The Bioscan 12(2): 789-792, 2017
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.com

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

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KEYWORDS
Sesamum indicum
Sesame phyllody
Vector
Orosius albicinctus
Phytoplasma

Received on :
15.03.2017

Accepted on :
18.05.2017

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ABSTRACT
Sesame is an important oilseed crop and India happens to be the largest producer. However, its productivity is quite low due to the infestation of insect pests and diseases and one of the important diseases is sesame phyllody transmitted by the leafhopper, *Orosius albicinctus* Distant. In North Karnataka, 4 species of leafhoppers were found to infest the crop and therefore, the present study was undertaken to detect and confirm the vector of the disease. Both symptomatic and asymptomatic plants were collected from the field and also the leafhoppers during *Kharif* – 2013. The total genomic DNA of the plants was extracted through CTAB method and phenol-chloroform extraction protocol was used for leafhoppers. Amplification of band size 1.8 kb and 1.2 kb was obtained from symptomatic sesame plant and its vector *O. albicinctus* using the primer pair P1/P7 and R16F2n/R16R2 respectively in first and second round PCR. The phytoplasma was detected only from stems of symptomless branch of infected plant while in symptomatic branches it was amplified from stems and flowers. Thus the status of leafhopper, *O. albicinctus* as a vector of phytoplasma that cause sesame phyllody was confirmed and this is the first time that phytoplasma has been detected from the vector *O. albicinctus* through molecular techniques.

INTRODUCTION

Sesame (*Sesamum indicum* Linnaeus) is an important oilseed crop grown in tropics and subtropics and it is also known by "queen of oil seeds". Around the globe, it is grown on an area of 7.73 million ha with the production of 6.11 million tons. Among the sesame growing countries, India ranks first in area (17.5 lakh ha) and production (8.93 lakh tonnes) but the productivity is quite low (368 kg/ha) compared to world average (489kg/ha) (Anonymous, 2012). In India, the crop is commonly known as "Till" and it is mainly grown in the state of Maharashtra, Uttar Pradesh, Rajasthan, Orissa, Andhra Pradesh, Madhya Pradesh, Tamil Nadu, West Bengal, Gujarat, Karnataka, Kerala, Bihar, Assam and Punjab and to a limited extent, in Tripura and Himachal Pradesh (Ghosh *et al*., 2013). In Karnataka, it is grown during *kharif* season with the total area of 0.87 lakh ha and production of 0.51 lakh tonnes (Anonymous, 2013). The crop is known to be infested by many insect pests and diseases which hampered its productivity and one of the important pests is leafhopper, which is known to transmit phyllody diseases caused by phytoplasmas. The disease has now been reported from India, Iran, Iraq, Israel, Burma, Sudan, Nigeria, Tanzania, Turkey, Uganda, Upper Volta, and Mexico (Akhtar *et al*., 2009). The first evidence of association of mycoplasma-like organism with the disease was obtained in the Upper Volta by Cousin *et al*. (1971). Prasad and Sahambi, 1982 reported that leafhopper *Orosius albicinctus* Distant is responsible for the transmission of phytoplasma that caused sesame phyllody from India and Esmailzadeh-Hosseini *et al*. (2007) and Omidi *et al*. (2010) also reported *Orosius albicinctus* as a vector of phytoplasma associated with sesame phyllody in Iran. However, Salehi and Izadpanah, (1992) reported leafhopper *Neoaliturus haematoceps* as a vector of sesame phyllody in Fars province of Iran. In North Karnataka, 4 species of leafhoppers (*Orosius albicinctus* Distant, *Amrasca biguttula biguttula* Ishida, *Hishimonas phycitis* Distant and *Balclutha incise* Matsumura) were found to infest the crop and therefore, the present study was undertaken to detect and confirmed the vectors of the sesame phyllody.

MATERIALS AND METHODS

To detect the phytoplasma from the infected plants and its vectors, both symptomatic and asymptomatic plants were collected from the phyllody infected sesame field during *Kharif* - 2013 and kept in - 20ºC for isolation of plant DNA. Symptomatic plant having both symptom and symptomless branch were also selected to know whether the symptomless branch also harbour phytoplasma. From both symptoms and symptomless branches, leaves, flowers, stems, seed capsules and roots were selected for detection of phytoplasma. Leafhoppers *viz*., *Orosius albicinctus* Distant, *Amrasca biguttula biguttula* Ishida, *Hishimonas phycitis* Distant and *Balclutha incise* Matsumura which were dominant during the research period were also collected from the field using insect

collection nets. These leafhoppers were preserved at -20°C for detection of phytoplasma following the extraction of DNA as mention by Esmailzadeh-Hosseini *et al.,* 2007. Total genomic DNA from asymptomatic and symptomatic plants were extracted by following CTAB (Cetyl Trimethyl Ammonium Bromide) method of Kollar *et al*. (1990) and Genomic DNA from leafhoppers was isolated using a phenol-chloroform extraction protocol (Barr *et al.,* 2009). The presence of phytoplasmas in infected plant samples and its vectors were determined by a PCR procedure using the universal phytoplasma primer pair P1/P7 in direct PCR followed by primer pair R16F2n/R16R2 in nested PCR (Lee *et al.,* 2004).

PCR amplification of Phytoplasma DNA

PCR was performed in 20 μL volume using primer pair P1/P7 followed by primer pair R16F2n/ R16R2. The reaction mixture composition was 2.0 μL 10X PCR buffer (supplied with the enzyme), 2.0 μL of 2.5mM dNTPs, 1.0 μl of 5pmol/μl forward and reverses primers, 0.5 μl Taq DNA polymerase (3U/ μl), 1.0 μl template, 12.5 μl of nuclease free water. For primer pair P1/P7, DNA amplification parameters were 32 cycles of initial denaturation for 2 min at 94ºC, denaturation for 45 sec at 94 °C, annealing for 2 min at 54.9ºC, extension for 3 min at 72 °C and final extension at 72ºC for 10 min. Amplification products were hold at 4ºC for infinity prior to gel electrophoresis. For primer pair R16F2n/ R16R2, similar programmme was followed except the annealing temperature of 2 min at 54.8ºC.

Gel electrophoresis

The PCR products were analysed in 1.0 per cent agarose gel electrophoresis in 1X TAE buffer containing 0.5 μg/ml of ethidium bromide. 2 μl of loading dye were mixed with 10μl of each PCR product and loaded in the well. 2μl of double digest DNA marker was loaded in one well as molecular weight standards. The electrophoretic gel was run at 100v for 45 minutes till the dye has migrated one-third of the distance in the gel. Migrated DNA was visualized using a UV trans-illuminator for DNA bands and photographed for documentation.

RESULTS AND DISCUSSION

The result revealed that phytoplasma DNA was detected by PCR primer pair P1/P7 and R16F2/R16R2 from the symptomatic sesame plants and also from the leafhopper, *O. albicinctus* Dist. A 1.8 kb fragment of 16S rRNA gene of phytoplasma was amplified in PCR using phytoplasmal primer pair P1/P7 from the phyllody infected samples. Using the same primer, phytoplasma DNA was detected only from stem of symptomless branch of infected plant but fails to detect from leaves, flowers, seed capsules and root (Fig.4) and from the symptomatic branch of infected plant, phytoplasma was detected from stem and infected flower but unable to detect from leaves and seed capsules (Fig.5). The primer pair P1/P7 successfully yield 1.8 kb PCR product at annealing temperature of 54.9°C from leafhopper *Orosius albicinctus* and fail to detect from *Hishimonas phycitis, Amrasca biguttula biguttula* and *Balclutha incise* (Fig. 6) and 1.2 kb PCR product at annealing temperature 54.8°C was obtained from infected plant as well as from leafhopper *O. albicinctus* using phytoplasma specific

Figure 1: Healthy plant

Figure 2: Sesame phyllody symptom

Figure 3: *Orosius albicinctus* Distant

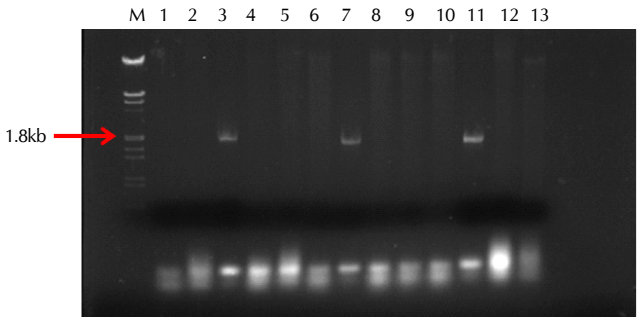

Figure 4: Phytoplasma strain amplified from stem of symptomless branch of phyllody infected plant with primer P1/P7 (1.8 kb) (M- marker, 1,5,9- Leaf, 2,6,10- flower, 3,7,11- Stem, 4,8,12- Seed capsule, 13- Root)

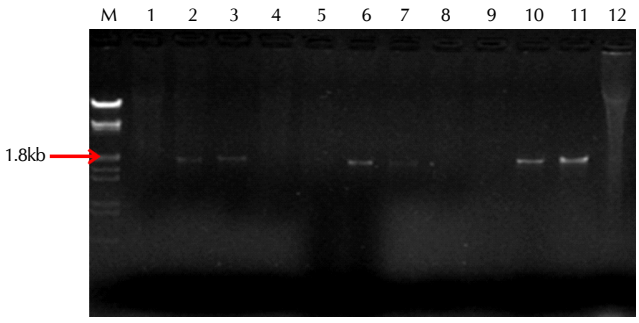

Figure 5: Phytoplasma strain amplified from infected flower and stem of infected part of phyllody infected plant with primer P1/P7 (1.8 kb) (M- marker, 1,5,9- Leaf, 2,6,10- flower, 3,7,11- Stem, 4,8,12- Seed capsule)

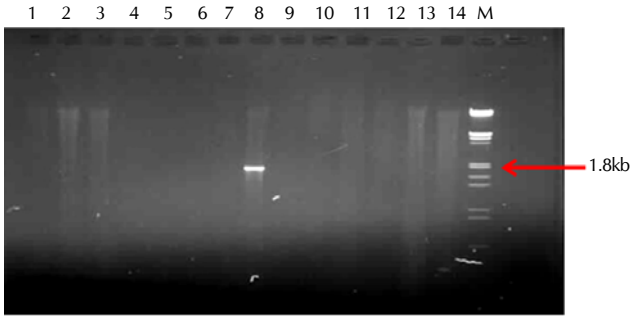

Figure 6: Phytoplasma strain amplified from leafhoppers (*Orosius albicinctus* Distant) with primer P1/P7 (1.8 kb) (1,2,3,4- *Hishimonas sp.,* 5,6,7,8- *Orosius sp.*, 9,10,11- *Amrasca sp.*, 12,13,14- *Balclutha sp.* M- Marker)

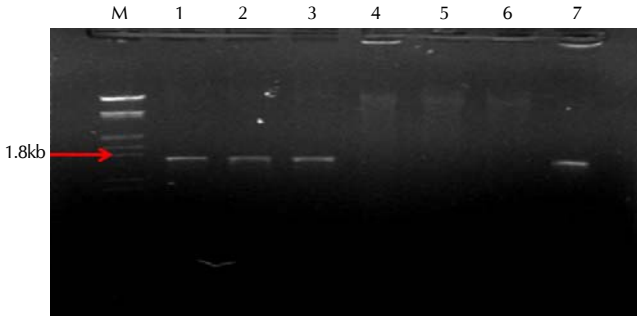

Figure 7: Phytoplasma strain amplified from infected plant and leafhoppers (*Orosius albicinctus* Distant) with primer R16F2n/ R16R2 (1.2 kb); (M- Marker, 1- Flower, 2- Stem, 3- *Orosius* sp., 4- Leaf, 5- Capsule, 6- Negative control, & 7- Positive control)

primer pair R16F2n/R16R2 in the second round PCR and unable to detected from asymptomatic samples and other leafhoppers *viz*., *A. biguttula biguttula*, *H. phycitis* and *B. incise* (Fig. 7). The results clearly revealed that the sesame phyllody disease was caused by phytoplasma and it is transmitted only by leafhopper, *Orosius albicinctus* and the stem of symptomless branch of infected plant also harbor phytoplasma. Similar results were also obtained by Al-Sakeiti *et al*. (2005), Akhtar *et al*. (2009) and Catal *et al*. (2013) in different places by using same primer pairs from the infected sesame plants. However, Sertkaya *et al*. (2007) detect phytoplasma from both infected plants and its vector *Orosius orientalis* (Matsumura) by using the universal phytoplasma primer pair P1/P7 in direct PCR followed by primer pair R16F2n/R16R2 in nested PCR in Turkey. Esmailzadeh-Hosseini *et al*. (2007) also detected phytoplasma from infected sesame and its vector *O. albicinctus* by using the phytoplasma universal primer pair R16mF2/R16mR1 in the first amplification followed by R16F2n/R16R2 in the second amplification with the 1.2kb 16SrDNA fragment. Similar results were also recorded by Khan *et al*. (2007 a) in Oman using primer pairs P1/16S-Sr followed by R16F2n/R16R2 which amplify 1.53 kb and 1.2 kb fragment respectively. However, Khan *et al*. (2007 b) and Un-Nabi *et al*. (2015) in India used phytoplasma universal primer pair P1/P6 and R16F2n/R16R2 for amplification of a fragment of 1.5 and 1.25 kb of 16SrRNA gene. Thus the status of leafhopper, *O. albicinctus* as a vector of phytoplasma that cause sesame phyllody was confirmed. Further, symptomless parts of phyllody infected plants also harbour phytoplasma and this is the first time that phytoplasma has been detected from its vector *O. albicinctus* through molecular technique in North Karnataka.

ACKNOWLEDGEMENT

The authors gratefully acknowledge the help rendered by Dr. P. U. Krishnaraj, Professor and Head, Dept. of Biotechnology, UAS, Dharwad for providing necessary facilities to conduct the work and Dr. C.A. Viraktamath, Department of Entomology, University of Agricultural Sciences, GKVK, Bangalore for identifying the leafhopper specimens.

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