Bulletin of Insectology ***64*** *(Supplement): S79-S80, 2011*
ISSN 1721-8861

Diversity among phytoplasma infecting various economically important plant species grown in India

Shri K. RAJ[1], Sunil K. SNEHI[1], Mohd S. KHAN[1], Govind P. RAO[2]
[1]*Plant Molecular Virology, National Botanical Research Institute, Lucknow-226001, India*
[2]*Division of Plant Pathology, Indian Agricultural Research Institute, New Delhi-110012, India*

Abstract

Phytoplasma have been identified by electron microscopy observations of infected tissues, by polymerase chain reaction amplification with specific primers, restriction fragment length polymorphism, and sequence analysis of 16S ribosomal DNA gene. Various phytoplasma diseases have been detected and studied in a variety of crops, ornamental, vegetables and weed plants. They are classified into three major phytoplasma groups.

Key words: diseases, electron microscopy, PCR, sequencing, phytoplasma.

Introduction

Phytoplasmas are intracellular obligate prokaryotes which lack cell wall. Phytoplasma genome is very small (680-1,600 kb). They are transmitted in plant by leafhoppers, and cause typical yellowing, stunting of whole plant, virescence, phyllody, proliferation of axillary buds, witches' broom and die back symptoms inducing severe yield losses in a variety of plants of horticultural, agricultural and ornamental importance.

Several plant species are being grown in India as cereal crops, oil crops, fruit crops, vegetables, medicinal and ornamental plants. The cultivation of these plants has been drastically affected by several diseases caused by various types of pathogens such as fungi, bacteria, viruses, and phytoplasmas. Among them the latter play an important role since they are associated with diseases which affect the biomass yield and the production of these plants. Efforts have been made for detection, identification and possible management of phytoplasma diseases naturally occurring in various plant species in India so that their growth and yield may be improved.

Materials and methods

During a survey carried out in the last decade, the typical phytoplasma symptoms: yellows, virescence, phyllody, little leaf, proliferation of axillary buds and witches' broom were observed in various plant species growing at the National Botanical Research Institute (NBRI) gardens, kitchen gardens and agricultural fields in and around Lucknow, India.

Little leaf of *Withania somnifera,* chili, brinjal, *Datura* spp., *Cajanus cajan, Catharanthus roseus,* desert rose (*Adenium* spp.), rose (*Rosa alba*), and *Hibiscus* spp.; spike disease of sandal (*Santalum album*); yellows of chrysanthemum and *Achyranthes aspera*; witches' broom of *Ziziphus spp*., *Cannabis sativa*, *Parthenium hysterophorus*, *Sesamum indicum*, marigold and malformation and twisting of floral spikes in gladiolus were studied.

For phytoplasmas detection and identification the samples were collected from the symptomatic plant species listed below, and total DNA was extracted from approximately 100 mg leaf tissues employing a phytoplasma enrichment procedure (Ahrens and Seemüller, 1992). Direct polymerase chain reaction (PCR) using the total DNA and P1/P6 (Deng and Hiruki, 1991) universal primers, specific to 16S rDNA gene of phytoplasma was performed. Further nested PCR was carried out using 1: 10 diluted amplicons from the first stage (P1/P6) and R16F2n/R16R2 primers (Gundersen and Lee, 1996). The amplicons of ~1.3 kb obtained from nested PCR were cloned and sequenced.

Phylogenetic analysis using MEGA version 4.0 software was then carried out to determine the phylogenetic relationships among detected phytoplasmas.

Results

Direct PCR using P1/P6 primers resulted in the expected size bands of ~1.5 kb in some leaf samples. The nested PCR with R16F2n/R16R2 primers resulted in ~1.3 kb bands from many samples collected from various plant species indicating the association of a phytoplasmas with the symptomatic plants.

The partial sequence of 16S ribosomal DNA gene of phytoplasma associated with several plant species was obtained, deposited in Genbank and compared with other phytoplasmas strains using the BLASTn tool.

Phytoplasmas related to '*Candidatus* Phytoplasma asteris' were identified in little leaf of *Withania somnifera* (DQ151998), spike disease of sandal (*Santalum album*) (DQ092357, EF198362), *Catharanthus roseus* phyllody (DQ097396, EF015464), *Achyranthes aspera* (EU573926), *Cannabis sativa* (EU439257), Chili (DQ343288), *Parthenium hysterophorus* (EU375485, EU375488), *Cajanus cajan* (DQ343287), *Sesamum indicum* (DQ431843), chrysanthemum witches' broom (DQ431842), little leaf of desert rose (*Adenium* spp.), (EF159729), marigold witches' broom (EU516321), periwinkle little leaf (EU727085), little leaf disease of rose (*Rosa alba*)

(FJ429364), gladiolus with twisting of floral spikes (GQ338824), malformation of gladiolus (FJ491455), yellows and little leaf disease of *Hibiscus rosa-sinensis* (FJ939287, FJ939288).

Phytoplasmas related to '*Candidatus* Phytoplasma ziziphi' were identified in symptomatic samples of *Ziziphus nummularia* (EU375487); *Ziziphus jujube* (EU366162); *Datura inoxia* (EU573925) and phytoplasmas related to '*Candidatus* Phytoplasma trifolii' were identified in brinjal little leaf (EU375486).

Discussion

Based on phylogenetic analysis phytoplasma strains have been classified into three phytoplasma 16Sr groups ie. '*Ca.* P. asteris' (16SrI group); '*Ca.* P. ziziphi'(16SrV group); '*Ca.* P. trifolii' (16SrVI group). These results indicated that a clear-cut diversity exist among phytoplasma strains infecting various crops in India.

Acknowledgements

The authors express their gratitude to the director, National Botanical Research Institute (NBRI), Lucknow for facilities and keen interest, and the Council of Scientific Industrial Research (CSIR), New Delhi, India for financial support.

References

AHRENS U., SEEMÜLLER E., 1992.- Detection of DNA of plant pathogenic mycoplasma-like organism by a polymerase chain reaction that amplifies a sequence of the 16S rRNA gene.- *Phytopathology*, 82: 828-832.

DENG S., HIRUKI D., 1991.- Amplification of 16S rRNA genes from culturable and nonculturable mollicutes.- *Journal of Microbiological Methods*, 14: 53-61.

GUNDERSEN D. E., LEE I-M., 1996.- Ultrasensitive detection of phytoplasmas by nested-PCR assays using two universal primer pairs.- *Phytopathologia Mediterranea*, 35: 144-151.

Corresponding author: Shri K. RAJ (e-mail: skraj2@rediffmail.com), Plant Molecular Virology, National Botanical Research Institute, Lucknow-226001, India.