First Report of '*Candidatus* Phytoplasma phoenicium' on almond in Southern Italy

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In spring 2017, phytoplasma suspected symptoms were reported on 25% of 15-yr-old almond plants, cv Filippo Ceo and Genco grafted onto GF677, in a commercial orchard (20 ha) located at Grottaglie, Apulia (South-East Italy). Among the symptoms, development of many axillary buds with small and yellowish leaves, and witches' brooms developing from the trunk, were the most frequent, followed by leaf rosetting, proliferation of slender shoots, tree decline, and dieback. Twenty-six leaf samples were collected in the symptomatic orchard, from both symptomatic (19) and asymptomatic (7) plants. Moreover, additional leaf samples (5) from asymptomatic almond orchards of the same varieties, located 80 km away from the infected fields at Valenzano (Province of Bari), were also collected. Leaf midribs were homogenized in extraction bags by using the Homex apparatus (Bioreba AG, Reinach, Switzerland), and total genomic DNA was extracted by using CTAB protocol (Abou-Jawdah et al. 2002). The nucleic acid pellet was washed with 70% ethanol, air-dried, suspended in 50 µl of nuclease-free water (Qiagen, Venlo, The Netherlands), and maintained at -80°C until use. Polymerase chain reaction (PCR) was carried out with phytoplasma 16S rRNA universal primer pair R16mF2/R16mR1, followed by nested PCR using R16F2n/R16R2 (Gundersen and Lee 1996), resulting in DNA amplicons of 1.41 and 1.23 kb, respectively. Moreover, another primer pair, groELF1/groELR1 and groELF2/groELR2, amplifying a region of

the *groEL* gene, and originating amplicons of 1.34 and 1.28 kb, respectively, were also used following the protocol described by Quaglino et al. (2015). Lyophilized DNA from '*Ca*. Phytoplasma phoenicium'-infected almond located in Lebanon (AF515636.1), was resuspended in nuclease free water, and used as positive control. All the symptomatic and 2 of 7 asymptomatic samples collected in the infected field located at Grottaglie, as well as the control DNA, resulted positive with all the tested primers. However, all the samples collected in Valenzano and 5 asymptomatic samples from Grottaglie were tested as negative. Therefore, these results confirmed that the observed symptoms were caused by a phytoplasma. Twelve nested-PCR products for both 16S rRNA and *groEL* coding region were purified, ligated into pGEM-T Easy Vector Systems (Promega, Madison WI, USA), and the plasmid DNA was sequenced (Genewiz, London, England). BLASTn analysis revealed that 16S rRNA gene sequence of Apulian phytoplasma strain shared 100% sequence identity with that of the reference strain of the species '*Ca*. P. phoenicium'(AF515636.1) (Verdin et al. 2003). The affiliation to this species was also confirmed by *groEL* gene sequence identity of 100% with that of '*Ca*. P. phoenicium' strain SA213 (KM275493) (Quaglino et al. 2015). The sequences obtained from the 12 samples for each coding region were identical, therefore two representative sequences were deposited in GenBank, under the accession numbers MK377252 and MK377253 for 16S rDNA, and MK387076 and MK387077 for *groEL* gene. Additional analysis of the 16Sr group/subgroup classification, based on *in silico* restriction fragment length polymorphism analyses using iPhyClassifier, confirmed that the Apulian phytoplasma strain was a member of the taxonomic subgroup 16SrIX-B, which include '*Candidatus* Phytoplasma phoenicium' strains (Zhao et al. 2009). '*Ca*. P. phoenicium', the causal agent of the almond witches' brooms, is a quarantine pathogen in the European Union, being included in the List A1 of the European Plant Protection Organization (EPPO) by September 2018. It may be rapidly spread to healthy stone fruits plantations, and a natural epidemic spread to peach and nectarine orchards has been reported in Lebanon (Abou-Jawdah et al. 2009). Recently, the pathogen has also been reported on apricots in Iran (Salehi et al. 2018). To the best of our

knowledge, this is the first report of '*Ca*. P. phoenicium' on almond in Italy, and its impact on stone fruit and other hosts production can be destructive (Abou-Jawdah et al. 2009; Salehi et al. 2018).

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