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Molecular characterization of temple tree rust caused by *Coleosporium plumeriae* Pat.

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Abstract

Temple tree (*Plumeria rubra*) is a cosmopolitan ornamental flowering and an avenue tree. Generally, *Plumeria* sp. is affected by several diseases, among them rust caused by *Coleosporium plumeriae* was found infecting the temple trees in Bengaluru, Karnataka, India with the severity of 80-100 per cent in the early June till end of August 2016-17. Initially, numerous orange coloured rusty pustules were seen on abaxial leaf and these pustules covered the entire leaf causing inward rolling of leaves, necrosis, senescence and finally leaf abscission. Microscopic observation of the rust pustules revealed the presence of golden pale yellow coloured uredospores that varied from globose to ellipsoidal in shape, ranging from 20-40 x 16-28 µm in size, borne on erumpent uredinia and no other spore stages were observed. Molecular identification of this pathogen (Indian isolate) through ITS rDNA region amplification, sequencing and phylogenetic analysis revealed 99 % sequence homology with the China isolate (Accession no. KF879087.1). ITS rDNA region partial sequence of the pathogen was deposited in NCBI, GenBank with accession no. MH656772.

Key words: *Plumeria rubra, Coleosporium plumeriae,* and ITS rDNA

Introduction

Temple tree (*Plumeria rubra*) is a cosmopolitan ornamental flowering plant commonly known as Hawaiin Lei flower or Indian temple tree (Family: Apocynaceae) which is indigenous to Mexico, Central America, Caribbean and Brazil. Most species include deciduous shrubs or small trees with large-leaved foliage, highly fragrant flowers of multiple colors which are used for the preparation of garlands, perfumes and primarily in making Lei, hence also called Lei flowers. About eight species in genus *Plumeria* are known in India.

The *Plumeria* sp. is incited by many diseases, of which rust caused by *Coleosporium plumeriae* was observed with high severity. *C. plumeriae* infects *P. rubra* (Thomas, 2014), *P. acuminata* (To-anun *et al.,* 2004) and *P. alba* (Baiswar, 2008). Further, *C. plumeriae*, is an autoecious rust fungus and infects only *Plumeria* sp. The rust of temple tree was originally reported in Santo Domingo, West Indies over 150 years ago. Later, its distribution was rapid but limited to Central America until 1980s and Thailand (To-anun *et al.*, 2004). Recently, this fungus was reported from Australia, Africa, Indonesia, Taiwan (Chung *et al.*, 2006), Malaysia (Holcomb *et al.*, 2010), Vietnam (Wang *et al.*, 2011), India (Baiswar *et al.,* 2008; Thomas, 2014) and China (Yang *et al.*, 2014). The temple tree rust was noticed at the College of Horticulture, Bengaluru, Karnataka, India during 2016-17 with 80-100 per cent severity in June, July and also till the end of August.

No molecular evidence has been reported on *C. plumeriae* from India. Hence, our investigation was planned on reconfirmation of morphological characters and molecular characterization of *C. plumeriae* that has been affecting the beauty of the temple tree.

Material and methods

Morphological characterization: Disease severity was recorded on *P. rubra* plants showing the typical symptoms of rust. The infected leaf tissue was teased using a sharp blade on a glass slide having a drop of sterile water and observed under binocular microscope, photographed using microscopic camera Nikon D-7000. Parameters like colour, shape and type of spores were recorded visually under microscope. Size of urediniospores (length and breadth in µm) was also measured.

Molecular characterization

Genomic DNA isolation: The rust infected *P. rubra* leaves showing the typical symptoms of *C. plumeriae* was collected in a paper bag. The presence of uredospores was confirmed by microscopic examination before the isolation of genomic DNA. Total genomic DNA from the rust infected leaf tissue was isolated using cetyl-trimethyl ammonium bromide (CTAB) method as explained by Pedley (2009) with slight modification with the addition of Proteinase K and a pinch of celite, in order to get good quality DNA. Further the isolated DNA was quantified using NanoDrop™ (Thermo Scientific™ 2000c) and was tested using Bio-rad agarose gel electrophoresis unit.

ITS rDNA amplification, sequencing and phylogenetic analysis: The template DNA was amplified using universal ITS-rDNA primers ITS4 and ITS5. Amplification was done in PCR tubes with a total reaction mixture of 25 µL containing the following components: PCR reaction mixture (1X)-(8.5µL), forward primer (ITS5-5'GGAAGTAAAAGTCGTAACAAGG-3') 10 pM/ µL (2.0 µL), reverse primer (ITS4-5'TCTCCGCTTATTGATATGC-3') 10 pM/ µL (2.0 µL), 5 ng template DNA (2.0 µL) and sterile water (10.5 µL). PCR reaction was programmed for 35 cycles with

Initial denaturation for 5 min @ 94 ºC, denaturation for 30 s @ 94 ºC, primer annealing for 30 s @ 48 ºC, primer extension 60 s @ 72 ºC and final extension for 8 min @ 72 ºC in Eppendorf™ Master cycler™ Nexus Gradient. The amplified PCR product was confirmed and visualized using 1 % agarose gel electrophoresis and ITS region was sequenced. The obtained sequence was subjected to NCBI-nucleotide blast analysis and phylogenetic relationship of *C. plumeriae* was obtained using MegaX software.

Results

Rust of temple tree caused by *C. plumeriae* was noticed during 2016-17 and the disease was seen in June, July and till the end of August. But the disease severity varied from 80-100 per cent and similar disease severity was observed during 2017-18. Fig. 1 shows symptoms of the disease on *P. rubra*. Initially, numerous orange coloured rusty pustules on the lower surface and corresponding yellowing on the upper surface of the affected leaves was observed (Fig. 2). With the advancement of disease, these pustules coalesced and covered entire leaf causing inward rolling of leaves, necrosis, senescence and severely affected leaves fell off by leaving the tree with bare branches (Fig. 3).

Fig. 1. General view of rust symptoms on *Plumeria rubra*

Fig. 2. Orangish rust pustules on abaxial surface and corresponding yellowish spots on adaxial surface of affected leaves

On microscopic examination, rust pustules revealed the presence of golden pale yellow coloured uredospores which varied from globose to ellipsoidal in shape and measured from 20-40 x 16-28 µm (Fig. 4) in size. No teliospore or the other spore stage were observed in our study.

Further, molecular identity of this pathogen was confirmed by

Fig. 3.1. Severe infection leads to inward rolling, necrosis and senescence of affected leaves

Fig. 3.2. Defoliation of affected leaves can be seen at the base of the plant

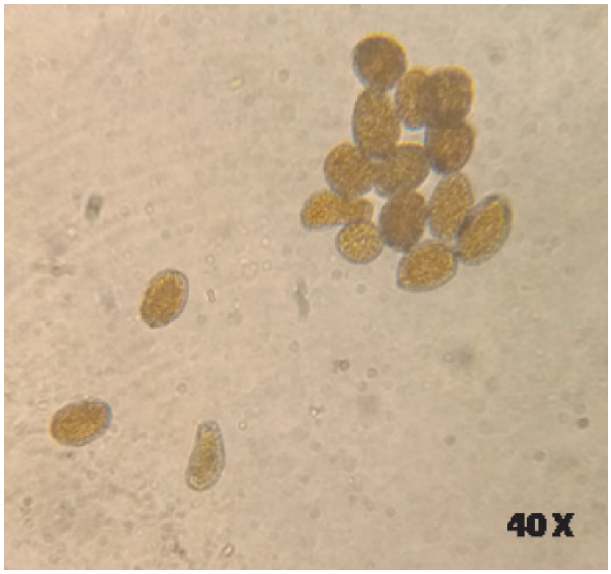

Fig. 4. Microscopic view of golden pale yellow coloured uredospores

DNA sequencing using ITS-rDNA primers (ITS4) and (ITS5). The obtained amplicon size of 787 bp was sequenced (Fig. 5) and aligned using ClustalW and Bioedit software's and phylogenetic analysis of the data done with MegaX programme. The results revealed that the studied Indian isolate was found to match with China isolate (Accession no. KF879087.1) of *C. plumeriae* from *P. rubra* with 99 per cent sequence homology (Fig. 6) and was close to Nigeria (MF769646.1), Guyana (MG907225.1) isolates also.

Discussion

The infected temple tree rust samples collected by Thomas (2014) from 2012 January to July 2013 in Kerala had reached 100 per cent premature defoliation due to *C. plumeriae* on both *P. rubra* and *P. alba*. Same disease was observed in Bengaluru, Karnataka, India during June, July and till the end of August 2016-17 on *P. rubra* with 80-100 per cent disease severity and complete premature defoliation was also mentioned by Thomas (2014). Microscopic examination of rust pustules revealed the presence of golden pale yellow coloured uredospores (globose to ellipsoidal in shape and ranging from 20-40 x 16-28 µm in size) and no other spore stages were observed, as reported by Laundon and Rainbow (1969). These spores are mostly spread through wind under wet-humid conditions and cause infections. (Hosagoudar, 1988). Thomas (2014) reported that most infections are caused by windborne uredospores that stick to moist leaves under wet and humid conditions and acts a secondary inoculum. Similar favourable conditions were observed in Bengaluru with an annual rainfall of 970-1000 mm, temperature ranging from 19-28°C and relative humidity of 89-92 per cent along with moist conditions on leaf surface which helps for the disease development.

Molecular characterization of *C. plumeriae* was the first attempt in India, however, isolation of genomic DNA using CTAB procedure from the fungi is well established as explained by

Fig. 5. ITS rDNA region amplification produced the amplicon size of 787 bp through PCR

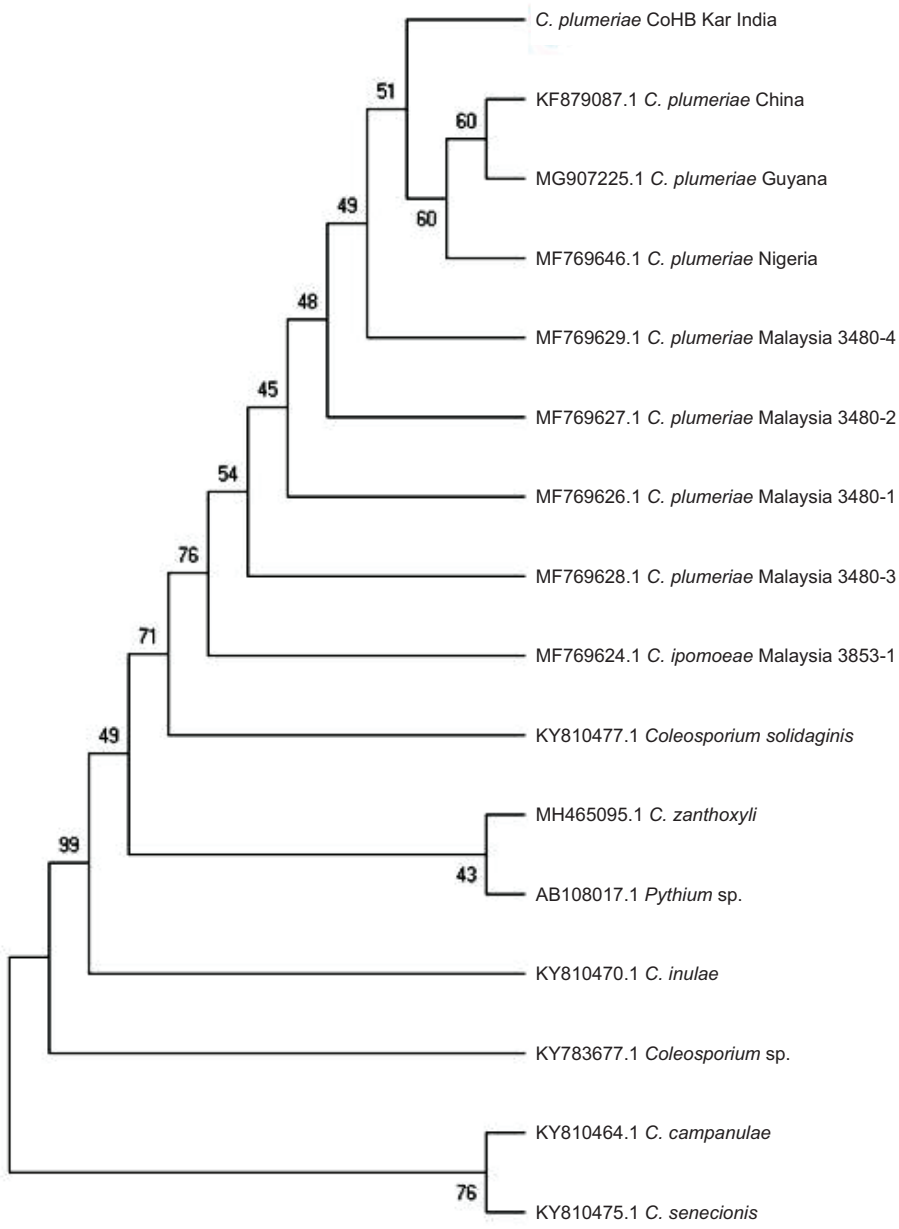

Fig. 6. Phylogenetic relationship of *Coleosporium plumeriae* of Indian isolate with other species of *Coleosporium*

Pedley (2009). The protocol was slightly modified *viz.,* addition of Proteinase K (0.3 mg/ mL) and a pinch of celite, which yielded a good quantity and quality DNA. This is due to Proteinase K which removes proteins and celite which act as abrasive and disrupt the fungal cell wall and releases the DNA easily into extracting solution. The phylogenetic relationship of *C. plumeriae* with other rust species was determined using neighbour joining and bootstrap method with 1000 replications in MegaX Software. Only one cluster was formed with respect to fungal hierarchy. The outgroup *Pythium* was far and the separate clusters were formed with Order: Uredinales and differ even at species level. *C. plumeriae* fell apart from other rust species of *Coleosporium* but it was closely associated and showed 99 % homology with *C. plumeriae* of China (Accession no. JQ303103.1). The pathogen under the study belongs to rust species was confirmed using universal eukaryotic ITS regions of fungi (Bruns *et al.*, 1991; White *et al.*, 1990) and the sequence has been deposited in NCBI, GenBank (Accession no. MH656772).

To our knowledge, this is the first fully described report on molecular characterization of *C. plumeriae* from India. Further, there is a need for investigation on occurrence and severity of disease, all spore stages, survival and spread mechanism of this pathogen. The integrated and ecofriendly disease management strategies helps to prevent survivability and severity of the pathogen.

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