Abstract

Detection of propagules of plant pathogens in plants, seed, vegetative propagating materials and in plant products is an essential component of disease management strategies. Detecting and identifying pathogens provides the basis for understanding their biology and selecting appropriate control strategies. Consequently, improved methods for disease and pathogen recognition and indexing are constantly being sought. It is a well known fact that conventional methods for identifying fungal 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 the 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 molecular and serological detection of foliar fungal pathogen *Bipolaris sorokiniana*(Sacc.) Shoemaker of host plant *Sorghum bicolor* (L) Moench, commonly known as sorghum (Jowar) causing spot blotch disease. Sorghum [*Sorghum bicolor* (L) Moench] is the one of the most important cereal crops in the world. It is the staple food grain for over 750 million people who live in the semi-arid tropics of Africa, Asia, and Latin America. Global pro-duction of sorghum is currently estimated to be 57.6 million tonnes, with Asian countries contributing 20% of the total production. Within Asia, India is the largest producer of sorghum grain.

Recently there have been severe signs of sorghum decline caused by *Bipolaris sorokiniana* resulting in decreased production of sorghum in villages of Kalimpong and Darjeeling. It is hence essential to detect this foliar disease at an early stage and also to minimize this disease using eco-friendly technologies by application of different bioinoculants.

In the present study, initially, several strains of the fungus were isolated from diseased leaves of *Sorghum bicolor* and *Triticum aestivum* which were morphologically identified as *Bipolaris sorokiniana*. Screening of resistance of ten different varieties against this pathogen was carried out. Polyclonal antibodies against the pathogen were raised in male albino rabbits and immunological assays were optimized for easy and early detection of the pathogen in sorghum leaf tissues. Cross reactive antigens (CRA) shared between plant and fungal pathogen were demonstrated following indirect immunofluorescence and immunogold labeling. Pathogen detection in infected leaf tissue was carried out using PTA-ELISA and Dot-immunobinding assay. Cellular localization of pathogen in 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. From this investigation it was noted that by using these immunotechniques the fungal disease could be detected as early as 24 hrs after inoculation whereas the disease symptoms appeared only after 48-72 hrs after inoculation.

For moleculat detection of the pathogen genomic DNA of *B.sorokiniana* isolated from infected leaves was purified and PCR amplification of 18s rDNA was done using specific primers. Amplified product (1190 bp) was sequenced and aligned against ex-type strain sequences of *B.sorokiniana* from NCBI GenBank using BLAST and phylogenetic analysis was done using MEGA4 software. RAPD PCR analysis and DGGE analysis of amplified genomic DNA were done.The evolutionary history was inferred using the UPGMA method. Amplification of ITS region of the rDNA can be considered as a rapid technique for identifying pathogens successfully in all cases. Diversity analysis among the different fungal isolates was carried out by genic and intergenic tubulin gene

sequencing, RAPD and DGGE techniques.

The main objectives of the present study was to determine the efficacy of different bioinoculants (PGPR, PGPF, AMF) on plant growth promotion and biocontrol of spot blotch disease of sorghum caused by *Bipolaris sorokiniana* along with determination of cell defense responses in rice plants associated with induction of resistance towards B. sorokiniana by microbial formulation. *In vitro* antagonistic effect of six potential Plant growth promoting rhizobacteria (PGPR) and seven selective Plant Growth Promoting Fungus (PGPF) gave positive results against the pathogen in terms of suppression of the mycelia growth of the pathogen in dual plate culture. Chitinase activity of the different *Trichoderma* strain was assayed which revealed higher amount of enzyme accumulation in T.harzianum and T.aspellum. Endochitinase gene sequence was done for these two Trichoderma isolates to analysis the diversity and uniqueness of this specific gene related to coffering resistance plants against pathogen. These PGPR and PGPF were mass multipled and applied to the sorghum plants to evaluate their effect on growth promotion and biochemical changes. Dominant arbuscular mycorrhizal fungi (AMF) present in the rhozosphere of sorghum plants were screened, mass multiplied used for application as bioinoculants.

Enhancement of growth was evaluated in terms of height and result revealed that growth promotion occurred in all varieties treated with bioinoculants though combined application of bioinoculants (PGPR+PGPF+AMF) showed better results in comparison with single treatment. Content of different biochemical component viz. protein, phenol, chlorophyll, total sugar, proline when estimated showed better results in case of treated set of plants. MDA and H_2O_2 content was found in higher amount in infected plants where establishment of disease of observed rather than healthy and bioinoculants treated plants. Activities of defense enzymes (chitinase, β-1,3-glucanase, phenylalanine ammonia lyase, peroxidase) following treatment were analysed. Enhanced increase in activities of defense enzymes were noticed in leaf where disease establishment was poor. HPLC profile of phenolic acids were also determined. Extraction of antifungal compounds from healthy and treated inoculated

sorghum leaves was done and then the compound was subjected to GCMS analysis. GCMS analysis of antifungal compounds reveals presence of sereval phenolic compounds which are related to plants defense mechanisms. Presence of phenolic compounds was further confirmed by estimation of phenol content and TLC plate bioassay. 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 leaves after application of bioinoculants.

Immunogold localization of defense enzymes (glucanase and chitinase) in sorghum leaves treated with bioinoculants was studied using transmission electron microscopy. Deposition of gold particles was observed near the cell wall, cytoplasms, mesophyll tissues of treated leaves. Induction of resistance in dcution of resistance in sorghum plants against spot blotch pathogen was confirmed using bioinoculants.