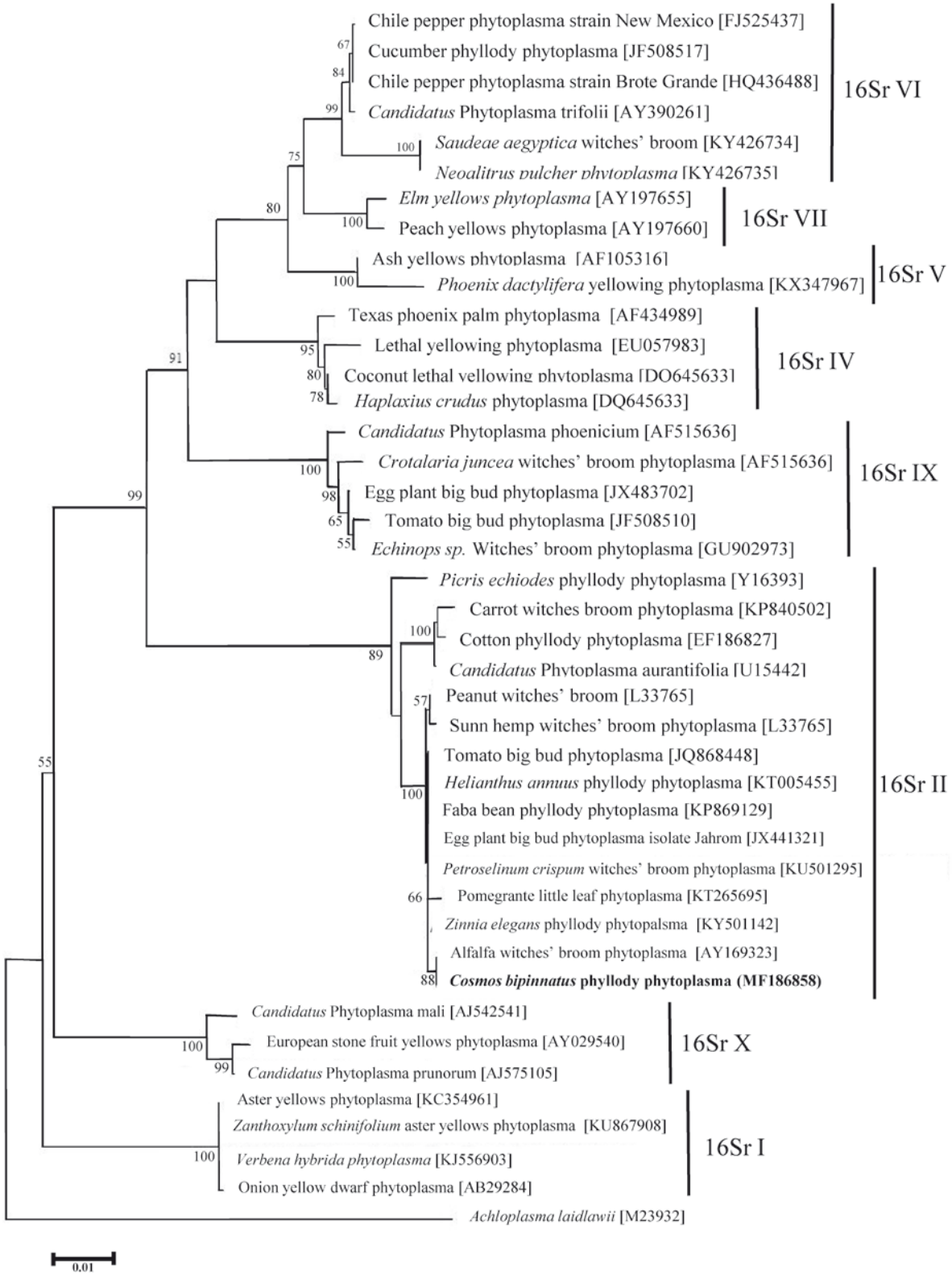

Fig 2. Phylogenetic tree inferred from partial 16S rDNA gene sequence from *Cosmos bipinnatus* phyllody phytoplasma isolate (marked by bold face type) and selected phytoplasma sequences. GenBank accession numbers are shown in brackets, and 16Sr groups are annotated to the right. *Acholeplasma laidlawii* was used as outgroup to root the tree. The tree was constructed by the neighbor-joining method using MEGA 6 software. Bootstrap values are shown at nodes with greater than 50% support

are of a global economic importance. In addition, phytoplasma-infected *C. bipinnatus* plants may pose an epimiological threat to the other ornamentals or nearby plant species. This is the first report on detection of a '*Candidatus* Phytoplasma aurantifolia'-related strain (16SrII group) affecting *C. bipinnatus* in Iran. Further studies will be required to identify the insect vector of the phytoplasma.

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