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Association of '*Candidatus* Phytoplasma phoenicium' with GF-677 witches' broom in Iran

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Abstract

Symptoms of GF-677 witches' broom (GFWB) disease were observed in Beedzard and Estahban areas in Fars province of Iran. Polymerase chain reaction (PCR) using phytoplasma-specific primer pair P1/P7 or nested PCR using P1/P7 followed by R16F2n/R16R2 amplified products of expected size (1.8 and 1.2 kbp, respectively) from symptomatic GF-677 trees. Restriction fragment length polymorphism (RFLP) analysis and sequence homology of 16S rDNA indicated that GFWB related to almond witches' broom phytoplasma.This is the first report of the natural occurrence of GFWB and the molecular characterization of the GFWB phytoplasma.

Key words: GF-677 witches' broom, '*Candidatus* Phytoplasma phoenicium', Iran.

Introduction

GF-677 (*Prunus amygdalus* x *Prunus persica*) with strong roots and a good potential for pests and diseases (Fasolo *et al.*, 1987) is one of the most suitable rootstocks for almond and peach used in calcareous soils to overcome lime-induced chlorosis (Kester, 1970; Fasolo *et al.*, 1987; Hartmann *et al.*, 1990). GF-677 cuttings were imported from France to Iran and planted in Beedzard, Estahban and Neyreez areas (Fars province) for further propagation and distribution to other Iranian stone fruit growing areas. During a survey conducted in 2009, witches' broom disease of GF-677 trees was observed in the Beedzard and Estahban regions. Because witches' broom is a typical symptom for phytoplasma infection, polymerase chain reaction (PCR) using phytoplasma specific primers was performed to detect phytoplasmas in symptomatic GF-677 trees. This paper describes the occurrence of witches' broom disease in GF-677 trees and the molecular detection and identification of the associated phytoplasma.

Materials and a ethods

Leaf samples from 20 witches' broom affected and 3 symptomless GF-677 trees from Beedzard and Estahban areas were collected. Total DNA was extracted from fresh midrib tissue using the small-scale procedure of Zhang *et al*. (1998) as modified by Abou-Jawdah *et al.* (2002). DNA samples were tested for phytoplasma infection by direct PCR using the universal phytoplasma primer pair P1/P7 (Schneider *et al*., 1995) which amplify a 1800 bp fragment of the 16S rRNA ribosomal operon the 16S-23S intergenic spacer region (SR) and a portion of the 5′ region of the 23S rRNA gene. To increase the sensitivity of assays, a nested PCR was performed using a 1: 40 dilution of P1/P7-primed PCR products as templates and primer pair R16F2n/R16R2 (Gundersen and Lee, 1996), which yielded a 1,200-bp fragment of the 16S rRNA gene. Eight microlitres (approximately 200 ng) of nested PCR products (1.2 kbp) from Beedzard and Estahban strains were individually digested with the restriction enzymes *Alu*I, *Hha*I *Hin*fI, *Hpa*II, *Mse*I, *Rsa*I, *Sau*3AI *Taq*I and *Tha*I (Roche), following the manufacturer's instructions. RFLP profiles were analyzed by electrophoresis of digested DNA in a 2% agarose gel, staining with ethidium bromide and visualization with a UV transilluminator. The profiles were compared with previously published data (Lee *et al*., 1998; Abou-Jawdah *et al*., 2002). Ribosomal DNA products amplified by R16F2n/R16R2 primer pair were ligated onto the pTZ57R/T vector and cloned into *Escherichia coli* DH5α cells using InsT/Aclone PCR Product Cloning Kit (Fermentas, Vilnius, Lithuania) according to manufacturer instructions and sequencing was performed by SEQ LAB (Germany). Using sequenced fragments BLAST search was performed to determine the closest phytoplasma relatives of Beedzard and Estahban strains.

Results and Xiscussion

Characteristic symptoms of the disease were internode shortening, chlorosis, reduced size of leaves especially in the broom, proliferation of slender upright shoots, witches' broom, stunting and dieback (figures 1 and 2). Up to 10% of the trees were found infected in Estahban area. With universal primer pair P1/P7 the target DNA fragments of approximately 1,800 bp were amplified by direct PCR from total nucleic acid samples extracted from 14 out of 20 witches' broom-affected GF-677 trees but not from the 3 symptomless samples, collected as negative controls. Nested PCR with primer pair P1/P7 followed by primer pair R16F2n/R16R2 yielded fragments of approximately 1.2 kbp from all witches' broom affected GF-677 trees tested but not from healthy control plants.

Figure 1. Internode shortening, chlorosis, reduced leaf size and stunting in a GF-677 tree from Neyreez, infected with a '*Ca*. P. phoenicium' related phytoplasma (middle), compared with healthy trees (right and left). (In colour at www.bulletinofinsectology.org)

Figure 2. Proliferation of slender upright shoots and witches' broom in a GF-677 tree from Neyreez, infected with a '*Ca*. P. phoenicium' related phytoplasma. (In colour at www.bulletinofinsectology.org)

After enzymatic digestion, Beedzard and Estahban strains showed identical profiles, undistinguishable from those of the almond witches' broom phytoplasma (AWBP) (Abou-Jawdah *et al*., 2002) '*Ca.* P. phoenicium'. Blast search showed that the 16S rRNA gene sequences of Beedzard (GenBank accession No. JF781493) and Estahban (GenBank accession No. F781494) isolates shared a 99% of identity with the 16S rRNA gene sequence of AWBP.

Based on disease symptoms and PCR amplification of the 16S rRNA gene with universal phytoplasma primers, GF- 677 witches' broom (GFWB) is associated with phytoplasma presence. RFLP analysis and sequence homology of 16S rDNA indicated that GFWB phytoplasma is molecularly indistinguishable from AWBP. Almond witches' broom is an important disease in Fars province of Iran (Salehi *et al*., 2006). Infected almond trees may be the source of GFWB phytoplasma. However, infection of GF-677 trees with AWBP suggests involvement of a vector. This is the first report of '*Ca.* P. phoenicium' associated with witches' broom disease of GF-677 trees in Iran.

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