Chapter 1
Introduction

Sorghum [*Sorghum bicolor* (L) Moench] is the fifth most important cereal crop in the world; and its wide range of other applications are now being explored with worldwide interest in renewable resources (Dahlberg *et al.,* 2011). It is the staple food grain for over 750 million people who live in the semi-arid tropics of Africa, Asia, and Latin America (Sreenivasa *et al*., 2010). Global production of sorghum is currently estimated at 57.6 million tonnes, with Asian countries contributing 20% of the total production. Within Asia, India is the largest producer of sorghum. The C4 cereals, like sorghum are originated from the tropics and can tolerate heat and drought conditions more effectively as compared with C3 plants (like wheat), which originated from temperate regions (Blum *et al.* 1990, Chapman and Carter 1976). Under arid environmental conditions, osmotic adjustment is imperative in the drought resistance of many C4 plants (Slatyer 1963) and may enable sorghum to grow when leaf water potential is low (Craufurd *et al*. 1993). Due to several morphological and physiological properties, sorghum is better drought resistant in comparison with maize (Purseglove 1972). Sorghum plants (C4) use nitrogen more efficiently than most C3-type crops and are more tolerant to drought and high temperature stresses compared to corn (Young and Long 2000). Lemaire *et al*. (1996) concluded that sorghum has greater ability to satisfy its nitrogen requirement. Better uptake of N_2 from the soil, gives this species an undeniable agronomic advantage over maize, due to its greater adaptation to growing condition in limiting in water and nitrogen.

About 100 countries grow sorghum, of which 66 cultivate it over more than 1000 ha or produce more than 1000 t. India has the largest sorghum area with

10.06 million ha. The second largest sorghum cultivating country is Nigeria, followed by Sudan, USA and Niger. More than 90% of the world's sorghum area lies in the developing countries, mainly in Asia and Africa. However, in terms of annual production, USA tops the list with 13.38 million t during 1999-2001, followed by India (8.23 million t), Nigeria (7.65 million t), Mexico (6.09 million t) and Argentina (3.16 million t).

However, fungi associated with sorghum are of serious concern due to their toxic potential. The risk of contamination by mycotoxins in sorghum is related to the kind of fungi associated. *Bipolaris sorokiniana*, a spot blotch pathogen which is a major threat for cereals like wheat and barley has also now a days shown its harmful effects on other members of the family Poaceae and the mostly affected one is sorghum. In case of our study area, the Darjeeling and Kalimpong hills, where the crop is cultivated in replacement of rice, *B. sorokiniana* (Sacc.) Shoemaker (syn. *Helminthosporium sativum* teleomorph: *Cochliobolus sativus*), is the main cause of spot blotch disease in barley (*Hordeum vulgare* L.) and wheat (*Triticum aestivum* L.) (Acharya *et al*., 2011). The symptom of spot blotch usually appears on the leaf, sheath and stem. Yield losses due to spot blotch vary from 16 to 35% in sorghum. *B. sorokiniana* is widely distributed in the areas where cereals are grown and forms a continuous genetic pool of isolates varying in virulence and aggressiveness to various cereals and grasses. The infection process on the leaves usually occurs through natural wounding, stomata or with the use of an appresorium-like structure through the cell wall. The presence of other hosts plays an important role in disease epidemic. The primary inoculum of *B. sorokiniana* comes from several sources such as weed hosts, soil, crop debris which enhances the disease level (Bashyal *et al.,* 2011). Recently, Chakraborty *et al*., (2016) have reported the serological and molecular characterization of *B. sorokiniana* causing spot blotch disease of wheat.

Traditionally, diagnosis of plant diseases has been based on recognizing characteristic symptoms presented by diseased plants and looking for the presence of pathogens on their surface (Kiraly et al., 1970; McIntyr and Sands, 1977). This, together with other observations and evaluation of the environmental conditions, generally allows us to be classified as causative

agent to be a virus-like organism, a bacterium, a fungus or some environmental factors.

The earliest serological techniques in plant pathology used polyclonal antisera prepared by centrifugation of clotted blood of immunized animals. For classical enzyme-linked immunosorbent assays (ELISA) , this is further refined to a antiserum fraction that is predominantly IgG, which is obtained by ammonium sulphate precipitation, followed by passage over ion-exchange cellulose column (Clark and Adams, 1977). Although polyclonal antisera are still used regularly, the use of monoclonal antibodies in plant pathology is becoming progressively more routine. The direct immunofluorescence assay has been used for the detection of fungi and plant pathogenic bacteria on plants or soil samples for nearly 20 years (Choo and Holland, 1970; Malajczuk, McComb and Parker, 1975). This assay uses pathogen-specific antibodies conjugated with fluorescent dye molecules. A revolution in serological detection of plant pathogens occurred when enzyme-linked immunosorbent assay (ELISA) was introduced into plant pathology (Engvall and perlmann, 1972; Clark and Adams,1977). ELISA has the advantages of sensitivity, economical use of antiserum, the production of quantifiable data and the capacity to handle large numbers of samples quickly.

Nucleic acid-based methods (using probes and/or PCR) have increasingly been used in recent years to develop diagnostic assays for plant pathogens (Schots *et al.*, 1994; Ward, 1994; Martin *et al.*, 2000). The ribosomal RNA genes (rDNA) possess characteristics that are suitable for the detection of pathogens at the species level. These rDNA are highly stable at the species level and exhibit a mosaic of conserved and diverse regions with the genome. Internal transcribed spacer (ITS) regions have been used successfully to generate specific primers capable of differentiating closely related fungal species. Phylogenetic species concept between five or more gene trees has been proposed by Taylor *et al.* (1999). Random amplified polymorphic DNA (RAPDs) analysis has attracted a lot of attention after its advent during the 90's. This marker system was developed by Welsh and McClelland (1990). Manulis *et al.,*(1994), applied RAPDs to the carnation wilt fungal

pathogen *Fusarium oxysporum* and they were able to identify specific banding patterns that were subsequently used as probes to distinguish between races of the pathogen. In another study, genetic relationships could be inferred among the wheat bunt fungi using RAPD markers (Shi *et al*., 1995). *Colletotrichum gloeosporioides* isolates from mango and cashew plants were separated in different groups based on their RAPD band profile (Serra *et al,* 2011). The PCR-DGGE method is mainly applied for the analysis of the genetic diversity of microbial communities without the need of any prior knowledge of the species (Portillo *et al.,* 2011). These molecular techniques which interrelations among spsecies combined with phenotypic characters, can lead to a reliable taxonomy that is reflective of phylogenetic relathionship. ITS sequences of rDNA analysis and universally primed polymerase chain reaction have been used to categorize the isolates of *Talaromyces flavus* and *Trichoderma* species (Chakraborty *et al.,* 2011).

It is in this context that the present study was designed for identification of *B. sorokiniana*, spot blotch pathogen of *Sorghum bicolor* (L.) Moench in hill regions of North Bengal based on the sequence analysis of ITS regions of the rDNA gene, RAPD and DGGE analysis with universal primer and development of rDNA markers for analysis of genetic variability.

Immunological and nucleic acid techniques, both offer considerable advantages over traditional methods. However, the choice between these newer methods will depend on several factors including the application involved, the skills of the worker, the costs, the facilities needed and how many samples are to be analyzed. Sometimes, a combination of diagnostic techniques is the best approach viz. immunocapture can be used to improve the sensitivity of PCR assays and overcome problems with inhibitors in the sample. Culturing for a short time can be combined with PCR-detection to increase the quantity of pathogen present, and to ensure that only viable microorganisms are detected (Schaad *et al.,* 1999).

At present for eco friendly and sustainable agriculture use of potential biocontrol agent to control disease severity and plant growth promotion is gaining popularity over inorganic

pesticides. Use of consortia of helpful bacteria and fungus can improve the health status as well as induce systemic resistance in these plants. Several instances has been reported where different bioinoculants have induced systemic resistance in several crop plants (Chakraborty *et. al*., 2006, De Meyer *et al.*1998; Yedidia *et al*. 1999; Meena *et al*. 2000; Oostendorp *et al*. 2001; Bargabus *et al*. 2004; Bharati *et al*. 2004). The use of biological fertilizers, especially plant growth promoting bacteria, are the most important strategy to increase production in sustained agricultural systems (Sharma, 2003). These bacteria are called plant growth promoting bacteria because of their great impact on crop growth (Vessy, 2003). Biocontrol agent as plant growth promoting fungi belonging to the genus *Trichoderma* are among the most commonly isolated soil fungi. Due to their ability to protect plants and contain pathogen populations under different soil conditions, these fungi have been widely studied and commercially marketed as biopesticides, biofertilizers and soil amendments. Arbuscular mycorrhizal (AM) fungi are common root symbionts of plants and ecologically important constituents of soil communities in many habitats (Smith and Read, 2008). AM fungi are recognized as high potential agents in plant protection and pest management. Bhattacharjee *et al*., (2016) found the positive effects of bioinoculants in their previous work: growth improvement and disease suppression in sorghum plants following application of bioinoculants. In recent studies Bhattacharjee *et al* (2017) tested two PGPR *Bacillus megaterium* and *Bacillus altitudinus* to determine their efficacy in promoting induction of resistance in selected cereals in their work: biochemical Responses in *Sorghum bicolor* and *Triticum aestivum* to spot blotch disease and induction of resistance by plant growth promoting rhizobacteria.

So it can be concluded that, inspite of being an important cereal crop of large area of India spot blotch disease in sorghum, pathogen diversity and management strategies of disease have not yet been studied well. Non judicial uses of chemical fertilizer and fungicide causes soil contamination, fungicide resistance and harmful effects to non-target organisms. In order to adopt eco-friendly and inexpensive alternate disease

management strategies, increasing use of biocontrol agents provide alternatives to use of chemicals for disease control.

The present study has therefore been undertaken with the following objectives:

Detailed studies of the growth, morphology, sporulation of different isolates of *Bipolaris sorokiniana* causing spot blotch disease of Sorghum plants.

Immune detection of *B.sorokiniana* is Sorghum leaf tissue using polyclonal antibody (PAb) raised against the pathogen.

Molecular identification of the pathogen using species specific primers and ITS-PCR.

Diversity screening of *B.sorokiniana* isolates based on various genic and intergenic regions of the genome including tubulin gene.

In vitro testing of selected biocontrol agents for suppression of fungal pathogens.

Determination of biochemical changes in Sorghum plants following induction of resistance using bioinoculants against foliar fungal pathogen with special reference to accumulation of proteins, phenolics and defense enzymes [Chitinase (CHT), β-1,3-Glucanase (GLU), Phenylalanine ammonia lyase (PAL) and Peroxidase (POX)].