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Association of a 16SrII group phytoplasma with dieback disease of papaya in India

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Abstract

During surveys, papaya plants showing dieback symptoms with yellowing and necrosis of leaves were collected from the Sugarcane Research Station campus, Gorakhpur, Eastern Uttar Pradesh, India in September 2010. DNA was extracted from infected and healthy plants and indexed in nested PCR with phytoplasma generic primers P1/P7 and R16F2n/R16R2. Nested PCR amplicons of 1.2 kbp were obtained from dieback symptomatic papaya samples. Following RFLP analysis, infected papaya samples exhibited identical *Hae*III and *Rsa*I profiles, which were typical of a 16SrII phytoplasma. None of the healthy papaya samples evaluated was found positive for the phytoplasmas. The amplification with phytoplasma primers and their RFLP profiling suggests that the dieback phytoplasma associated with papaya in India is a phytoplasma member of the 16SrII group ('*Candidatus* phytoplasma aurantifolia'). This is the first record of this phytoplasma associated with dieback disease of papaya in India.

Key words: Phytoplasma, papaya, 16SrII group, dieback disease, PCR and RFLP analysis.

Introduction

Carica papaya is one of the most common fruits grown in India and the area devoted to its cultivation has increased considerably in the last decade. *Carica papaya* (papaya) has numerous medicinal properties. A papaya paste was used traditionally for the relief of burns, cuts, rashes and stings. A compound known as papain is derived from the papaya fruit and has long been used as a natural antacid, for ulcer relief and to relieve constipation. The whole papaya fruit is an excellent source of dietary fiber, which is also necessary for digestive health.

Identification and molecular characterization of phytoplasmas in *C. papya* has been reported in many countries. So far, phytoplasmas belonging to 6 ribosomal groups have been identified in *C. papaya* plants from all over the world (White *et al*., 1998; Arocha *et al*., 2007, 2009; Chaturvedi *et al*., 2010; Alhudaib and Arocha, unpublished; Navarrete-Yabur *et al*., unpublished).

Based on the sequences retrieved from GenBank, identified phytoplasmas of papaya mainly belong to the 16SrI, 16SrII, 16SrIII, 16SrX, 16SrXII and 16SrXVII groups. The peanut witches' broom group of phytoplasmas is the prevalent group of phytoplasmas identified in *C. papaya*. White *et al*. (1998) assigned the taxon '*Candidatus* Phytoplasma australasia' for papaya yellow crinkle and papaya mosaic disease on *C. papaya* plants in Australia. So far only, Kumar *et al*. (GenBank Ac. No. HM449951) have reported association of the '*Ca.* P. asteris' (16SrI group) with papaya dieback disease on *C. papaya* in India.

The molecular characterization of phytoplasmas causing yellowing and tip necrosis of leaves followed by dieback symptoms on *C. papaya* in India was carried out.

Materials and methods

During surveys on phytoplasma diseases symptomatic *C. papaya* plants showing dieback disease along with yellowing and necrosis of leaves were collected from the Sugarcane Research Station campus, Gorakhpur, Eastern Uttar Pradesh, India in 2009-2010. DNA from symptomatic *C. papaya* plants was extracted and amplification of phytoplasma ribosomal DNA (rDNA) was done with the universal phytoplasma primer pairs P1/P7 (Schneider *et al*., 1995) and R16F2n/R16R2 (Gundersen and Lee, 1996). Reactions were performed in a Minicycler with initial denaturation at 94°C for 2 min, followed by 40 cycles consisting of denaturation at 94°C for 1 min, annealing at 52°C for 30 s and extension at 72°C for 30 s, with extension in the final cycle for 2 min. Total PCR volumes were 100 µl and contained 200 µM of each dNTP, 0.4 µM of each primer, 1 X DNA polymerase reaction buffer, 1 U *Taq* DNA polymerase (Boehringer) and 5 µl template DNA solution. Each reaction mixture was covered with 50 µ1 sterile mineral oil (Sigma). Five microlitres of each PCR product was subjected to electrophoresis in a 1.0% (w/v) agarose gel, stained with ethidium bromide and observed under UV illumination. Amplicons obtained with R16F2n/R16R2 primers were subjected to RFLP analyses with restriction enzymes *Hae*III and *Rsa*I to verify phytoplasma identity.

Results and discussion

The most peculiar symptoms observed on *C. papaya* plant includes yellowing, crinkling and leaf tip necrosis symptoms, drying of the upper leaves, which progresses to death of the entire plant (figure 1).

Figure 1. *Carica papaya* plant showing dieback symptom.
(In colour at www.bulletinofinsectology.org)

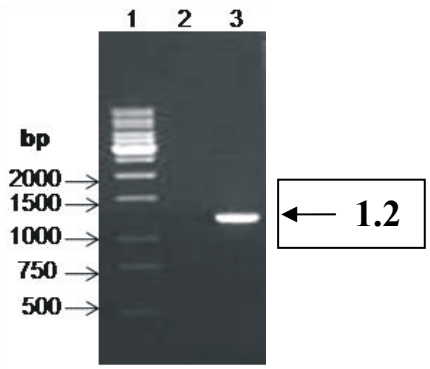

Figure 2. Lanes 1: 1kb ladder (MBI, Fermentas); lane 2: Symptomless papaya; lane 3: nested PCR amplicons from papaya infected leaf sample.

The 1.2 kb amplicon was obtained from nested PCR (figure 2). Neither by direct ('one-round') nor by nested PCR assays was DNA amplified from template DNA isolated from any of the healthy non-symptomatic samples. RFLP analysis with *Hae*III and *Rsa*I showed restriction profiles of phytoplasmas related to the 16SrII group. The amplification with phytoplasma nested primers and their RFLP profiling suggests that the dieback phytoplasma associated with papaya die-back disease in India is a member of the 16SrII group ('*Ca.* P. aurantifolia'). However, the only report available on association of phytoplasmas with *C. papaya* plants in India concluded that it belonged to the 16SrI group (Ac. No. HM449951). Therefore, this is the first record of a 6SrII group phytoplasma association with dieback disease of papaya in India. Dieback disease of papaya associated with phytoplasmas has already been reported from Australia (Ac. No. Y18215), Cuba (Arocha *et al*., 2005) and Ethiopia (Arocha *et al*., 2006). However, association of 16SrII group phytoplasmas on papaya has only been recorded from Ethiopia (Arocha *et al*., 2006) and Cuba (Ac. No. EU350564). This study confirms that the dieback disease of papaya is associated with two groups (16SrI and 16SrII) of phytoplasmas in India.

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