IDENTIFICATION OF PATHOGENS IN SEEDLINGS OF INDIAN SANDALWOOD AND SCREENING OF FUNGAL ENDOPHYTES AGAINST THE PLANT PATHOGENIC FUNGI

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Introduction

Indian sandalwood (*Santalum album* Linn.), an evergreen tree, indigenous to the Indian peninsula is known for its valuable heartwood worldwide. Sandalwood plantations are gaining importance throughout the Indian subcontinent demanding large-scale production and the establishment of nurseries with Quality Planting Material (QPM). However, sandalwood seedlings succumb to devastating diseases at nurseries leading to high mortality of the planting stock. Therefore, there is a dire need for the management of these diseases.

Materials and methods

ISOLATION OF PATHOGENS AND ENDOPHYTES

Pathogens were isolated from the diseased samples of sandalwood seedlings (Aneja, 2003) and endophytes were isolated from the disease escaped healthy seedlings of sandalwood as per standard isolation methods (Aravind et al. 2009).

IN VITRO SCREENING OF ENDOPHYTES FOR BIOACTIVITY AGAINST SELECTED PATHOGENS

A dual culture technique was used in which an endophyte and a pathogen were grown opposite each other in the same Petri plate using 5 mm diameter plugs collected from 7 day-old fungal cultures grown in potato dextrose agar (PDA).

Percent inhibition was assessed using the formula (Vincent, 1927):

$$\% \text{ inhibition} = \frac{C - T}{C} \times 100$$

in which ,

C is pathogen mycelia radius in the control plate (cm),

T is pathogen mycelia radius in pathogen-endophyte dual culture (cm).

Anthracnose disease caused by *Colletotrichum siamense*

Wilt disease caused by *Fusarium solani*

MOLECULAR CHARACTERIZATION OF PATHOGEN AND ENDOPHYTES

Genomic DNA was extracted from the mycelial mat of pure fungal isolates using the method described by Sathish et al., 2014. Molecular identification using the nuclear ribosomal DNA internal transcribed spacer (ITS) sequences were performed and those species which could not be resolved with ITS sequence were subjected to multi-locus gene (beta-tubulin (TUB2), glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene, chitin synthase 1 gene (CHS-1), actin gene (ACT) and glutamine synthetase (GS) genes) analysis and the sequences were deposited to GenBank.

Results

In our study, we isolated and identified phytopathogenic fungi such as *Fusarium solani* causing wilt disease with seedling mortality of 25 % and *Colletotrichum siamense* causing anthracnose disease with a disease incidence of 75 %. We identified and characterized a total of 90 fungal endophytic isolates from leaf, stem, and root tissues of disease escaped or apparently healthy seedlings of sandalwood. Total fungal endophytes isolated from the disease escaped sandalwood seedlings comprised 33.3 % *Colletotrichum siamense*, 26.6% *Diaporthe melonis*, 13.3% *Aspergillus sclerotiorum*, 13.3% *Fusarium oxysporum*, 13.3 *Paraphoma radicina*, 6.6% *Alternaria alternata* and 6.6% *Pestalotiopsis microspora*. Molecular analysis resolved the identification of the fungi to species level and the sequences were deposited to GenBank. Dual culture test assay revealed that the fungal endophytes *Aspergillus sclerotiorum* and *Diaporthe melonis* showed the highest percent inhibition of 63.08% and 61.54%, respectively against *Fusarium solani* and *Diaporthe melonis* and *Fusarium oxysporum* showed highest percent inhibition of 55.38 % and 67.69 % in case of the pathogen *Colletotrichum siamense* (Fig 1).

Figure 1
A. *Aspergillus sclerotiorum* and B. *Diaporthe melonis* against *Fusarium solani* . C. *Diaporthe melonis* and D. *Fusarium oxysporum* against *Colletotrichum siamense.*

References:

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