New Disease Reports

Characterisation of a phytoplasma associated with sunflower phyllody in Fars, Isfahan and Yazd provinces of Iran

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Sunflower (*Helianthus annuus*) is an important oil crop in Iran. During 2010-2012 surveys, symptoms of sunflower phyllody were observed in sunflower fields of Abarkooh, Yazd (Yazd province), Fasa (Fars province) and Nain (Isfahan province). Affected plants showed proliferation of abnormal heads, virescence, phyllody of main flowers and axillary buds along the stem (Fig. 1). Total DNA was extracted from 0.3 g of fresh suspected phyllody flowers of affected sunflower plants and also from healthy plants using the procedure of Zhang *et al.* (1998). Two universal primer pairs, P1/P7 (Deng & Hiruki, 1991; Schneider *et al.*, 1995) and R16F2n/R2 (Gundesen & Lee 1996), were used to target the phytoplasma 16S rRNA gene. Phytoplasma DNA fragments of ~1.8 and ~1.250 kbp were amplified, respectively, from direct and nested PCR assays from eight symptom-bearing sunflower plants (two samples per location) but not from symptomless plants. Eight P1/P7 DNA fragments amplified from Abarkooh, Yazd, Fasa and Nain phyllody-affected sunflower plants were separately cloned and sequenced. The 16S rDNA sequences shared 100% sequence identity with each other, with that of the Abarkooh isolate (ASP) being deposited in GenBank (Accession No. KJ016231).

BLAST search showed the highest sequence identity with phytoplasma sequences of phytoplasma members of the 16SrII group ('*Candidatus* Phytoplasma aurantifolia'). Phylogenetic analysis of the ASP 16S rDNA sequence and those of reference from other phytoplasma groups (MEGA version 5.0) showed that the ASP phytoplasma clustered with the 16SrII phytoplasma, closely related to '*Ca.* P. australasia' of subgroup 16SrII-D (Fig. 2). Computer-simulated restriction analysis with *Alu*I, *Bam*HI, *Bfa*I, *Bst*UI, *Dra*I, *Eco*RI, *Hae*III, *Hha*I, *Hin*fI, *Hpa*I, *Hpa*II, *Kpn*I, *Sau*3AI, *Mse*I, *Rsa*I, *Ssp*I, and *Taq*I enzymes using pDRAW32 software (http://www.acaclone.com) revealed that the RFLP patterns of the ASP phytoplasma (Fig. 3) were identical to those of '*Ca* P. australasia'. To our knowledge this is the first report of the association of a 16SrII phytoplasma with a sunflower phyllody disease in Iran. Mixed infections of phytoplasma groups 16SrII and 16SrVI ('*Ca*. P. ulmi') in a witches'-broom affected sunflower were previously reported from the Kerman province of Iran (Tazehkand *et al*., 2010). Since alfalfa and sugar beet witches'-broom caused by 16SrII related phytoplasmas are economically important diseases in the Abarkooh region (Salehi *et al*., 2005), the identification of a 16SrII phytoplasma affecting sunflower suggests that the 16SrII phytoplasma isolates limited to the same geographical location of Abarkooh may share similar epidemiological constraints.

References

Deng S, Hiruki C, 1991. Amplification of 16 S rRNA genes from culturable and non-culturable mollicutes. *Journal of Microbiological Methods* **14**, 53-61. http://dx.doi.org/10.1016/0167-7012(91)90007-D

Gundersen DE, Lee IM, 1996. Ultrasensitive detection of phytoplasmas by nested-PCR assays using two universal primer sets. *Phytopathologia Mediterranea* **35**, 144-151.

Salehi M, Heydarnejad J, Izadpanah K, 2005. Molecular characterization and grouping of 35 phytoplasmas from central and southern provinces of Iran. *Iranian Journal of Plant Pathology* **41**, 62-64.

Schneider B, Seemüller E, Smart CD, Kirkpatrick BC, 1995. Phylogenetic classification of plant pathogenic mycoplasma-like organisms or phytoplasmas. In: Razin S, Tully JG, eds. *Molecular and Diagnostic Procedures in Mycoplasmology,* Vol.1. Academic Press, San Diego, CA, USA, 369-380. http://dx.doi.org/10.1016/B978-012583805-4/50040-6

Tazehkand SA, Pour AH, Heydarnejad J, Varsani A, Massumi H, 2010. Identification of phytoplasmas associated with cultivated and ornamental plants in Kerman province, Iran. *Journal of Phytopathology* **158**, 713-720.

Zhang YP, Uyemoto JK, Kirkpatrick BC, 1998. A small-scale procedure for extracting nucleic acids from woody plants infected with various phytoplasmas for PCR assay. *Journal of Virological Methods* **71**, 45-50. http://dx.doi.org/10.1016/S0166-0934(97)00190-0

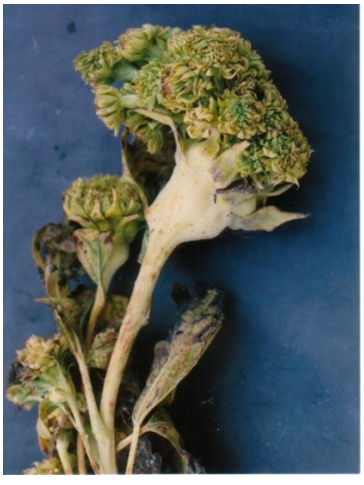

Figure 1

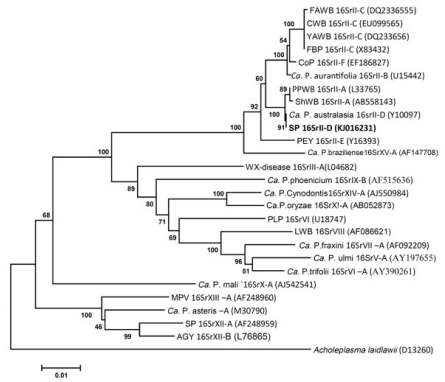

Figure 2

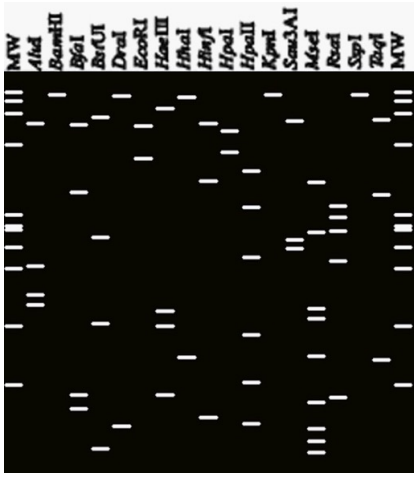

Figure 3

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