Occurrence of crown gall disease on Ficus benjamina in Fars and Isfahan provinces of Iran

Milad Aeinia, Hossein Mirzaeea, Seyed Mohsen Taghavia, Gholam Reza Khodakaramianb & Mehdi Amiri Mazhar

a Department of Plant Protection, College of Agriculture, Shiraz University, Shiraz, I.R. Iran
b Department of Plant Protection, College of Agriculture, Bu Ali Sina University, Hamedan, I.R. Iran

Published online: 05 Feb 2014.

To cite this article: Milad Aein, Hossein Mirzaee, Seyed Mohsen Taghavi, Gholam Reza Khodakaramian & Mehdi Amiri Mazhar (2014) Occurrence of crown gall disease on Ficus benjamina in Fars and Isfahan provinces of Iran, Archives Of Phytopathology And Plant Protection, 47:18, 2257-2262, DOI: 10.1080/03235408.2013.873256

To link to this article: http://dx.doi.org/10.1080/03235408.2013.873256

PLEASE SCROLL DOWN FOR ARTICLE

Taylor & Francis makes every effort to ensure the accuracy of all the information (the "Content") contained in the publications on our platform. However, Taylor & Francis, our agents, and our licensors make no representations or warranties whatsoever as to the accuracy, completeness, or suitability for any purpose of the Content. Any opinions and views expressed in this publication are the opinions and views of the authors, and are not the views of or endorsed by Taylor & Francis. The accuracy of the Content should not be relied upon and should be independently verified with primary sources of information. Taylor and Francis shall not be liable for any losses, actions, claims, proceedings, demands, costs, expenses, damages, and other liabilities whatsoever or howsoever caused arising directly or indirectly in connection with, in relation to or arising out of the use of the Content.

This article may be used for research, teaching, and private study purposes. Any substantial or systematic reproduction, redistribution, reselling, loan, sub-licensing, systematic supply, or distribution in any form to anyone is expressly forbidden. Terms &
Occurrence of crown gall disease on *Ficus benjamina* in Fars and Isfahan provinces of Iran

Milad Aeini*, Hossein Mirzaee*, Seyed Mohsen Taghavi*, Gholam Reza Khodakaramian and Mehdi Amiri Mazhar

*Department of Plant Protection, College of Agriculture, Shiraz University, Shiraz, I.R. Iran; Department of Plant Protection, College of Agriculture, Bu Ali Sina University, Hamedan, I.R. Iran

(Received 3 December 2013; accepted 4 December 2013)

*Ficus benjamina*, commonly known as weeping fig, Benjamin’s fig or Ficus tree is a species of flowering plant in the family of Moraceae. It is native to south and south-east Asia and Australia. Crown gall tumours were collected from branches of one-year-old weeping fig (*F. benjamina* L.) trees. A total of 50 strains of *Agrobacterium tumefaciens* were isolated from diseased Ficus plants and their morphological, molecular and biochemical characteristics were studied; pathogenicity tests on tomato, *F. benjamina* and *Bryophyllum daigremontianum* were also conducted. Based on the biochemical characteristics, pathogenicity test and PCR amplification of 730 bp fragment using VCR/VCF primers, the tested bacterial strains were identified as *A. tumefaciens*. This is the first report of crown gall on *F. benjamina* in Isfahan and Fars provinces of Iran.

**Keywords:** *Agrobacterium tumefaciens*; *Ficus benjamina*; occurrence; crown gall

**Introduction**

*Ficus benjamina* Linn. belonged to the family Moraceae (Burkill 1966). It is a medium-sized tree with several spreading branches from the base. The leaves are 2–5 cm wide and the bark is pale brown or greyish brown. The plant is known locally as “beringin, waringin and jejawi” (Holtum 1969). It is traditionally used as a stomachic, hypotensive and anti-dysentery agent (Trivedi et al. 1969). Neal (1965) describes *F. benjamina* as one of the most beautiful and graceful of figs. As the tree matures, its spread is generally broader than tall with a beautiful dense umbrella-like canopy of pendant branches that cascade down to the ground, hence the name, weeping fig (Rifflé 1998).

*F. benjamina* is widely distributed in the tropics (Baily 1963). It is native to a large area including India, southern China, south-east Asia, Malaysia, the Philippines, northern Australia and the islands of the South Pacific (Rifflé 1998).

The genus *Agrobacterium* (Conn 1942) is a member of the family Rhizobiaceae (Kersters & De Ley 1984) which has been included in the alpha-2 subclass of Proteobacteria on the basis of ribosomal characteristics (Woese et al. 1984). Until recently, the delineation of species within the genus *Agrobacterium* was based mainly on plasmid-borne pathogenicity characters and included *Agrobacterium radiobacter*, *Agrobacterium*
rhizogenes, Agrobacterium rubi and the type species A. tumefaciens (Skerman et al. 1980; Kersters & De Ley 1984).

Infection occurs at plant wound sites and involves the transfer of oncogenic DNA (T-DNA), which is located on Ti and Ri plasmids, from plasmid-harbouring (phytopathogenic) agrobacteria to the plant cell nucleus. The genes required for T-DNA processing and transfer reside in virulence (vir) regions (virA, virB, virG, virC, virD, virE and so on) that are also located on Ti and Ri plasmids (Sawada et al. 1995).

Crown gall was reported on fig trees as early as 1919, when extremely large tumours were observed on roots of Ficus aurea Nutt, growing in the Florida Everglades (Galloway 1919).

In the spring of 2011, crown galls (up to 5 cm in diameter) were observed in one-year-old weeping fig (F. benjamina L.) trees grown in a Fars and Isfahan provinces nursery (Figure 1).

Materials and methods

Isolations from galls of infected Ficus

Isolations were made from fresh galls on naturally infected Ficuses collected from late June through September 2010–2011. Galls were washed thoroughly in running tap water and blotted dried. Small sections of surface tissues were removed with a sterile scalpel, cut into smaller pieces in a drop of distilled water on a microscope slide and observed microscopically. About 5 to 10 sections of tissue were observed per gall, and when large masses of bacteria were seen, a loopful of the suspension was streaked on nutrient agar (NA) and A1 medium. All isolation plates were incubated 4–6 days at 28 °C, before typical colonies of Agrobacterium were selected for pathogenicity tests.

Physiological and biochemical tests

Physiological and biochemical features of the bacterial strains were determined by standard bacteriological methods including: Gram reaction, oxidase, growth at 35 °C, growth on 2% NaCl, action on Litmus milk, citrate utilisation, production of 3-ketolactose,
Pathogenicity tests

Strains were streaked on A1 medium to check for purity and to prepare inoculum for pathogenicity tests. Inoculations were made on two or more of the following hosts: *F. benjamina*, tomato (*Lycopersicon esculentum* Mill.) and *Bryophyllum daigremontianum*. For pathogenicity tests, plants were grown in a greenhouse at 30 °C under natural light. A suspension of the strain to be tested was made in sterile water. The concentration of the suspension was approximately $1 \times 10^9$ CFU mL$^{-1}$, as determined by measuring the optical density at 600 nm wavelength using a biophotometer. The suspensions were used to inoculate plants which were wounded at the third internode from the apex. Appearance of gall symptoms was assessed by visual inspection of the inoculated plants over a four-week-period.

DNA preparation

All strains were grown on NA medium at 25 °C for 3 days. A loopful of colony from each strain was suspended in sterile distilled water and their concentration were adjusted to 107 CFU (OD600 = 1). The bacterial suspensions were boiled for 8–10 min, cooled at room temperature and used as a template DNA for PCR experiment (Clerc et al. 1998).

Identification of *A. tumefaciens* with specific primers

Two oligonucleotides from VirC gene [primer VCF (sequence, 5′-ATCATTTGTAGCGACT-3′) and primer VCR (sequence, 5′-AGCTCAAACCTGCTTC-3′)] were selected for PCR and were purchased from Metabion Co., Germany. A universal primer set (VCF/VCR) for PCR analysis based on the sequences of the virC operon located on Ti and Ri plasmids was designed to detect these plasmids from phytopathogenic *Agrobacterium* strains (Sawada et al. 1995). The PCR reactions were performed in a Bio-Rad Icycler (USA) with 26 μl PCR mixture containing the following reagents: 2 μl of DNA template was transferred to 24 μl of a PCR mixture containing 50 pmol of each primer, 0.2 mM dNTP mix, 2 U of Taq DNA polymerase (Metabion Co., Germany) and 1.6 mM magnesium chloride. The PCR reaction was carried out for 35 cycles using the following procedure: template denaturation at 94 °C for 1.5 min, primer annealing at 60 °C for 1.5 minutes, DNA extension for 3.0 min at 72 °C and final extension at 72 °C for 10 min. The PCR products were electrophoresed on 1% TBE agarose gel at room temperature at 90 V cm$^{-1}$ for 1 h. Following staining with ethidium bromide, the gels were viewed and photographed under UV illumination.

Results and discussion

Crown gall is a neoplastic disease of plants which is caused by the Gram-negative bacterium, *A. tumefaciens* (Galsky et al. 1980). It is a common disease of dicot plants, including many woody shrubs and various herbaceous plants including many stone and pome fruit tree, grapevine, roses and some ornamental plants (Rhouma et al. 2006). Crown galls are often found at or just below the soil surface on the roots or crown region of plants (Ogawa et al. 1995). However, symptoms can develop on aerial parts.
of systemically infected plants. The ability of strains to cause crown gall depends on the presence of a Ti plasmid (pTi), a fragment of which (i.e. the T-DNA) is transferred during infection into wounded plant cells (Winans 1992). The purpose of this study is to isolate _A. tumefaciens_ from Isfahan and Fars Provinces of Iran and confirm their characteristics using biochemical, pathogenicity and PCR tests.

A total of 50 bacterial strains were isolated and purified from 50 diseased plant samples. All of the strains grew on A1 medium and produced large round, red colonies with white margines that are characteristics for _Agrobacterium_ (Figure 2). Morphological, physiological and biochemical test results showed that all strains were Gram-negative, rod-shaped, aerobic and grew at 35°C on A1 and 2% NaCl plates, alkalic on litmus milk and positive for oxidase, ferric ammonium citrate and 3-ketolactose reaction.

Figure 2. A1 medium and colonies that are characteristics of _Agrobacterium_ genus.

Figure 3. Formation of gall on stem of tomato artificially inoculated with _A. tumefaciens_.

_2260 M. Aeini et al._
Isolates were negative for citrate utilisation, gelatin liquefaction, starch hydrolysis, malonic acid, tartaric acid, PDA-CaCO3 (Potato dextrose agar) and mannitol-CaCO3. The isolates showed homogeneity in their physiological, biochemical and nutritional characteristics. All strains produced gall symptoms on inoculated tomato, *F. benjamina* and *B. daigremontianum* in greenhouse conditions after four weeks (Figures 3–5). A total of 50 isolates were tested with specific primers, VCF and VCR. All isolates amplified 730 bp fragment as expected (Figure 6). Based on the amplification of 730 bp fragment with VCR/VCF primers in PCR, biochemical and pathogenicity test, all of 50 strains isolated from Fars and Isfahan provinces were determined as *A. tumefaciens*.
Figure 6. Agarose gel electrophoresis of PCR products of A. tumefaciens strains with primers VCF and VCR. 100 bp DNA molecular marker. Right to left: 3–10 isolated A. tumefaciens. 2: positive control (A. tumefaciens); 1: negative control.

References