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Presence of phytoplasma infections in papaya (*Carica papaya* L.) Plants in Uttar Pradesh, India

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ABSTRACT

During survey of papaya fields in Meerut, severe symptoms of leaf yellowing, intervenial chlorosis, and curl apical necrosis were observed in approximately 25 per cent of plants in each of the papaya fields. DNA extracted from leaf, midrib and bark of symptomatic and healthy plants of papaya were preceded with universal primer pairs. Expected ~1600 bp fragments were amplified with primer pair P1/Tint in diseased plants. Infection of phytoplasma disease in papaya plants was confirmed by the PCR.

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INTRODUCTION

Diseases caused by phytoplasma occur worldwide in many economically important crops. Some of these diseases, especially those of woody plants, are lethal. The list of plants and insects known to harbour phytoplasma is continuously increasing, as is the number of taxonomically characterized phytoplasma strain (Seemuller *et al.*, 1998, 2002; Lee *et al.*, 2000).

Papaya is a popular fruit famous for its high nutritive and medicinal values. Papaya is a plant of family Caricaceae, is a native crop of Mexico, and was introduced in India in the 16th century. Now it has become popular all over India and is the fifth most commercially important fruit of the country produced 5,160,390 tonnes papaya in 2012 (FAOSTAT). In cultivation, it grows rapidly; fruiting within three years and the production of fruits is quite high per unit area. It is, however, highly frost-sensitive, limiting its production to tropical climates. Papaya can be used as a food, a cooking aid and in traditional medicine. Papaya fruit is a source of nutrients such as provitamin A, carotenoid, vitamin C, folate and dietary fiber. Papaya seed extract may have effects in toxicity-induced kidney failure. Papaya skin, pulp and seeds also contain a variety of phytochemicals, including lycopene and polyphenols.

Papaya (*Carica papaya*) is widely cultivated and affected by a number of phytoplasma diseases worldwide such as dieback, yellow crinkle and mosaic were recognized in Australia, Israel and Ethiopia (Guthrie *et al.*, 1998; Gibb *et al*., 1996, 1998; Liu *et al.*, 1996, Elder *et al*., 2002, Gera *et al.*, 2005; Arocha *et al.*, 2007a), bunchy top in cuba and Oman (Arocha *et al.*, 2007b; Perez *et al.*, 2010) and yellow crinkle and mosaic (Guthrie *et al.*, 1998). Lebsky *et al.* (2010) reported Application of scanning electron microscopy for diagnosing phytoplasmas in single and mixed (virus-phytoplasma) infection in papaya, from different Mexican

states. This paper extends the host range of phytoplasma in India.

MATERIAL AND METHODS

Sample collection :

A survey was conducted in Meerut, Bulandshahr and Allahabad district of Uttar Pradesh, India during October 2009 - July 2012. In March 2012 fresh samples of papaya showing phytoplasma-like symptoms as well non-symptomatic collected from Meerut were employed for nucleic acid extraction to verify phytoplasma presence.

DNA extraction :

Total nucleic acids were extracted from a 100 mg of leaf midribs and bark tissues from papaya plants samples by DNeasy plant mini kit method (Qiagen, GmbH, Germany), dissolved in sterile distilled water, and maintained at -20°C. The concentration of DNA in different plant samples were estimated using UV- spectrophotometer (UV-1800, Shimadzu) and good quality DNA was used in PCR reaction.

Polymerase chain reaction :

Two phytoplasma universal primer pairs designed from rRNA operon region P1/P7 (Deng and Hiruki, 1991) and P1/Tint (Smart *et al*., 1996) were used to prime phytoplasma. Direct Hot start PCR amplifications were carried out with universal primer pair P1/P7 (5'AAG AGT TTG ATC CTG GCT CAG GATT3'/5'CGT CCT TCA TCG GCT CTT3') and P1/Tint (5'AAG AGT TTG ATC CTG GCT CAG GAT T3'/ 5'TCA GGC GTG TGC TCT AAC CAG C3') separately in a thermocycler. Each 50 µl reaction containing 1X PCR buffer, 2mM $MgCl_2$, 200 µM of each dNTP (MBI, Fermentas), 100mM P1/P7 or P1/Tint primers and 1U of Taq DNA polymerase (MBI, Fermentas) consisting of denaturation at 94°C for 10 min, 35 cycles at 94°C for 30 sec, annealing at 55°C for 60 sec, extension at 72°C for 90 sec and a final extension step at 72°C for 10 min. Amplified DNA was separated by agrose gel electrophoresis in 0.8% agarose gel. Stained with ethidium bromide (EtBr), bands were visualized by using Gel-Doc. The Qiagen gel extraction kit was used to elute the amplicon from the agarose gel.

RESULTS AND DISCUSSION

The findings of the present study as well as relevant discussion have been summarized below :

Sample collection :

Severe symptoms of leaf yellowing, intervenial chlorosis, and curl apical necrosis were observed in approximately 25 per cent of plants in each of the papaya fields of Meerut, Uttar Pradesh (Fig. 1).

DNA extraction :

Estimation showed that total DNA extracted from leaf, midrib and bark of infected and healthy papaya plants was good quality and average concentration of DNA was 0.415µg/µl per samples. Infection of phytoplasma disease in papaya plants was detected by the PCR.

Fig. 1 : Symptoms of phytoplasma infection in papaya field (a) resembling leaf yellowing, intervenial chlorosis and (b) curl apical necrosis and internodes shortening

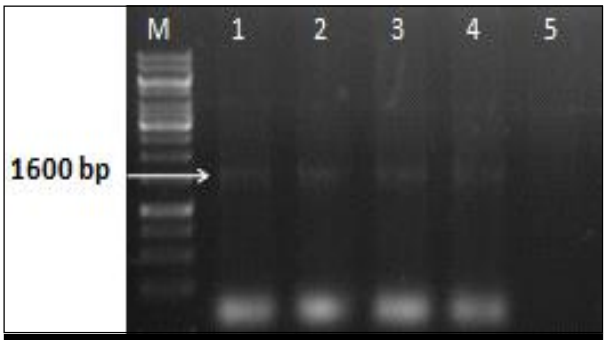

Fig. 2: Agarose gel electrophoresis of 16S rDNA PCR products obtained with primers P1/Tint in papaya healthy plants. Lane M: 1 kb DNA ladder (fermentas), Lane 1, 2, 3 and 4: ~1600 bp amplified product, lane 5-neative control (sterile distilled water)

Polymerase chain reaction :

DNA extracted from symptomatic and asymptomatic plants of papaya were preceded with primer pairs P1/P7 and P1/Tint. There was no amplification with primers P1/P7 but expected ~1600 bp fragments were amplified with primer pair P1/Tint in midrib and bark samples of all infected samples (Fig. 2). No amplification found in healthy plants and leaf samples of diseased papaya plants. Results revealed that all symptomatic plants collected were tested positive. Results of Direct PCR clearly indicate that severe symptoms of leaf yellowing in papaya caused by phytoplasma. P1/Tint amplified (1600bp) 16S rDNA region of phytoplasma associated to papaya plants was gel purified and sends for direct sequencing. Sequencing was not successful, due to low concentration of PCR amplified products.

It was the advent of the molecular biological revolution in the 1980s that saw the introduction of techniques such as nucleic acid purification and the polymerase chain reaction, with which the secrets of these fastidious bacteria begin to emerge (Weintraub and Jones, 2010). The amount of phytoplasma DNA is lower than 1per cent of total DNA extracted from tissue (Bertaccini, 2007). The success of PCR in detecting phytoplasma in field-collected samples largely depends on obtaining total nucleic acid preparations of good quality and enriched with phytoplasma DNA, but this has always been difficult (Firrao *et al.*, 2007).

Severe symptoms observed in papaya plants in present study differed from those associated with dieback disease of papaya reported from Gorakhpur, Western India (Rao *et al*., 2011) and the symptoms drying of upper leaves, proliferation of axillary shoots, reduction in leaf size and interveinal chlorosis observed in the papaya fields of Pune, Western India (Verma *et al*, 2012).

Presence of phytoplasma was confirmed in papaya plants by the amplification of approximately 1600bp fragment. Unsuccessful amplification with P1/P7 primers expressed the infection was in low titre, due to this sequencing of 16S rDNA phytoplasma associated to phytoplasma was failed. Therefore, there is an urgent need to develop effective control measures to halt spread of the disease to other areas of the country. Infected plants must be removed and destroyed so that the pathogen is not spread to healthy plants nearby.

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