ABSTRACT

It is a well known fact that conventional methods for identifying fungal plant pathogens rely on the interpretation of visual symptoms and/or the isolation, culturing and laboratory identification of the pathogen. The accuracy and reliability of these methods depend largely on the experience and skill of the person making the diagnosis. Diagnosis requiring culturing can be time consuming and can be impractical when rapid results are required. Hence newer methods that are increasingly being applied to the diagnosis of plant pathogens, include immunological methods, DNA/RNA probe technology and polymerase chain reaction (PCR) amplification of nucleic acid sequences.

The present study deals with the serological and molecular detection of foliar fungal pathogens (*Colletotrichum gloeosporioides* and *Pestalotiopsis disseminata*) of muga host plant *Persea bombycina* Kost, most commonly known as som plant causing leaf blight and grey blight diseases respectively. Muga sericulture is an integral part of North-Eastern India and is a major economic threshold. One of the main reason for decrease in muga culture is due to the various foliar diseases that affects the quality and quantity of the leaves and in turn affects the silk production. It is hence essential to detect these foliar diseases at an early stage and also to minimise these disease using eco-friendly technologies by application of different bioinoculants.

At the onset, two major foliar fungal diseases of som plant, leaf blight and grey blight was recorded, their causal organism isolated and their morphological characteristics studied to identify and understand them. Screening of resistance of eight different morphotypes against these pathogens was carried out. Polyclonal antibodies against these pathogens were raised separately in male white rabbits and immunological assays were optimized for easy and early detection of these pathogens in som leaf tissues. Cross reactive antigens (CRA) shared between som plant and fungal pathogens were demonstrated following indirect immunofluorescence and immunogold labelling. Detection of pathogen in infected leaf was carried out using Plate trapped antigen coated Enzyme Linked Immunosorbent Assay (PTA-ELISA)

and Dot-immunobinding assay. Cellular localization of pathogen in these infected tissues was also studied using indirect immunofluorescence technology as well as immunogold labelling. Early detection of infection in artificially inoculated leaves was studied using PTA-ELISA and Dot-blot technique. It was noted that using these immunotechniques the fungal diseases could be detected as early as 24hrs after inoculation whereas the disease symptoms appeared only after 90-120hrs after inoculation.

Molecular detection of the two major fungal pathogens, *C. gloeosporioides* (SOM/Cl/02) and *P. disseminata* (IPL/SOM/P/01) was carried out using 18S rDNA sequencing of their conserved region using ITS1/ITS4 primer pair. The BLAST query of the 18S rDNA sequence of the isolates against GenBank database confirmed the identity of the isolate SOM/Cl/02 as *Colletotrichum gloeosporioides* and IPL/SOM/P/01 as *Pestalotiopsis* sp. The sequences have been deposited in NCBI GenBank database, under the accession no KM491736 for *C. gloeosporioides* and KT697994 for *Pestalotiopsis* sp. Species specific identification of other isolates was done using specific primer pair for *C.gloeosporioides* –CgINT/ITS4. Diversity analysis among the different fungal isolates was carried out using RAPD and DGGE techniques.

In vitro antagonistic effect of two selective Plant growth promoting rhizobacteria (PGPR) and two selective Plant growth promoting fungus gave positive result against both the pathogens. These PGPR (*Bacillus pumilus* and *B. altitudinus*) and PGPF (*Trichoderma harzianum* and *T. asperellum*) were mass multiplied and applied to the som plants to evaluate their effects on growth promotion and biochemical changes. Dominant arbuscular mycorrhizal fungi (AMF) isolated from the rhizosphere of som plants were mass multiplied and used for application as bioinoculant for root colonization in som plants. Besides, these bioinoculants were also used as value addition with vermicompost.

Growth enhancement was evaluated in terms of height, no. of leaves and no. of branches. Results revealed that growth promotion occurred in all morphotypes of som plants following application of bioinoculants, singly or jointly and in different combinations. Activities of defense enzymes (peroxidase, phenylalanine ammonia

lyase, chitinase and β-1,3glucanase) following treatment were analysed. HPLC profile of phenolic acids were also determined. Enhanced increase in activities of these defense enzymes were also noted in leaf as well as root of two morphotypes of som plants (S5 and S6) that were grown in field condition after joint application of bioinoculants. Disease incidence was found to be decreased in treated plants in comparison with untreated control plants. Immunological tests like indirect immunofluorescence confirmed the induction of defense enzymes in both root and leaves after application of bioinoculants.

Immunogold localization of defense enzymes (glucanase and chitinase) in som leaves and roots following colonization with AMF and treated with bioinoculant was studied using transmission electron microscopy. Deposition of gold particles was observed near the cell wall of treated roots and leaves. Induction of resistance in som plants against foliar fungal pathogens was confirmed using bioinoculants.