Disease Note

FIRST REPORT OF A 16SrIX GROUP (PIGEON PEA WITCHES'-BROOM) PHYTOPLASMA ASSOCIATED WITH GRAPEVINE YELLOWS IN IRAN

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Association of 16SrXII-A phytoplasmas with grapevine yellows (GY) has been previously reported from Fars and Lorestan provinces of Iran (Salehi *et al*., 2014). During 2013-2014 surveys, symptoms resembling those of GY disease including thickening and downward rolling of leaves, vein chlorosis and necrosis, shoot dieback and stunting were observed in vineyards of Shiraz area (Fars province, Iran). Total DNA samples extracted from eight symptomatic and eight symptomless vines were tested for phytoplasma presence. In direct PCR using primers P1/P7 and nested PCR using primer pairs P1/P7 followed by R16F2n/R16R2 (Lee *et al*., 1998), respectively amplicons of *ca.* 1.8 and 1.2 kb were amplified from symptomatic but not from symptomless vines. Samples that yielded P1/P7 amplicons (4/8) were cloned and sequenced; obtained sequences showed 100% identity. A BLAST search using full length 16S rRNA gene sequence of a representative of these sequences (GenBank Accession No. KX011516) revealed that the sequence shared 99% homology with Indian *Brassica rapa* phyllody phytoplasma (HM988986), a representative of 16SrIX-C subgroup. Computer-simulated restriction analysis using *i*PhyClassifier showed that the RFLP profile of the Shiraz GY (SGY) phytoplasma 16S rDNA sequence was identical (similarity coefficient 1.00) to *Picris echioides* yellows phytoplasma (Y16389) representative of subgroup C of the 16SrIX group. Phylogenetic analysis revealed that SGY phytoplasma clustered with-16SrIX group phytoplasmas closer to Indian *B. rapa* phyllody phytoplasma, a 16SrIX-C member. To our knowledge, this is the first report of natural infection of grapevine by a 16SrIX phtoplasma in Iran.

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Disease Note

FIRST REPORT OF *RAMULARIA COLLO-CYGNI* INFECTING BARLEY IN SOUTH AFRICA

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Ramularia collo-cygni B. Sutton & J.M. Waller, the causal agent of Ramularia leaf spot, is reported for the first time in South Africa. The fungus was isolated from barley (*Hordeum vulgare* L.) leaves grown in the Western Cape Province. Infected plants, with typical Ramularia leaf spot symptoms of small brown rectangular lesions, some surrounded by a yellow halo, visible on both sides of the leaf, were first observed in September 2015. Brown spots were also visible on dead leaves. Microscopic investigation revealed immersed mycelium, with hyaline, septate, branched hyphae colonizing the mesophyll. Dense white to pinkish fungal colonies with a vinaceous reverse, were isolated on potato dextrose agar from barley leaves harvested from four fields in the Overberg region. BLAST searches of the Internal Transcribed Spacer (ITS) region of five isolates (GenBank accession Nos. KX156947-KX156951) resulted in 100% similarity with *R. collo-cygni* reference sequences. The presence of *R. collo-cygni* DNA was furthermore confirmed *in planta* in four different symptomatic barley leaf samples, following DNA extractions and PCR reactions as described by Havis *et al.* (2006). *R. collo-cygni* isolate IPP 494 (Balz, 2010) DNA, supplied by Proff. von Tiedemann and Karlovsky at the Georg-August University in Göttingen, Germany, was used as positive control. *Pyrenophora teres* and *Alternaria alternata* DNA were included as negative controls, because of their association with barley leaves and the similarity in barley leaf symptoms caused by *P. teres*. DNA from isolate IPP 494 and the positive barley samples resulted in a 426 base pair PCR product when visualised on an agarose gel following gel electrophoresis. No PCR products were observed for *P. teres*, *A. alternata* and the no template control. To our knowledge, this is the first report of *R. collo-cygni* in barley leaves in South Africa.

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