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DETECTION OF 'CA. PHYTOPLASMA PHOENICIUM' IN PERIWINKLE (*Cathranthus roseus*) PLANTS IN UTTAR PRADESH, INDIA

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Abstract

Cathranthus roseus also known as periwinkle, an ornamental plant contains several medicinal values, was found with the symptoms of little leaf and witches' broom at Shahjahanpur location with the incidence of up to 8%. The phytoplasma etiology was confirmed through scanning electron microscopy examination in all the four-leaf samples. Molecular analysis through PCR with universal primer pairs P1/P6 followed by nested PCR with R16F2n/R16r2 primers yielded ~1.2kbp amplicons in all the four symptomatic leaf samples. One amplicon was eluted, purified, sequenced, and used in BLASTn searches, which showed maximum identity of periwinkle isolate with several isolates of 16SrIX group of phytoplasma. Further, phylogenic analysis and *in silico* RFLP confirmed the association of 16SrIX-C subgroup phytoplasma in little leaf and witches broom plants which is the first report from India.

Keywords: Little Leaf. Periwinkle. Phytoplasma. SEM. Witches' Broom.

1. Introduction

Phytoplasmas can cause diseases in many ornamental plants and responsible for serious economic losses around the globe. The phytoplasma diseases have become the major constraints in profitable plants production and receiving international importance because of unspecific symptoms (Rao et al. 2017). The phytoplasma is transmitted through infected seed materials and insect vectors (Tiwari et al. 2021). The disease can be diagnosed by scanning electron microscope, PCR, and sequencing of 16SrRNA gene. Apart from this restriction fragments length polymorphism (RFLP) may be utilized to classify the phytoplasma on group and subgroup level (Zhao et al. 2009).

The periwinkle (*Catharanthus roseus*), a natural and perennial plant belonging to the *Apocyanaceae* family grown in various parts of the globe including India because of its medicinal importance. The plant known to have ajmalicine, serpentine, and reserpine alkaloids. This plant has been used to study the phytoplasma-plant interactions by several workers from India and abroad (Chen and Lin 2011; Barbosa et al. 2012; Rao et al. 2017). 16Sr I, II, III, IX, and XV group of phytoplasma on periwinkle plant is reported from Egypt (Omar et al. 2008), India (Chaturvedi et al. 2009; Madhupriya et al. 2016), Brazil (Barbosa et al. 2012) and China (Chao et al. 2019). In the present investigation, association of the 16SrIX-C group with periwinkle plants has been confirmed.

2. Material and Methods

In 2017-2018 little leaf, and witches broom symptoms were observed in several locations of Shahjahanpur district. Total of four-leaf samples along with one non-symptomatic samples were collected for further analysis. The leaf samples of infected and healthy plants were kept in glutaraldehyde (2.5%) with sodium cacodylate buffer at 4°C for 48hrs. The sample were dehydrated in alcohol followed by absolute acetone or hexamethyldisilane. The sample were later dried in Carbon dioxide and coated with palladium.

Total DNA was isolated from the four symptomatic leaves and one non-symptomatic leaf sample (Ahrens and Seemuler 1992). Isolated DNA was subjected to PCR with universal primer pair P1/P6 (Deng and Hiruki 1991) followed by nested PCR with R16F2n/R16r2 primers (Gundersen and Lee 1996). The PCR products were visualized on 1% agarose gel. The amplified products were eluted, purified through the gel elution kit, and purified products were sequenced. Sequences were aligned through the DNA baser V4.2 program and the phylogenetic tree was prepared through NJ method of MEGA 6.0 (Tamura et al. 2013). For classification at subgroup level, the R16F2n/R2 region sequences were used *in silico* analysis by pDraw32 tool (http://www.acaclone.com) and obtained image were compared with the reference strain.

3. Results and Discussion

During the visit to several nursery and kitchen gardens at Shahjahanpur locations (Cantonement area, Tilhar, Sadar, Botanical Garden of GF College, Reti road), the symptoms of little leaf, and witches broom symptoms were recorded at the Cantonment area (Figure 1) with an incidence of 8%.

Figure 1. Little leaf and witches' broom sympyoms in periwinkle plants at Shahjahanpur location.

Presence of phytoplasma inside the phloem tissue of symptomatic samples were detected in scanning electron microscopic examination. It was appeared as rounded shaped in the sieve element of the phloem tissue, ranging from 260.5 nm to 291.2 nm. But some of them were also detected in sieve tube, which indicate their movement through phloem tissue (Figure 2). The results confirmed the presence of phytoplasma in little leaf and witches broom samples and also suggest that the SEM technique is very useful in the detection of phytoplasma in phloem tissue.

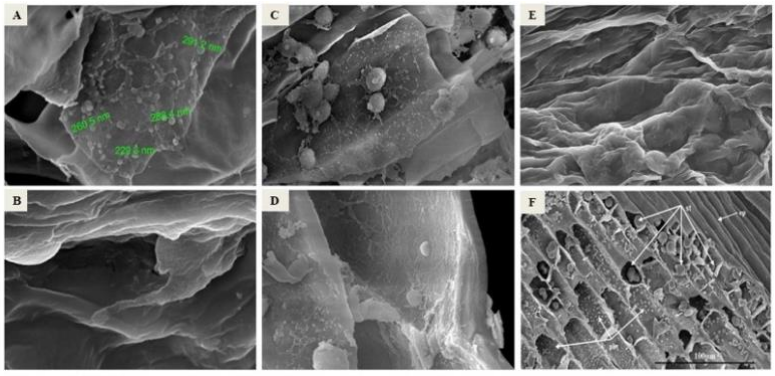

Figure 2. SEM image of the phloem tissues of symptomatic and asymptomatic *Catharanthus roseus* plant. A – phytoplasmas witches broom sample; B – non symptomatic sample; C – little leaf sample; D – non symptomatic sample; E – negative control; F – reference image of *phytopasma* infected tomato phloem cells, Lebsky and Poghosyan 2014.

The isolated DNA from symptomatic leaf samples were yielded ~1.2 kbp amplicon in all the four samples along with positive control and it was absent in non-symptomatic samples collected from the same location. The one amplified product was eluted, sequenced and sequences were submitted to the GenBank with the accession number MT274665. The query 16S rDNA sequence (MT274665) shares 99.1% similarity with that of the '*Candidatus* Phytoplasma phoenicium' reference strain (GenBank accession: AF515636) and the results was further supported by phylogenetic analysis where the present study isolate showed closest relationship with the 16SrIX group of phytoplasma and made a distant relationship with other group of phytoplasma (Data not shown). The *in silico* RFLP analysis through the pDraw32 software assigned the 16SrIX-C subgroup (Figure 3) of phytoplasma with the symptoms of little leaf and witches' broom which is the first report from India.

Figure 3. A – virtual RFLP patterns from *In silico* digestion of R16F2n/R16R2 fragments of the phytoplasma infecting periwinkle isolate MT274665 with 17 restriction enzymes; B – references strain of 16SrIX-C subgroup Acc. Y16389.

Earlier from India association of 16SrI-B, I-C, II with little leaf, phyllody symptoms in periwinkle is reported (Chaturvedi et al. 2009; Madhupriya et al. 2016); however, in the current study, we are reporting the 16SrIX-C subgroup association with little leaf and witches broom symptoms in periwinkle plant, which is new report from India. *Ca.* P. phoenicium group has been earlier recorded on periwinkle from Brazil (Barbosa et al. 2012). The existence of *Ca.* P. phoenicium group in India is not new and has been confirmed on *Carboratus edulis* (Shukla et al. 2014), but periwinkle as a new host of *Ca.* P. phoeniciumis being reported in the present study.

4. Conclusions

Periwinkle (*C. roseus*) found with the symptoms of little leaf and witches' broom at Shahjahanpur was due to 16SrIX-C subgroup of phytoplasma. The new host of 16SrIX-C subgroup from India may be because of vectors in studied area.

Authors' Contributions: TIWARI, N.N.: acquisition of data, analysis and interpretation of data; JAIN, R.K.: analysis and interpretation of data, and critical review of important intellectual content; TIWARI, A.K.: drafting the article. All authors have read and approved the final version of the manuscript.

Conflicts of Interest: The authors declare no conflicts of interest.

Ethics Approval: Not applicable.

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