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MOLECULAR DETECTION OF PHYTOPLASMA FROM PHYLLODY INFECTED SESAME AND ITS VECTOR *OROSIUS ALBICINCTUS* DISTANT

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ABSTRACT

Asymptomatic and symptomatic plants were collected from the phyllody infected sesame field during *kharif* 2012. From the infected plants, leaves, flowers, stems, seed capsules and roots were selected both from symptoms and symptomless branch. Leafhoppers were also collected from the same field with insect collection net. Total genomic DNA from asymptomatic and symptomatic plants was extracted following CTAB (Cetyl Trimethyl Ammonium Bromide) method with some modifications and from leafhoppers with phenol- chloroform extraction protocol. Amplification of band size 1.8 kb and 1.2 kb was obtained from symptomatic plant and its vector *Orosius albicinctus* using phytoplasma specific primer pair P1/P7 and R16F2n/ R16R2, respectively in first and second round PCR. The phytoplasma could be detected only from stems of symptomless branch of infected plant while in symptomatic plant's branch it was amplified from stem and flower. Thus the status of leafhopper, *O. albicinctus* as a vector of phytoplasma that cause sesame phyllody was confirmed. Further, it was observed that the symptomless parts of phyllody infected plants also harbour phytoplasma.

Key words: *Sesamum indicum*, sesame phyllody, *Orosius albicinctus*, phytoplasma, CTAB, symptomatic and asymptomatic plant parts, stems, flowers, branches

Sesame (*Sesamum indicum Linnaeus*) is an important oilseed crop cultivated extensively from tropical to the temperate zones. It is affected by many pests and diseases, of which the important one is leafhopper, *Orosius albicinctus* Distant which transmits phyllody disease. A virus is a possible cause which was later confirmed as mycoplasma like organisms (MLOs) and recently termed as phytoplasmas (Das and Mitra, 1998). This disease takes a heavy toll resulting in significant yield losses. The first evidence of association of mycoplasma-like organism with the disease was obtained in the Upper Volta by Cousin *et al.* (1971). This has been further confirmed in Thailand, Israel and India. Affected plants remain partially or completely sterile, resulting in total loss of yield, and as much as 10-100% incidence had been recorded in India (Brar and Ahuja, 1979), with yield loss due to phyllody in India estimated to be about 39-74%. Confirmation studies on phyllody and its vector are important and hence the present study.

MATERIALS AND METHODS

Symptomatic and asymptomatic plants were collected from the phyllody infected sesame field during *kharif* 2012 and kept at -20 °C for isolation of plant DNA. Symptomatic plant having both symptom and symptomless branch were also selected to know whether the symptomless branch harbour phytoplasma. From these plants, samples of symptoms and symptomless branches, leaves, flowers, stems, seed capsules and roots were drawn. Leafhoppers (*Orosius albicinctus* Distant, *Amrasca biguttula biguttula* Ishida, *Hishimonas phycitis* Distant and *Balclutha incise* Matsumura) were also collected from the field using insect collection nets. These leafhoppers were killed and stored in 75% alcohol at -20°C for detection of phytoplasma following the extraction of DNA.

Total genomic DNA from asymptomatic and symptomatic plants was extracted following CTAB (Cetyl Trimethyl Ammonium Bromide) method of Kollar *et al.* (1990) with some modifications. Genomic DNA from leafhoppers was isolated using a phenol-chloroform extraction protocol (Barr *et al.*, 2009). The PCR using primer pair P1/P7 in the first amplification followed by R16F2n/ R16R2 in the second amplification was performed to detect phytoplasma in the infected sesame samples and leafhopper vectors. For PCR amplification, 32 cycles were conducted in an automated thermal cycler (Eppendorf Thermocycler). PCR was carried out in mixtures containing 2 µl each of 10X PCR buffer and 2.5mM dNTPs mixture, 1 µl each of Primer P1 (5pM) and P7 (5pM), 0.5 µl of Taq

Polymerase (3u/µl), 1 µl of Template DNA (1µl) and 12.5 µl of sterile distilled water making the volume of 20 µl/tube. The following conditions were used: denaturation at 94°C for 45 sec, annealing for 2 min at 54.9°C (first round) and 54.8°C (second round), primer extension for 3 min at 72°C and final extension for 10 min at 72°C. After completion of the reaction, the products were kept at 4°C, prior to gel analysis. The PCR products were electrophoresed at 100v for 45 min through 1% agarose gel, stained in ethidium bromide and visualized under UV trans-illuminator for DNA bands and photographed for documentation.

RESULTS AND DISCUSSION

Phytoplasma DNA was detected with PCR primer pair P1/P7 and R16F2/R16R2 from the symptomatic sesame plants and vector, *O. albicinctus*. A 1.8 kb fragment of 16S rRNA gene of phytoplasma was amplified in PCR using phytoplasmal primers pair P1/P7 from the phyllody sample. Using the same primer phytoplasma DNA was detected only from stem of symptomless branch (Fig. 1) and stem and infected flower from the symptomatic branch of infected plant (Fig. 2). Amplification of band size 1.8 kb and 1.2 kb was obtained from *O. albicinctus* using phytoplasma specific primer pair P1/P7 and R16F2n/R2 respectively in first and second round PCR. Phytoplasma DNA was not detected from asymptomatic sesame samples and other leafhoppers *viz.*, *A. biguttula biguttula*, *H. phycitis* and *B. incise* (Fig. 3, 4).

These results clearly indicate that the sesame phyllody disease was caused by phytoplasma and it is

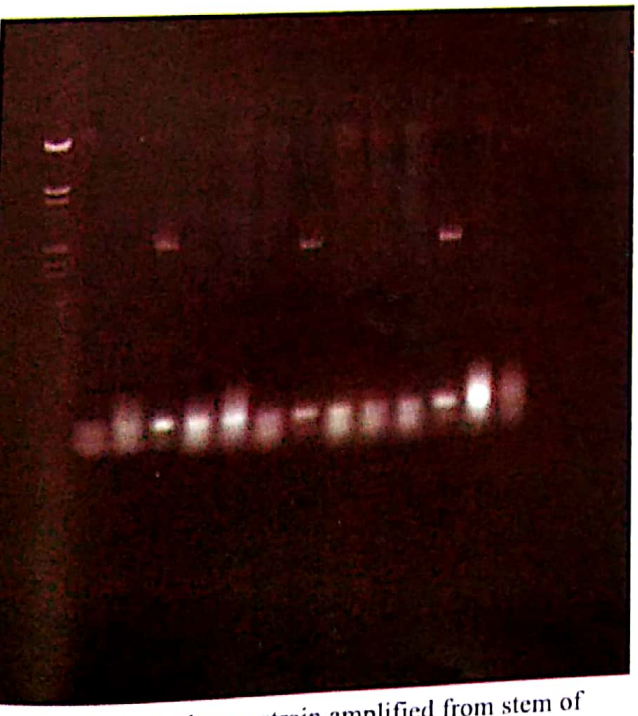

Fig. 1. Phytoplasma strain amplified from stem of symptomless branch of phyllody infected plant with primer P1/P7 (1.8 kb) (M- marker, 1,5,9- Leaf, 2,6,10- flower, 3,7,11- Stem, 4,8,12- Seed capsule, 13- Root).

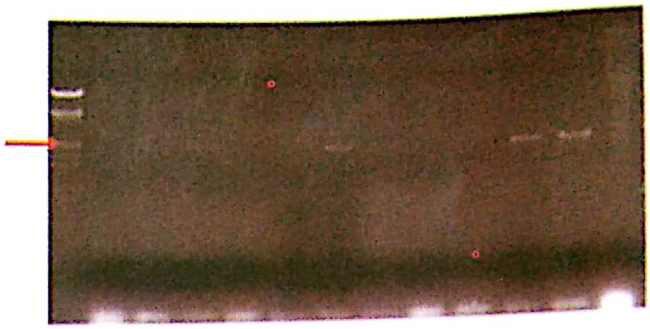

Fig. 2. Phytoplasma strain amplified from infected flower and stem of infected part of phyllody infected plant with primer P1/P7 (1.8 kb) (M- marker, 1,5,9- Leaf, 2,6,10- flower, 3,7,11- Stem, 4,8,12- Seed capsule)

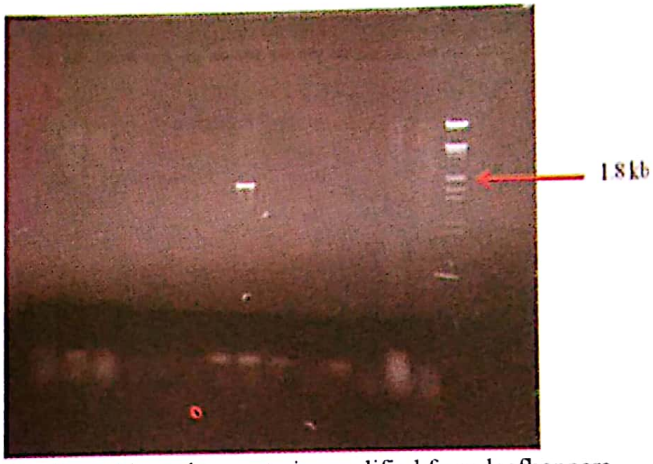

Fig. 3. Phytoplasma strain amplified from leafhoppers (*Orosius albicinctus* Distant) with primer P1/P7 (1.8 kb) (1,2,3,4- *Hishimonas sp.*, 5,6,7,8- *Orosius sp.*, 9,10,11- *Amrasca sp.*, 12,13,14- *Balclutha sp.* M- Marker)

Fig. 4. Phytoplasma strain amplified from infected plant and leafhoppers (*Orosius albicinctus* Distant) with primer R16F2n/ R16R2 (1.2 kb) (M- Marker, 1- Flower, 2- Stem, 3- *Orosius* sp., 4- Leaf, 5- Capsule, 6- Negative control, & 7- Positive control)

transmitted by leafhopper vector, *O. albicinctus*. Similar results had been obtained using the same primer pair by Al-Sakeiti *et al.* (2005), Sertkaya *et al.* (2007) and Akhtar *et al.* (2009). Esmailzadeh-Hosseini *et al.* (2007) also detected a 1.2-kb 16 S rDNA fragment of phytoplasma DNA from infected sesame and *O. albicinctus* using primer R16F2n/R16R2. Similar results had been obtained by Khan *et al.* (2007a) in Oman and Khan *et al.* (200 b) in India.

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