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Mixed infection of begomovirus and phytoplasma in *Hibiscus rosa-sinensis* with leaf curling and little leaf symptoms

Shoeb Ahmad[1], NN Tiwari[2], RK Jain[3], AA Khan[4]
[1, 4] Department of Botany, GF PG College, Shahjahnapur, Uttar Pradesh, India
[2, 3] Department of Biotechnology, Anand Engineering College, Agra, Uttar Pradesh, India

Abstract
Hibiscus rosa-sinensis (family- Malvaceae) is an ornamental perennial shrub and native of warm temperate, subtropical and tropical regions. Symptoms associated with leaf curling and little leaf of *H. rosa-sinensis* were observed at Sadar bazaar of Shahjahanpur, UP, India, during June 2018. Total DNA extracted from symptomatic and asymptomatic plants and further it was subjected to PCR/nested-PCR assay using phytoplasma 16S rDNA primers as well as it was also subjected to generic PCR with universal begomovirus coat-protein (CP) gene primers. Expected size of amplicons was obtained from symptomatic plants in both PCR assays but there was no any amplifications observed from asymptomatic plants. On the basis of PCR and gel electrophoresis analysis, mixed infection of begomovirus and phytoplasma was confirmed in *H. rosa-sinensis* from Uttar Pradesh, India.

Keywords: *Hibiscus rosa-sinensis*, phytoplasmas, begomoviruses, nested-PCR

Introduction
Hibiscus rosa-sinensis (family Malvaceae) is flower loving ornamental plants that are native to warm temperate, subtropical and tropical regions throughout the world. The local common name of this plant is gudhal. It has a collection of medicinal property and is widely used for pain treatment. A disease with incidence affecting approximately 3% of plants was observed in gudhal growing nurseries at Sadar Bazar, Shahjahanpur district during 2018. Symptoms included leaf curling and little leaf with upward leaf curl as well as distortion, stunted growth.

A number of phytoplasmas diseases have been reported in gudhal leaf yellowing and phyllody from India witches' broom from Brazil associated with the presence of phytoplasmas belonging to groups 16SrVI and 16SrXV respectively (Khasa *et al.*, 2016; Montano *et al.*, 2011) [2, 3]. However, similar symptoms were also reported by begomoviruses, (Huang *et al*., 2020) [1]. Disease shows symptoms similar to those induced by phytoplasmas and causes enormous economic loss to the flowering growing farmers. Earlier single pathogenic infection on gudhal plants are reported but not any mix infection reported across the world. To identify the pathogens present in symptomatic gudhal a PCR/nested PCR method was used for the detection of phytoplasmas and begomovirus.

Materials and Methods
Three samples of symptomatic Hibiscus were collected from Sadar bazaar of Shahjahanpur district in 2018 alongwith with one non symptomatic sample. Total DNA was isolated from symptomatic and non-symptomatic Hibiscus samples according to the methods describe by Ahrens and Seemüller (1992). The PCR and nested PCR reactions were performed with the universal phytoplasma primer pairs P1/P7 and R16F2n/R16r2 respectively. The denaturation temp for first PCR reaction was 55ºC for 1 min and 56 ºC for R16F2n/R16r2 primer. The initial denatutation was 94 ºC for 5 min, followed by 94 ºC for 45sec (34 cycles) and extensions at 72 ºC for 2min. The final extension was 72 ºC for 10 min. For the detection of begomovirus, universal primer pair AVIF/AVIR as described by Khan *et al* (2014) [4]. The amplified products were visualized on gel electrophoresis.

Results
During visit of different gardens, nurseries for the collections of ornamental plants with phytoplasma infection, Hibiscus plants with little leaf and leaf curling symptoms was observed at sadar bazaar of Shahjahnapur district in 2018 (Fig 1). The incidence of the diseases was upto 3%. The little leaf symptoms indicated the possibilities of phytoplasma association, however the leaf curling suggested the association of begomovirus. Total DNA were isolated from three symptomatic leaves alongwith one non symptomatic leaf, and subjected to PCR. The PCR with P1/P7 did not yield any amplicon in symptomatic and non-symptomatic leaves. The PCR products were diluted with 1:20 with distilled water and used as template DNA in nested PCR with R16F2n/R16r2 primers which yield 1.2kbp in three symptomatic leaf samples, however it was absent in non-symptomatic (Fig 2). This confirm the association of phytoplasma with symptomatic Hibiscus plant. The isolated DNA were now subjected to PCR with begomovirus universal primer pair which also yielded 750 bp amplicon in all the three symptomatic leaves and it was absent in non-symptomatic, this confirm the association of begomovirus with leaf curing and little leaf symptoms of Hibiscus plant (Fig 3). According to Arocha *et al* (2009) virus and phytoplamsa can be affect same plant species. Association of phytoplasma and begomovirus in same plant species is not new and it has been reported on eggplant with yellow leaf symptoms from Meerut, India (Singh *et al* 2015) [5]. Biswas *et al* (2013) [6] observed the phytoplasma and *Corchorus golden mosaic virus* in jute plant. Matus *et al*

(2008) [7] found the mixed infection in *Vitis vinifera.* In the present investatigation mixed infection of begmovorrus and phytoplasma was detected in Hibiscus plant through PCR and Nested PCR which might be first report from India.

Fig 1: Non Symptomatic or healthy Hibiscus plant, little leaf and leaf curling symptom in Hibiscus plant at Shahjahnapur

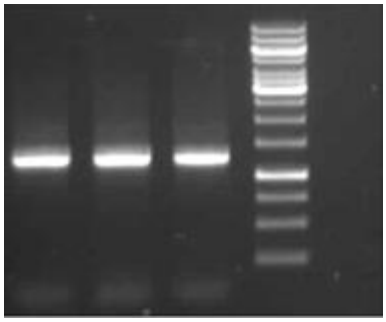

Fig 2: Nested PCR results of Hibiscus plant. Lane 1-3 symptomatic hibiscus plants, lane M ladder, Lane 4 non symptomatic

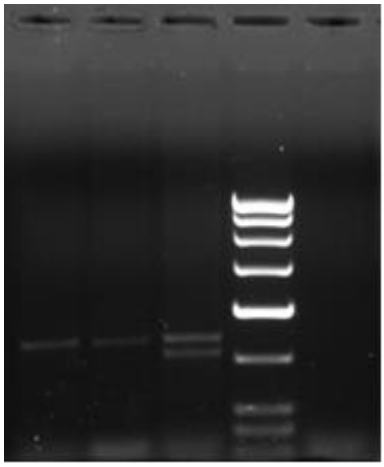

Fig 3: Agarose gel electrophoresis of PCR products obtained from *Hibiscus* samples showing positive amplicon of ~800 bp in (lane 1-3) and non-symptomatic (lane 4) M = Lambda DNA digested with *Hin*dIII and *Eco*RI.

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