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Polyclonal antibodies for the detection and identification of Fars alfalfa witches' broom phytoplasma

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Abstract

Alfalfa witches' broom (AlWB) is a destructive phytoplasma disease in Iranian southern and central provinces. Antiserum was raised by injection of partially purified Fars AlWB phytoplasma (FAlWBP) from 20 g infected alfalfa tissue into a rabbit. The specificity of the polyclonal antibody against phytoplasmas was shown by its reaction with diseased, but not with healthy alfalfa upon dot immunobinding assay (DIBA) tests. Phytoplasmas associated with Borazjan fababean phyllody and Chahbahar periwinkle phyllody were also shown to be serologically related to FAWBP. No serological relationship was found between FAlWBP and phytoplasmas associated with Yazd AlWB, witches' broom disease of lime, Khafr almond witches' broom, carrot witches' broom, sesame phyllody, eggplant big bud and tomato big bud.

Key words: phytoplasma purification, dot immunobinding assay, Iran.

Introduction

Iranian alfalfa witches' broom (AlWB) disease has been found in economically significant amounts in central and southern provinces (Salehi *et al*., 1995; Salehi *et al*., 2000). Fars (Iran) AlWB phytoplasma (FAlWBP) has been characterized using both biological and molecular methods (Salehi *et al*., 1995, Salehi *et al*., 2005). On the basis of full length 16S rRNA gene and SR sequences, FAlWBP is classified in the pigeon pea witches' broom (16SrII) group and is not distinguishable from agents of witches' broom disease of lime (WBDL) in Iran (Salehi *et al.,* 1998), and of the pigeon pea witches' broom related phytoplasma in Iran (Salehi *et al.,* 2005) The objectives of the present study were to partially purify the FAlWBP, to produce antiserum, to achieve serological detection of FAlfWBP and to investigate serological relationships among FAlfWBP and a selection of other phytoplasmas including WBDL phytoplasma using dot immunobinding assay (DIBA) tests.

Materials and methods

Infected alfalfa from Juyom (about 250 km south east of Shiraz, Fars province) was used for partial purification of the FAlfWBP phytoplasma. The nine following strains maintained and propagated in a red line of periwinkle (*Catharanthus roseus*) were also included in this study: Borazgan fababean phyllody (BFBP), carrot witches' broom (CWB), Chahbahar periwinkle phyllody (CPP), Khafr almond witches' broom (KLWB) sesame phyllody (SeP), witches' broom disease of lime (WBDL), Yazd AlWB (YAlWB), Zarghan eggplant big bud (ZEBB), and Zarghan tomato big bud (ZTBB).

The procedure of Clark *et al*. (1983) with modifications of Saeed *et al*. (1993) was used for partial purification and preparation of an antiserum. Infected alfalfa from Juyom (about 250 km south east of Shiraz,) was used for partial purification of FAlfWBP. Twenty grams of infected tissue (stem and leaf) were homogenized in cold GMS at a ratio of 1 g tissue to 5 ml GMS. The extract was filtered and then centrifuged at 2000 *g* for 15 min. This was followed by high-speed centrifugation at 39000 *g* for 1 h and resuspension of the pellet in 1.5 ml GMS buffer. The resuspended pellet was then incubated for 1 h at 4°C with 4 ml of undiluted antiserum prepared against healthy alfalfa extract. After another low and high speed centrifugation as described above, the final pellet was resuspended in 1 ml of PBS buffer (0.01 M phosphate buffer saline) and the resuspended pellet was used for immunization and production of polyclonal antiserum against FAlfWB.

A white rabbit was injected intramuscularly in legs and subcutaneously in the neck with an emulsion of equal volumes of partially purified FAWBP and Freund's incomplete adjuvant. Four injections were given at intervals of 7 days. The rabbit was bled 4 times at daily intervals beginning 5 week after primary immunization. This antiserum after absorption with healthy plant sap was used for serological detection and identification.

Dot immunobinding assays (DIBA) were used for serological detection of FAlfWBP and for investigation of possible serological relationships between FAlfWBP and a selection of other phytoplasmas using the Hibi and Satio (1985) procedure. Appearance of well defined purple spots on nitrocellulose sheets were regarded as positive reactions.

Results and discussion

After absorption with healthy plant sap, the antiserum prepared against partially purified FAlfWBP distinguished healthy and FAlfWB infected plants using DIBA tests. All symptomatic and 6 of 15 symptomless alfalfa plants from an infected alfalfa field in Juyom showed positive reactions. BFBP and CPP strains were shown to be serologically related to the FAlfWB phytoplasma. No serological relationship was found between FAlfWB and phytoplasmas associated with CWB, KALWB, SeP, WBDL, YAlWB, ZEBB and ZTBB (figure 1).

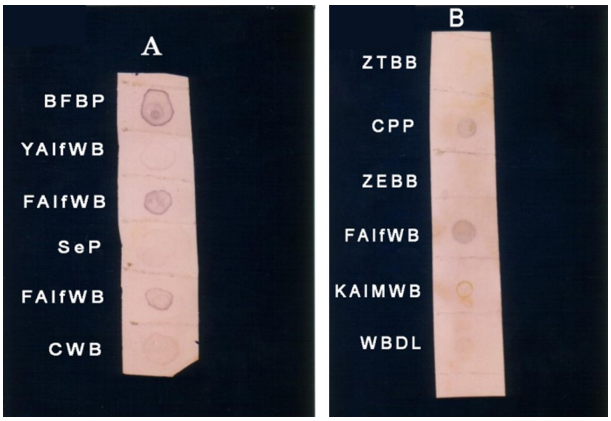

Figure 1. (A and B). Reaction of several Iranian phytoplasma strains to Fars alfalfa witches' broom antiserum in dot immunobinding assay. Phytoplasma abbreviations: BFBP, Borazgan fababean phyllody; CWB, carrot witches' broom; CPP, Chahbahar periwinkle phyllody; FAlWB, Fars alfalfa witches' broom; KALWB, Khafr almond witches' broom; SeP, sesame phllody; WBDL, witches' broom disease of lime; YAlWB, Yazd afalfa witches' broom; ZEBB, Zarghan eggplant big bud; ZTBB, Zarghan tomato big bud.

A polyclonal antibody against AlfWB was raised for the first time. Although the antibody was prepared by injection of partially purified phytoplasma, it exhibited a good degree of specificity and the background of non-specific reaction was considerably low. Use of this antiserum proved that BFBP and CPP phytoplasmas were related to FAlfWB phytoplasma, while YAlfWB phytoplasma was not serologically related to FAlfWB phytoplasma despite their common host and similar symptoms. Likewise, CWB, KALWB, SeP, WBDL, YAlWB, ZEBB and ZTBB phytoplasmas were serologically not related to FAlfWB phytoplasma. On the basis of 16S rRNA gene and SR sequence analysis CPP, BFBP, CWB, WBDL, YAlWB, ZEBB and ZTBB are related to 16SrII but KALWB and SeP phytoplasmas to 16SrIX Salehi *et al*., 2005). This study showed that sequence-based related phytoplasmas can be differentiated using polyclonal antibodies and serology is a useful method for separating different '*Ca.* Phytoplasma' species when they have over 97.5% similarity on 16S ribosomal gene as reported previously (IRPCM, 2004).

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