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Molecular characterization of a *Candidatus* Phytoplasma aurantifolia-related strain associated with *Zinnia elegans* phyllody disease in Iran

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Abstract *Zinnia elegans* plants showing symptoms of phyllody, virescence, witches' broom, little leaf and yellowing were observed in Bandar Abbas, Hormozgan Province, Iran. Analysis showed that the phytoplasma shares a 99% sequence identity with "*Candidatus* Phytoplasma aurantifolia" and is a strain related to this species. To the authors' knowledge, this is the first report of the occurrence of a 16SrII phytoplasma infecting *Zinnia elegans* flowers.

Keywords *Zinnia elegans* · 16SrII · Phyllody and witches" broom · Hormozgan

Phytoplasmas are non-helical, cell wall-less phyto-pathogenic bacteria, belonging to the Mollicutes class (Firrao et al., 2005) that can cause devastating yield loss in diverse low and high value crops worldwide. For instance, lime witches' broom disease (16SrII group phytoplasma) has been considered as one of the most destructive diseases in southern Iran, with aapproximate losses of US$ 27 million in 2007 (Mardi et al. 2007). These plant pathogens inhabit the sieve cells of phloem tissue and induce disease symptoms such as virescence, phyllody, sterility of flowers, proliferation of auxiliary or axillary shoots, abnormal elongation of internodes, generalized stunting and unseasonal yellowing or reddening of the leaves (Du Toit 2014).

Zinnia, belonging to family Asteraceae, is an annual plant and notable for its solitary long-stemmed flowers coming in a variety of bright colors. *Zinnia elegans* is the most familiar species, which originates from Mexico and is thus a warm-hot climate plant. In Iran, especially in the southern region, this species is widely planted as ornamental plants in municipal lands and parks.

In December 2016, typical symptoms of phytoplasma disease, including phyllody, virescence, witches" broom, little leaf and yellowing, were observed in *Zinnia elegans* planted in Bandar Abbas, Hormozgan province, Iran. The aim of this study was to identify the causal agent of this *Z. elegans* associated disease in Bandar Abbas, Iran.

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Samples of both symptomatic and symptomless *Z. elegans* plants were collected from Bandar Abbas, Iran. Total DNA was extracted separately from 1 g of flowers and leaves of five symptom-bearing (Fig. 1a, b) and two symptomless (Fig. 1c) using an adjusted cetyltrimethylammonium bromide (CTAB) method (Doyle and Doyle 1990). Both direct and nested PCR assays were performed for detection of phytoplasma DNA using phytoplasma universal 16S rDNA primer pairs P1/P7 (Deng and Hiruki 1991; Schneider et al. 1995) and R16F2n/R16R2 (Gundersen and Lee, 1966), amplifying fragments of ca. 1800 bp and 1250 bp, respectively (Deng and Hiruki, 1991). Following sequencing of the PCR products obtained, these sequences were checked and aligned using the softwares DNAstar and ClustalX. Phylogenetic analyses were conducted by neighbor joining (NJ) methods using MEGA 6.0 software (Tamura et al. 2013). The 16S rDNA gene sequences for other phytoplasmas used in the comprehensive phylogenetic analyses were retrieved from GenBank.

PCR products of 1800 and 1250 bp were obtained following direct and nested PCR from all five symptom-bearing plants but not from symptomless plants and PCR products from two plants were directly sequenced and deposited in GenBank (Acc. No. KY501142; KY501143). BLAST analysis of the 16S rDNA sequences revealed that the phytoplasma associated with *Zinnia elegans* phyllody (ZeP) shared 99% identity with phytoplasmas in the 16SrII phylogenetic group, "*Candidatus* Phytoplasma aurantifolia" such as Alfalfa witches" broom phytoplasma (KT634120). The phylogenetic neighbour-joining tree (MEGA 6 software) based on the partial 16S rDNA sequences (1260 bp) of the ZeP phytoplasma and several reference phytoplasmas supported these results and showed that the ZeP phytoplasma belongs to the '*Candidatus* Phytoplasma aurantifolia' clade (Fig. 2).

In Iran, group16SrII phytoplasmas have been identified as causal agent of 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 and bell pepper big bud. Association between phytoplasmas and *Zinnia elegans* were previously reported by Wang and Hiruki (2001) and Singh et al. (2011) who demonstrated that "*Candidatus* Phytoplamsa asteris"-related strains could infect this species in Canada and India, respectively. However, to our knowledge, this is the first report of an association between "*Candidatus* Phytoplasma aurantifolia"-related strain and *Z. elegans* in Iran or worldwide.

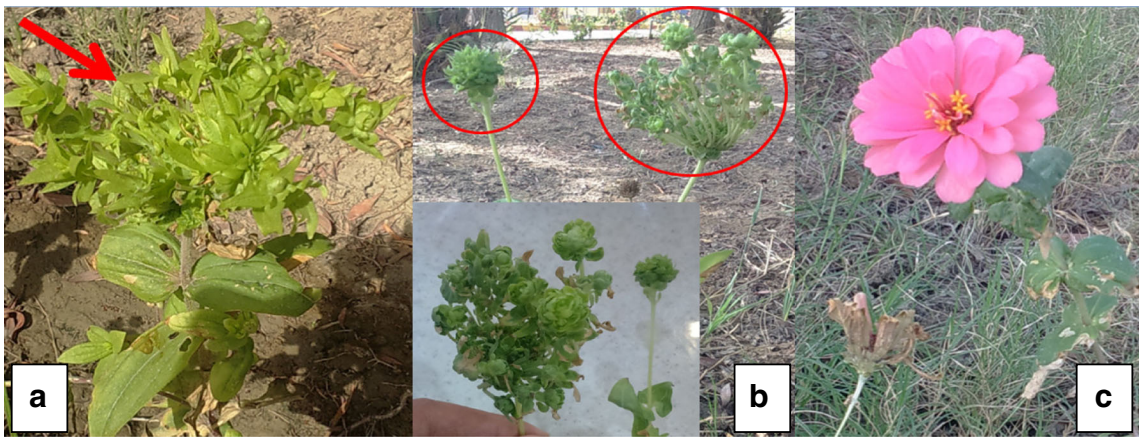

Fig. 1 Symptoms of witches" broom, little leaf and yellowing (shown by red arrow) (**a**), virescence and phyllody (shown in red circle) (**b**) in comparison with healthy *Zinnia elegans* (**c**)

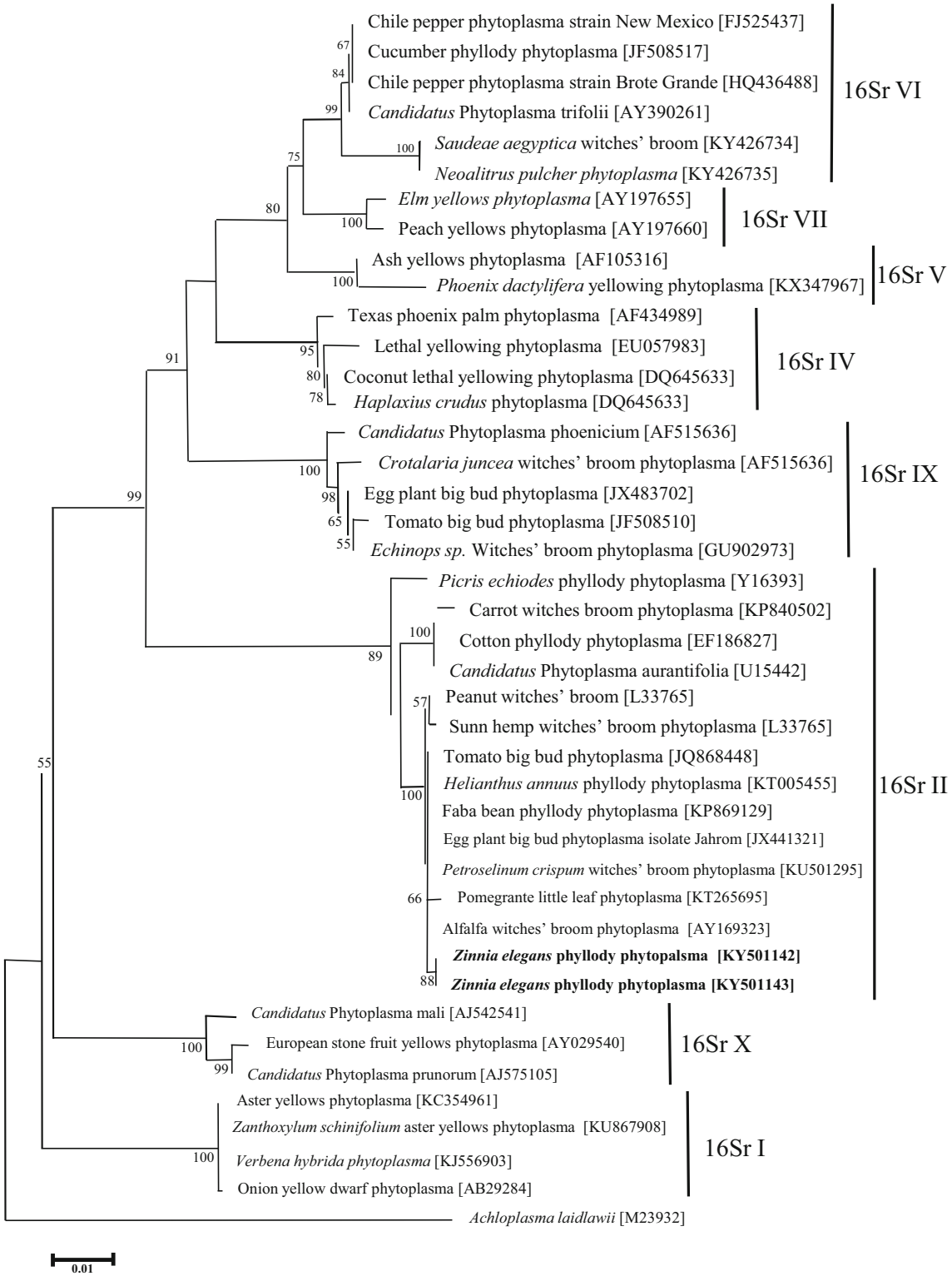

Fig. 2 Phylogenetic tree of partial 16S rDNA gene sequence from *Zinnia elegans* phyllody phytoplasma isolates (marked by bold face type) and selected phytoplasma reference sequences. GenBank accession numbers are shown in brackets, and 16Sr groups are annotated to the right. *Acholeplasma laidlawii*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

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