



First report of a '*Candidatus Phytoplasma trifolii*'-related strain associated with rapeseed witches' broom in Iran

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Rapeseed (*Brassica napus*) is an economically important oilseed crop grown in many countries worldwide. During recent years rapeseed cultivation has developed in regions of Iran. In April 2018, symptoms similar to phytoplasma-associated diseases including witches' broom (Fig. 1), stunting and little leaves (Fig. 2) were observed in rapeseed in several areas of Hormozgan province, Iran.

To identify the association of phytoplasma with the diseased plants, total genomic DNA was extracted from midribs of eight symptom-bearing and two symptomless plants using the CTAB method. The samples were analysed for phytoplasma DNA by direct PCR using the phytoplasma universal primer pair P1/P7 and nested PCR using primers P1/P7 (first round) followed by R16F2n/R16R2 (second round) (Gundersen & Lee, 1996). Amplicons of the expected sizes (1800 and 1250 bp) were amplified with direct and nested PCR in all symptom-bearing plants but not from symptomless plants or sterile distilled water used as negative controls. A positive nested PCR product was directly sequenced and deposited in GenBank under accession no. MH430092. A BLAST search using the sequenced fragment showed that the phytoplasma associated with rapeseed witches' broom (RsWB) had 100% nucleotide identity with the corresponding sequence of several members (KX773529, MG788318 and AM260486) of the 16SrVI phytoplasma group and 99.8% identity with the type strain, '*Candidatus Phytoplasma trifolii*' (AY390261). The virtual RFLP revealed that the RsWB phytoplasma belonged to the 16SrVI-A subgroup.

There are several reports regarding the association of aster yellows group (16SrI) phytoplasmas with rapeseed (e.g. rapeseed phyllody phytoplasma) in Iran (Salehi *et al.*, 2011) and other countries (Maliogka *et al.*, 2009; Olivier *et al.*, 2010). To our knowledge, this is the first report of natural infection of rapeseed by a 16SrVI phytoplasma in Iran and most probably in the world. Phytoplasmas belonging to the 16SrVI-A have been previously reported on several economically vegetable crops such as tomato and cucumber in Iran (Esmailzadeh Hosseini *et al.*, 2015; Jamshidi *et al.*,

2014). Therefore, the infected volunteer rapeseed around tomato and cucumber fields may act as an alternative host for this phytoplasma.

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Figure 1



Figure 2

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