

## Alternate hosts of alfalfa witches' broom phytoplasma and winter hosts of its vector *Orosius albicinctus* in Yazd-Iran

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### Abstract

Alfalfa witches' broom (AWB) vectored by *Orosius albicinctus* leafhopper is one of the most important alfalfa diseases in Iran especially Yazd province. During 2007-2009, overwintering hosts of *O. albicinctus* and weed hosts of phytoplasmal agent of AWB phytoplasma were evaluated. Based on the results of this study, *O. albicinctus* leafhopper were collected on *Cynodon dactylon*, *ordeum murinum*, *Digitaria sanguinalis*, *Echinochloa crus-galli*, *Lolium temulentum*, *Setaria viridis*, *Tamarix ramosissima* and *Seidlitzia rosmarinus* during winter season and these plants are reported as overwintering hosts of this phytoplasma vector. Direct PCR using P1/P7 and nested PCR using P1/P7 and R16F2n/R16R2 primer pairs showed phytoplasma infection of *Prosopis farcta* and *Cardaria draba*. Restriction fragment length polymorphism analysis of nested PCR products and indirect ELISA test using AWB polyclonal antibody identified peanut witches broom group member phytoplasmas in *Prosopis farcta* and *Cardaria draba* which were identical to each other and to Yazd AWB phytoplasma.

**Key words:** *Prosopis farcta*, *Cardaria draba*, 16SrII group, Yazd, Iran.

### Introduction

Alfalfa witches' broom (AWB), vectored by *Orosius albicinctus* D. leafhopper is one of the most important and destructive diseases of alfalfa in Iran, especially in the Yazd province (Salehi *et al.*, 1995). Knowledge of alternate hosts and vector is important in order to study the epidemiology and to proceed control measures for AWB disease. The aims of this study were to identify herbaceous plant species that in nature harbour the phytoplasma disease agent of AWB or acts as the winter hosts of *O. albicinctus*.

### Materials and methods

During surveys in 2007-2009, winter hosts of *O. albicinctus* and weed hosts of phytoplasmas associated with AWB disease were evaluated. Samples were taken from 40 weed species with suspicious symptoms in 10 infected alfalfa fields or adjacent areas in Mehriz, Ardakan and Abarkouh regions in Yazd province. Total DNA was extracted from 1 g of midrib tissue of weed samples following the protocol described by Zhang *et al.*, (1998). Total DNA samples were subjected to direct PCR using P1/P7 (Deng and Hiruki, 1991; Schneider *et al.*, 1995) and nested PCR using primer pairs P1/P7 (first step) and R16F2n/R16R2 (Gundersen and Lee, 1996) (second step). Primer pair P1/P7 amplifies a 1800-bp fragment of the ribosomal operon which includes the 16S rRNA gene, the 16S-23S intergenic spacer region (SR), and a portion of the 5' region of the 23S rRNA gene whereas primer pair R16F2n/R16R2 amplifies 1200 bp of the 16S rRNA gene. Each 25 µl PCR reaction mix contained 100 ng of total DNA, 2.5 µl 10X PCR buffer, 0.8 U Taq polymerase, 0.2 mM

dNTPs, 1.5 mM MgCl<sub>2</sub> and 0.4 µM of each primer. PCR was performed for 35 cycles using the following conditions: 1 minute (2 minutes for the first cycle) denaturation step at 94 °C, 2 minutes for annealing at 55°C and 3 minutes (10 minutes for the last cycle) at 72°C for primer extension. For identification of associated phytoplasmas, nested PCR products (1,200 bp) were digested with *AluI*, *HinfI*, *MseI*, and *RsaI* restriction enzymes and digestion profiles were compared with those of known phytoplasmas. Furthermore, ELISA test using polyclonal antibody prepared against alfalfa witches' broom from Yazd (Esmailzadeh-Hosseini *et al.*, 2003) was used for serological detection of phytoplasmas in suspected weeds. In order to identify winter hosts of *O. albicinctus*, specimens were collected by D-Vac aspiration from plant species and weeds in and around the 15 AWB affected alfalfa fields and sorted by their gross morphology.

### Results and discussion

From different species of herbaceous plants, *Prosopis farcta* (Banks & Soland.) Macbr and *Cardaria draba* (L.) Desv. showed phytoplasma-type symptoms (figures 1 and 2). *Prosopis farcta* and *Cardaria draba* proved to be positive in direct and nested PCR and PCR products of expected size (1,800 and 1,200 bp, respectively) were amplified. Five of 13 samples of *Prosopis farcta* and 3 of 10 samples of *Cardaria draba* tested were positive. Restriction fragments length polymorphism (RFLP) analysis of nested PCR products (1,200 bp of 16SrRNA) indicated that phytoplasmas associated with *Prosopis farcta* and *Cardaria draba* are similar to each other and with Yazd AWB agent, a peanut witches' broom group related phytoplasma (Lee *et al.*, 1998). Elisa test showed serological relationship of *Prosopis farcta* and



**Figure 1.** Small laves, shortened internodes, proliferation of axillary buds and bushy growing habit in *Prosopis farcta* in Esfandabad (Abarkouh, Yazd province). (In colour at [www.bulletinofinsectology.org](http://www.bulletinofinsectology.org))



**Figure 2.** Dwarfing, virescence, phyllody and infertile flowers in *Cardaria draba* in Banadak Sadat (Mehriz, Yazd province). (In colour at [www.bulletinofinsectology.org](http://www.bulletinofinsectology.org))

*Cardaria draba* phytoplasmas with Yazd AWB phytoplasma (YAWBP). RFLP analysis and serological relationship indicated that *Prosopis farcta* and *Cardaria draba* are hosts of YAWBP. *O. albicinctus* leafhoppers were collected on *Cynodon dactylon* L. (Pers.), *Hordeum murinum* L., *Digitaria sanguinalis* (L.) Scop., *Echinochloa crus-galli* (L.) P. Beauv., *Lolium temulentum* (L.), *Setaria viridis* (L.) P. Beauv., *Tamarix ramosissima* Ledeb and *Seidlitzia rosmarinus* (Ehrh.) Bge. during winter season and these plants are reported as overwintering hosts of the phytoplasma vector. In consequence, on the basis of this study eradication of weed species growing next to alfalfa farms is recommended.

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