Chapter 2
Review of Literature

2.1. Brief History of Sorghum

Sorghum is among the oldest of cereals and fifth important in the world (Arnon, 1964) Sorghum (*Sorghum bicolor* L. Moench) is belonging to the Tribe *Andropogonae* of the family *Poaceae*. The genus *Sorghum* has been classified into five subgenera: *Eusorghum*, *Chaetosorghum*, *Heterosorghum*, *Para-sorghum* and *Stiposorghum* (Garber 1950). Although this classification is convenient, however it does not stand for evolutionary relationships (Dillon et al. 2004). The *Eu-sorghum* comprises the cultivated species *S. bicolor* (L.) Moench and its subspecies are *drummondii, arundinaceum*, and wild species includes *S.* x *alum* Parodi, *S. halepense* (L.) Pers. and *S. propinquum* (Kunth) Hitchc (deWet 1971). The United States is the world's largest producer followed by India. Sorghum grain is the leading cereal grain on African continent and Nigeria is the world second largest producer of the grain (ICRISAT, 2002). The plant is drought resistant and is therefore an extremely important commodity that provides necessary food and feed for millions of people living in semi-arid environment worldwide. In many parts of the world, sorghum has traditionally been used in food products and various food items (Badi and Ollis, 1986). Fifty percent of sorghum is grown directly for human consumption. It is one of the major staple foods in Africa, Middle East and Asia. Sorghum is an important animal feed used in countries like United States, Mexico, South America and Argentina. Good quality sorghum is available with nutritional feeding value that is equivalent to that of corn. The grain is higher in protein and lower in fat content than corn. Varieties with waxy endosperm are sources of starch having properties

similar to tapioca (Dalton and Mitchell, 1959). In West Africa, Nigeria has emerged as a pioneer in the industrial utilization of sorghum. A wet-milling process similar to that used in cornstarch manufacture is used for sorghum. The starch is made into dextrose for use in foods (Moench, 1999).

2.2. Spatial Distribution of Sorghum

Sorghum cultivation is distributed throughout the world. In Asia, it is grown in China, India, Korea, Pakistan, Thailand and Yemen. Australia and USA grow the crop too. In Southern and Eastern Africa, the sorghum-growing countries are Botswana, Eritrea, Kenya, Lesotho, Madagascar, Malawi, Mozambique, Namibia, Somalia, South Africa, Swaziland, Tanzania, Zambia and Zimbabwe. In West and Central Africa, the crop is grown in Benin, Burkina Faso, Burundi, Cameroon, Central African Republic, Chad, Egypt, Gambia, Ghana, Guinea, Guinea- Bissau, Ivory Coast, Mali, Mauritania, Morocco, Niger, Nigeria, Rwanda, Senegal, Sierra Leone, Sudan, Togo, Tunisia and Uganda. In Latin America, the sorghum -growing countries are Argentina, Brazil, Colombia, El Salvador, Guatemala, Haiti, Honduras, Mexico, Nicaragua, Peru, Uruguay and Venezuela. In Europe, it is grown in France, Italy, Spain, Albania and Romania Bantilan, 2003).

The area, production and yield of sorghum in different states of India are given in Table 2.1. The area under sorghum has declined over time. During the last three decades (1972/73-2000/01), all the major sorghum-growing states except two (Maharashtra and Karnataka) experienced a measurable decline in area under sorghum. The rate of decline in sorghum area in India in the 1990s (3.08% per year) was much faster than in the 1980s (1.57% per year). Decline in sorghum production in India during 1972/73-2000/01 was 0.42% per year. The yield of sorghum in all these states has increased during the same period. During 1972/73-2000/01, annual growth in sorghum yield in India was 1.44% while the highest growth in yield was observed in Gujarat (2.44%) and Maharashtra (2.24%). During the 1990s, the highest growth in yield was observed in Gujarat (5.87% per year), followed by Rajasthan (3.43% per year). For the two sorghum niche states of Maharashtra and Karnataka, production increased from 4 to 6 million t during the period (Bantilan,

Table 2.1. Area, production and yield of sorghum in different states of India

State	1972-75	1981-84	1991-94	1998-2002
Area ('000 ha)				
Andhra Pradesh	2709.9	2102.2	1057.2	721.6
Gujarat	970.6	956.6	444.6	206.1
Karnataka	2037.3	2205.7	2159.2	1885.0
Madhya Pradesh	2122.7	2138.0	1363.9	690.6
Maharashtra	5718	6588.7	5857.0	5019.8
Rajasthan	971.7	968.3	714.6	588.4
Tamil Nadu	665.3	688.7	500.8	402.6
India	16139.3	16469.0	12703.5	10012.3
Production ('000 t)				
Andhra Pradesh	1363.9	1326.4	815.6	559.2
Gujarat	321.4	544.7	267.6	190
Karnataka	1578	1726.3	1842.7	1707.7
Madhya Pradesh	1598	1747.7	1277.3	575.9
Maharashtra	2577.7	4740.7	5351.3	4388
Rajasthan	337.3	451.7	243.1	153.8
Tamil Nadu	504	492	508.3	403.9
India	8826.3	11578.0	10773.3	8272.0
Yield (kg ha-1)				
Andhra Pradesh	506.7	630.0	770.0	779.3
Gujarat	333.3	570.0	616.7	896.7
Karnataka	763.3	783.3	856.7	906.0
Madhya Pradesh	750.0	816.7	936.7	828.7
Maharashtra	436.7	720.0	906.7	875.3
Rajasthan	350.0	463.3	330.0	363.6
Tamil Nadu	760.0	710.0	1013.3	1001.7
India	543.3	706.7	846.7	826.0

2003).

2.3. Uses of sorghum

Rooney and Waniska (2000) provide a detailed overview of the uses of sorghum in food and industry. Worldwide, sorghum has been used for human food, animal feed, building material and fencing (House 1985, Doggett 1988). Traditionally, sorghum is used in unfermented and fermented breads, porridges, couscous, rice like products, snacks, and malted alcoholic and non-alcoholic beverages in many African and Asian countries. Sorghum can be used to produce foods that are gluten free and in this respect the potential for new food uses exists for both the US and Europe. Broomcorn is a classical example of industrial use of sorghum in Europe (Berenji and Kisgeci 1996). The demands of ecological and natural products have led to renewed interest in old fashioned, biodegradable, wooden-handled brooms, which have had a

positive effect on broomcorn production. The use of sorghum as forage crop is gaining importance in many region of the world (Zerbini and Thomas 2003). Sweet sorghum stalks consist of sugars, mainly sucrose that amounts up to 55% of dry matter and in glucose (3.2% of dry matter). They also contain fiber contents like cellulose (12.4%) and hemicelluloses (10.2%) (Billa *et al*. 1997). Sugar in biomass of Sweet sorghum is readily fermentable and thus it can be considered as a tremendous raw material for fermentative hydrogen production. Although sorghum has been thoroughly investigated as an energy crop for bioethanol and methane production (Jackman 1987, Richards *et al*. 1991, Mamma *et al.* 1996), it can also be used as a potential source for hydrogen production. Sorghum biomass could be fully exploited for hydrogen production since both soluble and complex carbohydrates can be utilized, either in a single step or separately after extraction.

2.4. The Indian Sorghum Economy

India is the second largest producer of sorghum worldwide, and has the largest area under the crop. It occupies around 11 million ha in the semi-arid regions of the country and is the third most important food grain. Sorghum straw is widely used as fodder and it often gains importance over grain in certain regions, particularly where growing conditions are unfavorable. Sorghum is grown both as a rainy-season (Jun-Oct) and post rainy-season (Sep-Jan) crop. The rainy-season crop is grown over about 53% of the area and it contributes about 65% of the total production. The postrainy-season crop covers the rest of the area and production. The major states growing rainy-season sorghum are Karnataka, Madhya Pradesh, and Maharashtra, which together share 63% of the area and 77% of the production (Figures 2.1 and 2.2, see Appendix). Maharashtra, Karnataka, and Andhra Pradesh together share 93% of the area under postrainy-season sorghum. While the rainy-season crop coincides with the main monsoon spell, postrainy-season sorghum is grown on residual soil moisture and scanty rain during the crop season. Postrainy-season sorghum grain maturing under dry weather is of high quality, valued for food, and fetches a high price. The rainy-season production system is notable for its wide use of high yielding hybrids, which occupy about 75-80% of the

Fig 2.1. Distribution of *Bipolaris sorokiniana*: (a) Global and (b) in India (Acharya *et al*., 2011)

area. Hence, the productivity of rainy-season sorghum is about 60% higher than that of the postrainy-season crop. The highest rainy-season yield was recorded in Maharashtra at 1.7 ton hectare-1 with some of its major districts yielding 1.9-2.61 hectare-1.

2.5. Important diseases of sorghum

Grain sorghum continues to be an economically important crop worldwide, fitting into production schedules and helping to control nematodes of both cotton and soybeans. However, numerous diseases of grain sorghum can be found wherever the crop is grown. Some of these diseases have been investigated in the past and several are listed below which are either continuing to be important pathogens that affect yields or are very common and provoke interest from producers.

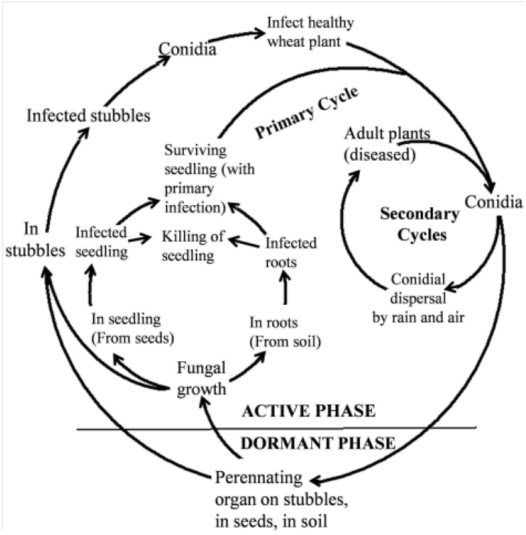

Fig 2.2. Disease cycle of *Bipolaris sorokiniana* (Acharya *et al*., 2011)

2.6. Spot blotch pathogen

Almost 90% of all the world's food crops are grown from seeds (Schwinn, 1994). Seed are widely distributed in national and international trade, and germplasm is also distributed and exchanged in the form of seeds in breeding programmes. Due to their high mobility, seeds are a highly effective means for disseminating plant pathogens over long distances. Numerous examples exist in agriculture literature for the international spread of land diseases as a result of the importation of seeds that

were infected or contaminated with pathogens (Agarwal and Sinclair, 1996). In sorghum (*Sorghum bicolor*), covered smut (*Sphacelotheca sorghi*), head smuth (*Sphacelotheca reiliana*) and long smut (*Tolyposporium ehrenbergii*) have been reported to be the most destructive pathogens, causing heavy losses in third world countries (Frowd, 1980). *Peronosclerospora sorghi,* the downey mildew pathogen in sorghum and maize, and *Sclerospora graminocola* in pearl millet transform the floral primordial into vegetative leafy structures causing 30 to 70% losses seed production in the semi-arid tropics (Williams,1984). A yield loss of 58 to 70% of hybrid sorghum and millet with 60 to 76% ergot severity has been reported in most sorghum and millet growing countries (Thakur and Chahal, 1987). Besides, these losses in potential yield, mold fungi which grow on the seed substratum produce mycotoxins which are hazardous to man and animals (Halt, 1994). Commercially, discolored sorghum seeds caused by fungi are of poor quality (Castor and Frederikser, 1980: Gopinath and Shetty, 1987), reducing their acceptability and thus, the market value of the produce. Grain mold causes crop loss by reducing seed size and weight, the food value and keeping quality of grains (Gopinath, 1984: Bandyopadhyay, 1986). Many of the diseases that cause reduced yields in sorghum have seed borne phases. Seed borne inoculums therefore, has severe implications for yield, seed production and distribution systems, trade, human nutrition and germplasm. The management of these pathogens during the seed-borne phase is considered to be the cheapest disease control strategy (Shenge, 2007). However, effective management can only be implemented effectively if the pathogens are correctly identified. It is in view of this that the current study aimed at detecting seed borne pathogens on farmer saved sorghum seeds at Samaru, Zaria, North-Western Nigeria (Abdulsalaam S and Shenge KC, 2011).

Now a days spot blotch has been a serious problem in north-eastern as well as in north western parts of India. Yield losses due to spot blotch vary from 16 to 35% in sorghum. *Bipolaris sorokiniana* (Sacc.) Shoemaker (syn. *Helminthosporium sativum* teleomorph: *Cochliobolus sativus*), a hemibiotrophic phytopathogenic fungus is a wellknown cause of spot

blotch disease in barley (*Hordeum vulgare* L.) and wheat (*Triticum aestivum* L.). *B. sorokiniana* usually induces symptoms on the leaf, sheath and stem. Yield losses due to spot blotch vary from 16 to 33% in barley (Clark, 1979). *B. sorokiniana* is widely distributed in the areas where cereals are grown. *B. sorokiniana* forms a continuous genetic pool of isolates varying in virulence and aggressiveness to various cereals and grasses (Duveiller and Altamirano, 2000). 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 (Yadav, 1981). 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. The grass weeds as collateral hosts of *B. sorokiniana* in rice–wheat system are considered as a possible reason for perceived increase in *Helminthosporium* leaf blight and cause major losses to the crop (Hobbs and Morris, 1996). Phenolic compounds are formed in response to the ingress of the pathogens and their production is considered as part of active defence response (Nicholson, 1992). Numerous studies suggest that low molecular weight phenols, such as benzoic acids and the phenylpropanoids, are formed in the host at initial response of infection. Rapid accumulation of phenols at the infection site function to slow the growth of the pathogen and allow activation of phytoalexins or other stress related substances (Matern and Kneusel, 1988). However, these are not studied against the *Bipolaris sorokiniana* in different host systems. Present study was undertaken to find the role of weed and other hosts which are grown with barley and provide shelter to pathogen during the season. Role of phenolic compounds against spot blotch pathogen was also explored to find out the level of resistance. This would help in developing management strategies in future (Bashyal *et al.,* 2011).

2.6.1. Yield Loss

The destructive capacity of this pathogen is evident from the reports around the world. Grain yield reductions due to spot blotch are variable but are of great significance in warmer areas of South Asia (Saari, 1998; Sharma and Duveiller 2004). On

an average, a South Asian country loses 20% of crop yield through leaf blight disease (Saari, 1998). Grain yield loss due to spot blotch in South Asia ranged from 4% to 38% and 25% to 43% in the year 2004 and 2005 respectively and the number of kernels per spike as well as thousand-kernel weight were reduced respectively by 10% and 15% in 2004 and 11% and 18% in 2005 (Sharma *et al*., 2006). Yield loss was estimated to be 18-22% in India (Singh *et al*., 1997), which can be devastating for farmers in the Eastern Gangetic Plains, who frequently have small holdings with little land or profitability (Joshi *et al*., 2007). In Nepal, under rice-wheat cropping system, spot blight severity went up to 100% and 70% in 2004 and 2005 respectively (Sharma and Duveiller 2007). Spot blotch has been reported to cause 15% grain yield reduction in Bangladesh (Alam *et al.,* 1998) and China (Xiao *et al.,* 1998). The pathogen also causes grain yield losses up to 10, 15, and 20% through common root rot and seedling blight in countries like Scotland, Canada, Brazil etc. (Murray *et al*., 1998) .

2.6.2. Symptoms

Symptoms mainly develop on sub-crown internodes, stem, leaves, awns, glumes and seeds. The main symptom caused by the pathogen is spot blotch, which is nothing but the disease of leaves. The early lesions on leaves are 1-2 mm long, small and dark brown in colour. There is no sign of chlorotic margin at the initial stage of infection. In the later stage in case of a susceptible genotype the small lesions extends very rapidly and ultimately reach into several centimetres. When the infection occurs into the spikelet; it results into shriveled grain and the embryo end of the seed becomes dark in colour. Diseased seedlings develop dark brown lesion on the coleoptiles, crowns, stems and roots. Death of the seedlings may occur before or soon after emergence. Common root rot is distinguished by dark brown to black necrotic lesion on roots, subcrown, internodes and basal portion of the stem. At severity, multiple lesions often coalesce to form large areas of necrosis (Jones and Clifford 1983; Mathre, 1987). Plants with common root rot produce fewer tillers and fewer kernels per year.

2.6.3. Epidemiology

Foliar blight development and severity of the disease is directly related to the minimum tillage or surface seeding, irrigation, low soil fertility, sowing

density, crop growth stage, late rain during crop cycle, heat stress during grain filling as a result of late planting, high temperature in the field and relative humidity favouring long duration (>12 hours) of leaf wetness. Even at the end of the monsoon and in absence of rainfall, high relative humidity arising from high levels of soil residual moisture along with foggy days allows long hours of wetness on leaf blades that can last until late January in Indo-Gangetic Plains, creating ideal conditions for the establishment and multiplication of wheat pathogen. In Brazil, Reis (1991) suggested that, for foliar blight outbreaks to occur, wheat leaves must remain wet for >18 h at a mean temperature of 18˚C or higher. Moderate to warm temperatures (18˚C to 32˚C) favours the growth of *B. sorokiniana*. In Asia, Nema and Joshi (1973) reported that infection was more rapid and more severe at 28°C than at lower temperatures. Area under disease progress curve values (AUDPC), conducted during 26th November 2002 to 26th December 2003 for calculating the epidemiological study of disease development, increased significantly as a function of sowing time. The higher values of AUDPC/day or AUDPC/ degree day under late-sown conditions are most likely caused by heat stress, which enhanced HLB development. Delayed seeding for wheat, grown after rice in eastern India and Nepal also results in higher losses of grain yield and total kernel weight due to foliar blight (Duveiller et al., 2005).

2.6.4. Disease Cycle

B. sorokiniana is a saprophyte and survives primarily as thick walled conidia. The sexual stage is not important in the disease cycle. The pathogen perennates both externally as conidia and internally as mycelium in the seeds, as well as in infected crop residues, volunteer plants, secondary hosts and free dormant conidia in the soil (Reis, 1991). However, the role of infected seed as a primary source of inoculums appears to be important and according to Shaner (1981), it is the main source of inoculums of leaf blight pathogens. Along the germination of the diseased seeds, the perennating organs of the causal organism become active. This is the starting point of the disease. It germinates completely in four hours, and then appressoria forms at the juncture of epidermal cell wall after eight hours and hyphae from initially infected cells enter adjacent

cells in 24 hours, which results in the granularisation of the host cytoplasm. Then fungus is transmitted to the plumules and coleoptiles tips with an efficiency reaching upto 87% (Reis and Forcelini 1993). Maximum development of symptoms appears when the leaves remain wet for more than 18 hours with a mean temperature greater than 18˚C (Couture and Sutton 1978). Under favourable conditions, hypha produces conidiophores, which emerge out through stomata of the host tissue. The emerging conidiophores produce a succession of conidia, which are transmitted by rain splashes and wind, thus building up polycyclic epidemics. Conidia on germination produce germ tube, which is surrounded by thick mucilaginous substrata. This mucilaginous substratum enables the germinating conidia to remain adhered to the host surface. The germ tube then swells to produce appresorium from which infection hyphae are developed. The infection hyphae then enter the host tissue either through stomata or by rupturing through epidermis. Immediately after the entrance in the host tissue, the infection hypha divides rapidly and ramifies along the intercellular spaces of the mesophyll tissue (Acharya *et al.,* 2011).

B. sorokiniana expanded its area of colonization with the warming of the temperature towards temperate areas because high humidity with temperature between 22-25oC during growing season enhance damage caused by spot blotch (Kaur and Nanda, 2001). Conidial populations of *B. sorokiniana* have extensive variability in morphological and physiological traits (Christensen and Davies, 1937; Tinline, 1960). Characterization of inherent variability is fundamental for the study of host parasite interactions in order to develop appropriate strategies for plant breeding programmes and long-term management of disease. Analysis of the diversity of plant pathogens have been revolutionized by the molecular techniques particularly the Polymerase Chain Reaction (PCR). Among these PCR based molecular markers, Random Amplified Polymorphic DNA (RAPD) markers provide a mechanism for swiftly and easily characterizing isolates in terms of polymorphism of primer – defined DNA fragments and genetic polymorphism in phytopathogenic fungi for inter and intraspecific variability among populations from different and from

the same geographic regions. There has been a limited effort on molecular study of Indian isolates of *Bipolaris sorokiniana* obtained from different cereal hosts and no information was available on these aspects from Western part of India. Previously study was undertaken to assess genetic variability of *B. sorokiniana* isolates obtained from different cereal hosts (bread wheat, barley, durum, triticale and rye) from Punjab (Bala *et al.,* 2015).

Several studies revealed that the plants of *Oryza sativa, Halianthus annus, Arachis hypogea* and *Cicer arientenum* were non host of *Bipolaris sorokiniana,* which are in contrast to the results which are reported in USDA ARS Fungal Database http:// nt.ars-grin.gov/fungaldatabases, where *Cicer arientenum* and *Oryza sativa* are reported as a host of *B. sorokiniana*. The results on these crops still need confirmation as these crops along with other weed grass species in the wheat field helps in survival of this pathogen This pathogen infects number of both cultivated and wild plants. Eighteen monocotyledonous plants have been identified as its hosts More than 29 species of Graminae and other crops in Northeastern China, 65 species of Graminae in Yellow and hai river region and 17 plant species in Guandong province are reported as the hosts of this pathogen. Keeping in view the wide host ranges of this fungus and on the basis of these preliminary studies further detailed investigations needed to be done which will ultimately help in sorting the spot blotch management. As one of the strategy to manage the disease is to rotate the crops with oat, rye, legumes or flax to reduce source of fungal spores from residue (Asad *et al.,* 2011) (Table 2.2).

Disease diagnosis and pathogen identification by conventional methods, which involve isolating the pathogen and characterizing it by inoculation tests, are labour-intensive and time-consuming. Over the past few decades, immunological and molecular diagonistic methods have increasingly received attention as an alternative or complement to conventional methods (Schaad *et al.,* 2003). Serological methods (enzyme linked immunosorbent assay) are routinely used in several laboratories for these purposes because they allow sensitive and simultaneous analysis of many samples in a single micro plate. One major drawback of serological assays,

however, is false positives caused by cross-reaction of antibiotics with plant debris or unrelated organisms (De Haan *et al*., 2000). Molecular methods based on polymerase chain reaction (PCR) are being used more frequently for detecting fungal pathogens in plant tissues (Bonants *et al*., 1997; Zhang *et al*., 1999; Kong *et al.,* 2003; Shen *et al*., 2005; Wang *et al*., 2006), owing to increased specificity and sensitivity compared with more traditional techniques. Also, large numbers of samples can be processed in a short time by these methods.

Disease diagnosis is an art as well as a science. We use the scientific method to perform and interpret tests for the detection of pathogens. The art lies is synthesizing information on symptom development, case history, and results of laboratory tests to determine the most likely causes of disease. Understanding the difference between the terms 'diagnosis' and 'detection'. Which are often mistakenly used interchangeably, is crucial. Detection of a pathogen does not necessarily prove that it caused the disease at hand. For example, one can usually culture, or induce by incubation, several pathogens from a plant that are not actively causing the observed symptoms. Considering complex etiology of plant disease it can be stated that the 'phenomenon of "one cause-one disease" is rare in nature'. The diagnostician must identify the various causal factors and determine their relative importance. This task can be difficult, as different factors may cause similar symptoms and different symptoms may be caused by the same factor. Just a positive test result does not prove that the pathogen detected caused the disease, a negative result does not definitely rule out the presence of a particular pathogen. The test itself may fail, or improper selection of plant tissues can lead to erroneous conclusions. Attempts to use culture techniques to recover a vascular pathogen, such as *Frusarium oxysporum* f. sp. *lysopersici*, from waited tomato foliage rather than vascular tissues would give erroneous negative results. False negative results can occur with any assay, and the diagnostician must be aware of the likelihood of their occurrence. Obviously, the choice of diagnostic test (s) can greatly influence the diagnosis. Many factors influence the interpretation of test results and the value of the final diagnosis. The quality and case history of the sample

Table 2.2. Sources (host and location) of *B. sorokiniana* isolates

Isolate No	Colony colour	Colony margin	Host	Variety	Location
1.	White	Smooth	Barley	PL 426	Ludhiana
2.	Offwhite	Smooth	Barley	PL 481	Ludhiana
3.	Dull black	Irregular	Barley	PL 419	Ludhiana
4.	Black and white	Irregular	Bread wheat	PBW 343	Ludhiana
5.	Greenish white	Irregular	Barley	BL 286	Ludhiana
6.	Greenish white	Smooth	Barley	RD 2700	Ludhiana
7.	Offwhite	Irregular	Barley	PL 481	Ludhiana
8.	Black	Smooth	Triticale	TL 2708	Ludhiana
9.	Black and white	Irregular	Triticale	TL 2708	Ludhiana
10.	White	Smooth	Barley	RD 2503	Ludhiana
11.	White	Irregular	Barley	RD 2715	Ludhiana
12.	Grey	Smooth	Barley	PL 426	Ludhiana
13.	Black	Smooth	Barley	RD 2703	Ludhiana
14.	Black	Irregular	Barley	BS 169	Ludhiana
15.	Off white	Irregular	Bread wheat	WH 147	Gurdaspur
16.	Dull black	Smooth	Bread wheat	HP 1633	Gurdaspur
17.	Dull black	Smooth	Durum	PDW 291	Abohar
18.	Grey	Smooth	Triticale	TL 3358	Ludhiana
19.	Black and white	Smooth	Bread wheat	PBW 373	Abohar
20.	Black	Smooth	Bread wheat	WH 896	Fazilka
21.	Dull black	Smooth	Durum	PDW 291	Ludhiana
22.	Black and white	Irregular	Bread wheat	H P 1633	Fazilka
23.	Black and white	Smooth	Bread wheat	WL 1562	Fazilka
24.	Black	Smooth	Bread wheat	PBW343	Fazilka
25.	Dull black	Smooth	Rye	Rye	Ludhiana
26.	Dull black	Irregular	Rye	Rye	Ludhiana
27.	Black	Smooth	Bread wheat	HD 2687	Abohar
28.	Grey	Smooth	Bread wheat	HW2021	Abohar
29.	Grey	Smooth	Triticale	TL 3358	Ludhiana
30.	Dull black	Irregular	Triticale	TL 3358	Ludhiana

provided, available resources, and expertise of the diagnostician all play a role in the accuracy of the diagnosis. Sample quality is of paramount importance and is probably the most common limiting factor to accurate diagnosis. The best diagnosis would be one for which the affected plant(s) could be observed both in the field and in the laboratory. Unfortunately, expense, time and logistic usually preclude field visits by the diagnostician. Case history information provided with a specimen can be more importance to the diagnosis than the specimen itself. This is particularly true when biotic agents play a minor role in the disease. Indeed, many plant specimens submitted to doagnostics labs are afflicted with abiotic problems, such as nutritional imbalances, chemical injury, temperature or moisture extremes, or air pollution. Most abiotic problems must be diagnosed from case-history information and symptom directions in the literature. Inadequate or incorrect information often leads to an inaccurate diagnosis.

The competent diagnostician must be able to synthesize information about many different aspects of Plant health and know the plant in health as well as disease. The diagnostician has historically been a generalist rather than a specialist, well versed not only in pathology, but also in agronomy, horticulture, entomology, soil science and weed science. To understand and interpret modern diagnostic techniques, he or she is must also have working knowledge of immunology and molecular biology. In addition to familiarity with pathology, the diagnostician must develop expertise in production practices, response of plants to environmental factors, and characteristics of specific cultivars or hybrids of many different crops. To this extent the individual must become a crop specialist. The difficulties inherent in becoming both a specialist and a generalist in a field that deals with hundreds of plant species and exponentially more pathogens or abiotic agents are obvious. Pathogens more readily detected or identified by various techniques which are listed in Table 2.3

2.7. Immunodetection of phytopathogens

Recent trends in detection of plant pathogens include the development of more rapid diagnostic techniques with high specificity for the target organism (Chakraborty, 1988). These techniques

can be used to detect fungi, bacteria and viruses present in low amounts in and on plant tissues and, therefore, in many cases the pathogen can be detected at an earlier stage of disease development than was previously possible. Some of these rapid sensitive techniques are enzyme linked immunosorbentassay (ELISA), immunoflurescence (IF) and polymerase chain reaction (PCR). Commercial developments of such techniques are expensive.

Before the development of serological techniques, laborious and time-consuming assays such as transmission to indicator host were used for the routine detection of viruses in many diagnostic labs. Early serological techniques were less sensitive and more time-consuming than those more widely used today. Conventional techniques for viral detection have largely been replaced by the more rapid and sensitive modern techniques, however, some of the conventional techniques still have a place in the diagnostic lab.

The diagnosis of bacteria diseases has been aided by many contributions from both basic and applied research, directed to the rapid detection and identification of plant pathogenic bacteria. Many selective and semi-selective media have been developed. Serology continues to become more successful as sensitivity increases and assay time decreases. Several test formats similar to those developed for fungi and viruses have been developed for bacteria, including multiwall ELISA, dot blot and immunoflurescence. Fatty acid analysis has also prevented to be a reliable method of identification, and new techniques, such as nucleic acid probes and PCR, have extended the limits of specificity. Many techniques that differ in sensitivity, specificity, reliability and cost are available for the detection of plant pathogens. The most desirable methods for diagnosticians are those that give the least number of false negative or false positive over time and among workers, are relatively rapid, cost-effective and detect the broadest range of pathogens. Sensitivity is not a priority in the diagnostic lab as it is in the regulatory labs or indexing programmes, although a technique must be sensitive enough to detect a pathogen in symptomatic tissue. A diagnostician depends heavily on reliable, accurate, standardized assays. Rapidity is important, although techniques that may taken an entire day

to complete but do not require diagnosticians full attention are preferably acceptable. Cost is always a consideration, and diagnostic labs will invest in technology that is applicable to the greatest number of pathogens.

Airborne spores of fugal plant pathogens have commonly been detected and enumerated by microscopic examination of surfaces on which spores have been impacted (Aylor, 1998; Hunter *et al*., 1999).however, technological advances of fungal diagnostics in which either antibody or nucleic acid probes are used offer the potential for developing rapid systems for detecting and quantifying airborne spores of fungal plant pathogens. An immunoassay system developed by Spore View (Chaparral Diagnostics, Burlington, Vt.) utilizes passive deposition of ascospores of *Venturia inaequalis,* the causal agent of apple scab, on a membrane surface. Similarly, studies to develop an antibody based immunoassay for early detection of *Sclerotiana sclerotium* (Jamaux and Spire, 1994), a major fungal pathogen of oilseed rape (*Brassica napus),* have

Table 2.3: Serological and molecular techniques for detection of plant pathogens

Plant Pathogens	**Serological and Molecular techniques**
Fungi, Bacteria, Viruses	Multiwell ELISA
Fungi, bacteria	Flow-through
Fungi	Dipstick
Fungi, bacteria, Viruses	Dot-Blot
Fungi, viruses	Tissue print
Fungi, bacteria, virus inclusions	Immunofluorescence
Viruses	Serologically specific electron microscopy (SSEM)
Viruses, bacteria	Agglutination
Fungi, bacteria, viruses, viroids	Nucleic acid hybridization
Fungi, bacteria, viruses, viroids	Dot blot/Squash blot
Fungi, bacteria, viruses, viroids	Polymerase chain reaction (PCR)
Viruses	Tissue print hybridisation
Bacteria	Fatty acid analysis
Viruses	dsRNA analysis
Viroids and viruses	Polyacrylamide gel electrophoresis (PAGE)
Bacteria	Nutritional test kits
Fungi, bacteria, nematodes, virus inclusions	Light microscopy
Fungi, bacteria	Culture
Fungi	Baiting
Viruses, viroids, fungi, bacteria, nematodes	Host inoculation
Viruses	Leaf dips
Nematodes	Extraction/Identification

relied solely on passive deposition of ascospores on rapeseed petals and subsequent mycelia growth. Different immunological formats used for detection of plant pathogenic fungi are listed in Table 2.4 and 2.5.

2.8. Molecular Detection of phytopathogens

The presence of nucleic acids (DNA/ RNA) is one of the important characteristics of all living organisms. These characteristics of nucleic acids and other organelles of organisms have been studies for detection, identification and differentiation of microbial plant pathogens. Today in 21st century scientists are becoming increasingly able to diagnose and manage diseases at the molecular level. Molecular methods offer an entirely new approach to the plant diseases diagnosis, however many a times molecular methods may be an

Table 2.4: Different immunological formats used for detection of pathogenic fungi

Serological formats	Pathogen	Crop
PTA indirect, Dot Blot	Armillariamellea	Tea
PTA indirect	*Bipolariscarbonum*	
PTA indirect, Dot blot	*Corticumtheae*	
PTA indirect, Dot blot	*Exobasidiumvexans*	
PTA indirect	*Fomeslamaoensis*	
PTA indirect	*Glomerellacingulata*	
PTA indirect	*Pestalotiopsistheae*	
PTA indirect, Dot blot	*Poriahypobrumea*	
PTA indirect, Dot blot	*Roselliniaarcuata*	
PTA indirect	*Sphearostilberepens*	
PTA indirect, Dot blot	*Ustulinazonata*	
PTA indirect	*Fusariumcalmorum*	Wheat
DAC	*Gauemannomycesgraminis*	
PTA indirect	*Pseudocercosporellaherpotrichoides*	
PTA Indirect, Dot blot	*Bipolarissorokiana*	
PTA Indirect	*Penicilliumislandicum*	Rice
PTA indirect	*Erysiphegraminis*f.sp.*hordei*	Barley
PTA indirect	*Phomaexigua*	Potato
PTA indirect	*Phytophthoranicotianae*	Tomato
PTA indirect	*Fusariumoxysporum*	Soybean
PTA indirect	*Fusariumgraminearum*	
PTA indirect	*Phomopsislongicolla*	
DAC	*Phytophthoramegasperma*	
PTA indirect	*Sclerotiumrolfsii*	
PTA indirect	*Thielaviopsisbasicola*	Cotton
DAC	*Colletotrichumfalcatum*	Sugarcane
DAC	*Ustilagoscitaminea*	
DAC	*Sclerotiniasclerotiorum*	Sunflower
PTA indirect	*Phytophthorafragariae*	Strawberry
PTA indirect	*Botrytis cinerea*	Grapes
PTA indirect	*Leptosphaeriakorrae*	Turf grass
PTA ELISA, Dot Blot	*Macrophominaphaseolina*	Mandarin

improvement over conventional microbiology testing in many ways. Over the last ten years much effort has been devoted to the development of methods for detecting and identifying plant pathogens based on DNA/RNA probe technology and PCR amplification of nucleic acid sequences. Perhaps the greatest advantage these techniques have over conventional diagnostic methods is the potential to be highly specific. They can distinguish between fungal species and within a single species (Ward, 1995 and Ward and Adams, 1998). DNA-based diagnostics are also used to determine whether the pathogen is resistant or sensitive to particular fungicides and to determine its virulence characteristics. The rapid development in the fields of molecular plant pathology has provided new insights into the genetic and structural features of a large number of pathogens. These results obtained through intensive basic research are further leading to improvement in diagnosis procedures. As more information becomes available on fungal genomics and gene function, the scope for using molecular-based diagnostic will also increase. Automation and high-density oligonucleotide probe arrays (DNA

Table 2.5: *In situ* analysis of phytopathogenic fungi using immunofluorescence

Crop	**Pathogen**	**Immunofluorescence assay**
Barley (grain)	*Alternariasp*	Indirect
Barley (grain)	*Aspergillussp*	Indirect
Tea (leaf)	*Bipolariscarbonum*	Indirect
Tea (leaf)	*Corticumtheae*	Indirect
Grapevines (wood)	*Eutypaarmeniacea*	Direct, Indirect
Tea (leaf)	*Exobasidiumvexans*	Indirect
Tea (root), soil	*Fomeslamaoensis*	Indirect
Soybean (root), soil	*Fusariumgraminearum*	Indirect
Soybean (root), soil	*Fusariumoxysporum*	Indirect
Cotton (root), soil	*Fusariumvasinfectum*	Direct
Cashew (root)	*Ganodermalucidum*	Direct
Tea (leaf)	*Glomerellacingulata*	Indirect
Elm (wood)	*Ophiostomaulmi*	Indirect
Tea (leaf)	*Pestalotiopsistheae*	Indirect
Barley (grain)	*Penicilliumsp.*	Indirect
Barley (grain), soil	*Penicilliumcyclopium*	Indirect
Soil	*Phaeolusschweinitzii*	Indirect
Potato (tuber)	*Phomaexigua*	Indirect
Soil	*Phytophthoramegasperma*	Indirect
Soil	*Phythophthoracinnamomi*	Direct, Indirect
Cabbage (root), soil	*Plasmodiophorabrassicae*	Indirect
Soybean (root), soil	*Sclerotiumrolfsii*	Indirect
Tea (root), soil	*Sphaerostilberepens*	Indirect
Tea (root), soil	*Ustulinazonata*	Indirect

chips) also hold great promise for characterizing microbial pathogens.

2.8.1. Hybridization-Bases Nucleic Acid Techniques

The characteristic genetic constitutions of individual organisms are due to many generations of mutations and recombinations. It is generally accepted that closely related organisms share a greater nucleotide similarity than those are distantly related. Techniques based on nucleic acid hybridization involve the identification of a highly specific nucleotide common to given strain or isolate of the microbial plant pathogen species, but absent in other strains or isolates or species and this selected sequence of the organism is used to test for the presence of the target organism. Likewise, a highly conserved sequence present in all strains or species in a genus may be employed to probe for the presence of any member of that genus. The selection of a specific sequence as a probe is distinctly derived from the sequential data and screening of related organisms to determine its specificity.

Detection of microbial plant pathogens by nucleic acid hybridization techniques is based on the formation of double-stranded (ds) nucleic acid molecules by specific hybridization between the single-stranded (ss) target nucleic acid sequence (denatures DNA or RNA) and complementary single-stranded nucleic acid probe. Sequences of either RNA or DNA have been used as probes. If the probe strand in the duplex is labeled with a detectable marker like ^{32}P, information of the duplex can be assayed after removal of unhybridized sequences. Hybridization reaction may be performed in solution (solution hybridization), in situ (in situ hybridization) and on solid filter supports (filter hybridization). The filter and in situ hybridization methods have been more frequently employed for detection of microbial pathogens.

Detection of fungal plant pathogens by employing nucleic acid (NA)-based techniques provides certain distinct advantages over immune detection methods. The fungus-like and fungal pathogens are complex antigens, the nature of which may vary, depending on the stage in their life cycle. The antisera produced against one type of spores or mycelium formed at a particular stage may not actively react with spores or mycelium produced at all stages in the life cycle of the pathogen. However, the presence or

absence of spore-bearing structures or the slow growing nature of some fungal pathogens will not affect their detection by NA-based techniques, since the nature of the genomic elements remains constant, irrespective of the stages of life cycle of the pathogen to be detected. It is possible to detect, identify, differentiate and quantify the fungal pathogens concerned, using appropriate DNA probes, even in the case of pathogens that are not amenable for detection by other methods. For example, fungal pathogens causing nonspecific, generalized rotting and death of plants and obligate fungal pathogens that cannot be cultured may be rapidly detected and differentiated by employing suitable probes (Narayanasamy, 2011).

2.8.2. Polymerase Chain Reaction (PCR)

The polymerase chain reaction (PCR) allows the amplification of millions of copies of specific DNA sequences by repeated cycles of denaturation, polymerization and elongation at different temperatures using specific oligonucletides (primers), deoxyribonucletide triphosphates (dNTPS) and a thermostable Taq DNA polymerase in the adequate buffer (Mullis and Faloona, 1987). The amplified DNA fragments are visualized by electrophoresis in agarose gel stained with EtBr, SYBR Green or other safer molecule able to intercalate in the double stranded DNA, or alternatively by colorimetric (Mutasa *et al.,* 1996) or flurometric assays (Fraaije *et al*., 1999). The presence of a specific DNA band of the expected size indicated the presence of the target pathogen in the sample. Advances in PCR-based methods, such as real-time PCR, allows fast, accurate detection and quantification of plant pathogens in an automated reaction. Main advantages of PCR techniques include high sensitivity, specificity and reliability. Moreover, it is not necessary to isolate the pathogen from the infected material reducing the diagnosis time from weeks to hours, and allowing the detection and identification of non-culturable pathogens (Capote *et al.,* 2012). This is particularly useful in studying systemic infections, or in the early detection of disease, before symptoms are visible. Compared to culturing, molecular methods are relatively fast, results are often possible within one or two days of sampling. They are potentially more reliable than identification of visual

symptoms, as they do not rely on the skills needed to distinguish suitable differences in disease symptoms (McCartney *et al*., 2003).

The products of PCR can be used for three different purposes: (i) as a target for hybridization, (ii) for the direct sequencing of the DNA to determine strain variations and (iii) as a specific probe. PCR approach for the detection of a bacterial pathogen in diseases plant was first applied by Rasmussen and Reeves (1992). PCR assay may be preferred by researchers because of several advantages over conventional methods involving isolation and examination of cultural characteristics. The pathogen(s) need not be isolated in pure culture before detection in infected plant materials. It is enough, if the pathogen DNA is extracted. High levels of sensitivity and specificity, in addition to simplicity, have made the PCR-based assays as the technique of choice for routine and large scale application in quarantine and certification programs.

Although PCR is a highly sensitive technology, the presence of inhibitors in the plant tissues and soil, greatly reduces its sensitivity. The inhibitors are believed to interfere with the polymerase activity for amplification of the target DNA. Another problem with the conduct of PCR arises from the possible DNA contamination leading to false negative results. Hence, it is essential to adopt stringent conditions during all operations and to have proper negative controls. Further, it would be desirable to allot separate dedicated areas for pre and post PCR handling. The DNA based detection methods have yet another limitation. In addition to determining the presence or absence of the pathogen in the plants or in the environment, the pathogen detection system has the principal goal of ascertaining the viability of pathogen propagules. In the event of positive result, it is necessary to know whether the pathogen detected poses a threat to crop production, public health or food safety. The lack of discriminating viable from dead cells is a pitfall commonly recognized, while applying nucleic acid-based systems, including PCR and microarrays (Keer and Birech, 2003; Call, 2005). Development of the method involving enrichment culturing (BIO-PCR) prior to PCR, addresses this problem to some extent (Schaad *et al*. 2003). Designing suitable primers is a critical step in PCR assay. Generally, short sequences (100-1,000 bp) are more

efficiently amplified and resolved by agarose electrophoresis. Specific primers are derived from sequences or either amplified or cloned DNA (cDNA) or RNA from target pathogen species or strains or isolates. Primer specificity for target sequences is affected by many factors which include primer length, annealing temperature, secondary structures of target and primer sequences. Ribosomal genes and the spacers between them provide targets of choice for molecular detection and phylognetic investigations, since they are present in high copy numbers, contain conserved as well as variable sequences and can be amplified and sequenced with universal primers based on their conserved sequences (Bary *et al*., 1991; Bruns *et al*., 1991; Stackebrandt *et al*., 1992; Ward and Gray, 1992).

A PCR assay, based on the amplification of 5.85 rDNA gene and ITS4 and ITS5 primers, was employed for the rapid detection and identification of economically important *Phytophthora* spp. belonging to six taxonomic groups. The pathogens detected include *P. cactorum, P. capsici, P. cinnamomi, P. citricola, P. citrophthora, P. erythroseptica, P. fragariae, P. infestans, P. megasperma, P. mirabilis* and *P. Palmivora* (Liew *et al.,* 1998).

Sometimes, PCR using a single pair of primers does not always give a sufficiently specific or sensitive test. This can be overcome by using DNA probes in conjunction with PCR (Mutasa *et al*., 1995). A second approach is to use nested primers. Here, after an initial PCR, the product is subjected to a second round of PCR using primers which recognise a region within that amplified by the first pair (Foster *et al.,* 2002, Schesser *et al*., 1991). However, this procedure is more labour-intensive, more costly and more prone to contamination that the single primer pair method. Another approach to overcoming sensitivity and specificity problems is by using antibodies in conjunction with PCR, ie. Immune-PCR or immunocapture PCR. However, although this approach has been used to detect a few plant pathogenic viruses (Jacobi *et al.,* 1998, Shamloul and Hadidi., 1999) and phytoplasmas (Pollini *et al.,* 1997), we are not aware of any examples of its use to detect fungi. Where there is a need to detect several different pathogens simultaneously, multiplex PCR, involving several pairs of costs, but care is needed to optimize the

conditions so that all of the different amplicons can be generated efficiently (McCartney *et al.,* 2003).

2.8.3. PCR-ELISA

This serological-based PCR method uses forward and reverse primers carrying at their 5' and biotin and an antigenic group (e.g. fluorescein), respectively (Landgraf *et al.,* 1991). PCR amplified DNA can be immobilized on avidin or streptavidin-coated microtitre plates via the biotin moiety of the forward primer and then can be quantified by an ELISA specific for the antigenic group of the reverse primer (e.g. anti-fluorescein antibody detected by colorimetric reactions). PCR-ELISA method is as sensitive as nested PCR. In addition, it does not require electrophoretic separation and/ or hybridization, and can be easily automated. All reactions can be performed in 96 well microtitre plates for mass screening of PCR products making them very suitable for routine diagnostic purposes. This procedure has been used for detection and differentiation of *Didymella bryoniae* from related *Phoma* species in cucurbits (Somai *et al.,* 2002) and for detection of several species of *Phytophthora* and *Pythium* (Bailey *et al.,* 2002).

2.8.4. PCR-DGGE

This 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). DGGE (Denaturing Gradient Gel Electrophoresis) and its variant TGGE (Temperature Gradient Gel Electrophoresis) use chemical gradient such as urea (DGGE) or temperature (TGGE) to denature and separate DNA samples when they are moving across an acrylamide gel. In PCR-DGGE target DNA from plant or environmental samples are firstly amplified by PCR and then subjected to denaturing electrophoresis. Sequence variant of particular fragments migrate at different positions in the denaturing gradient gel, allowing a very sensitive detection polymorphisms in DNA sequences. In addition, PCR-DGGE primers contain a GC rich tail in their 5' end to improve the detection of small variations (Myers *et al.,* 1985). The bands obtained in the gel can be extracted, cloned or reamplified and sequenced for identification, being even possible to identify constituents that represent only 1% of the total microbial community. These

techniques are very suitable for the identification of novel or unknown organisms and the most abundant species can be readily detected.

This method is however time consuming, poorly reproducible and provides relative information about the abundance of detected species. Interpretation of the results may be difficult since the micro heterogeneity present in some target genes may appear as multiple bands in the gel for a single species, leading to an overestimation of the community diversity. Furthermore, fragments with different sequences but similar melting behaviour are not always correctly separated. In other cases, the analysis of complex communities of microorganisms may result in blurred gels due to the large number of bands obtained.

A PCR-DGGE detection tool based in the amplification of the ITS region has been recently applied to detect multiple species of *Phytophthora* from plant material and environmental samples (Rytkonen *et al*., 2012). Other authors have used this technique to compare the structure of fungal communities growing different conditions or envirionments, e.g. to study the impact of culture management such as biofumigation, chemifumigation or fertilization on the relative abundance of soil fungal species (Omirou *et al.,* 2011; Wakelin *et al.,* 2008).

2.8.5. Fingerprinting

Fingerprinting approaches allow the screening of random regions of the fungal genome for indentifying species -specific sequences when conserved genes have not enough variation to successfully identify species (McCartney *et al*., 2003). Fingerprinting analyses are generally used to study the phylogenetic structure of fungal populations. However, these techniques have been also useful for identifying specific sequences used for the detection of fungi at very low taxonomic level, and even for differentiate strains of the same species with different host range, virulence, compatibility group or mating type.

2.8.5.1. Restriction fragment length polymorphism (RFLP)

RFLP involves restriction enzyme digestion of the pathogen DNA, followed by separation of the fragments by electrophoresis in agarose or polyacrylamide gels to detect differences in the size of fungal species. Polymorphisms on the

restriction enzyme cleavage sites are used to distinguish fungal species. Although DNA restriction profile can be directly observed by staining the gels, southern blot analysis is usually necessary. DNA must be transferred to adequate membranes and hybridized with an appropriate probe. However, the southern blot technique is laborious, and requires large amounts of undegraded DNA. RFLPs have been largely used for the study of the diversity of mycorrhizal and soil fungal communities (Thies, 2007; Kim *et al*., 2010; Martinez-Garcia *et al*., 2011). Although used for differentiation of pathogenic fungi (Hyakumachi *et al.,* 2005) this early techniques has been progressively supplanted by other fingerprint techniques based in PCR.

PCR-RFLP combines the amplification of a target region with the further digestion of the PCR products obtained. PCR primers specific to the genus *Phytophthora* were used to amplify and further digest the resulting amplicons yielding a specific restriction pattern of 27 different *Phytophthora* species (Drenth *et al*., 2006). PCR-RFLP analysis of the ITS region demonstrated the presence of different anastomosis group (AG) within isolates of *Rhizoctonia solani* (Pannecoucqe and Hofte, 2009); it also allowed the differentiation of pathogenic and non pathogenic strains of *Pythium myriotolum* (Gomez-Alpizar *et al.,* 2011). In other cases, the analysis of the ITS region by this technique failed in differentiating closely related species (e.g., clade 1c species such as *Phytophthora infestans* and *P. mirabilis*) (Grunwald *et al.,* 2011).

2.8.5.2. Random amplified polymorphic DNA (RAPD) analysis

RAPD (random amplified polymorphic DNA), AP-PCR (arbitrarily primed PCR) and DAF (DNA amplification fingerprinting) have been collectively termed multiple arbitrary amplicon profiling (MAAP; Caetano-Annolles, 1994). These three techniques were the first to amplify fragments from any species without prior sequences information. These three techniques produce markedly different amplification profiles, varying from quite simple (RAPD) to highly complex (DAF) patterns. The key innovation of RAPD, AP-PCR and DAF is the use of a single arbitrary oligonucleotide primer to amplify template DNA without prior knowledge of the target sequence. The amplification of nucleic acids with

arbitrary primers is mainly driven by the interaction between primer, template annealing sites and enzymes, and determined by complex kinetic and thermodynamic processes (CaEtano-Annolles, 1997).

The RAPD protocol usually uses a 10 bp arbitrary primer at constant low annealing temperature (generally 34-37^{o}C). RAPD primers can be purchased as sets or individually from different sources. Although the sequences of RAPF primers are arbitrarily chosen, two basic criteria indicated by Williams *et al*. (1990) must be met; a minimum of 40% GC content (50-80% GC content is generally used) and the absence of palindromic sequence (a base sequence that reads exactly resolve on 1.5-2.0% agarose gels and stained with ethidium bromide. Most RAPD fragments result from the amplification of one locus, and two kinds of polymorphism occur: the band may be present or absent, and the brightness (intensity) of the band may be different. Band intensity differences may result from copy number re relative sequence abundance (Devos and Gale, 1992) and may serve to distinguish homozygote dominant individuals from heterozygotes, as more bright bands are expected for the former.

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). Manuslis *et al*., (1994), applied RAPDs to the carnation wilt fungal pathogen *Fusarium oxysporum* f.sp. *Dianthi* 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).

2.8.5.3. Amplified fragment length polymorphism (AFLP)

AFLP analysis (Vos *et al*., 1995) consists in the use of restriction enzymes to digest total genomic DNA followed by ligation of restriction half-site specific adaptors to all restriction fragments. Then, a selective amplification of these restriction fragments is performed with PCR primers that have in their 3' end the corresponding adaptor sequence and selective bases. The band pattern of the amplified fragments is visualized on

denaturing polyacrylamide gels. The AFLP technology has the capability to amplify between 50 and 100 fragments at one time and to detect various polymorphisms in different genomic regions simultaneously. It is also highly sensitive and reproducible. As with other fingerprinting techniques, no prior sequence information is needed for amplification (Meudt and Clarke 2007). The disadvantages of AFLPs are that they require high molecular weight DNA, more technical expertise than RAPDs (ligation, restriction enzyme digestions and polyacrylamide gels) and that AFLP analysis suffer the same analytical limitations of RAPDs (McDonald 1997).

Depending on the primers used and on the reaction conditions, random amplification of fungal genomes produces genetic polymorphisms specific at the genus, species or strain levels (Liu *et al.,* 2009). As a result, AFLP has been used to differentiate fungal isolates at several taxonomic levels e.g. to distinguish *Cladosporium fulvum* from *Pyrenopeziuz brassicae* species (Majer *et al*., 1996), *Aspergillus carbonarius* from *A. ochraceus* (Schmidt *et al.,* 2004), and *Colletritrichum gossypii* from *C. gossypii* var. *cephalosporioides* (Silvar *et al*., 2005); also to differentiate *Monilinia laxa* that infect apple trees from isolates infecting other host plants (Gril *et al*., 2008); and to separate non-pathogenic strains of *Fusarium oxysporum* from those of *F. commune* (Stewart *et al*., 2006). AFLP markers have also been used to construct genetic linkage maps e.g. of *Phytophthora infestans* (VanderLee *et al.,* 1997). Specific AFLP bands may also be used for SCAR markers development used in PCR-based diagnostic tests. Using SCAR markers Cipriani *et al.,(*2009) could distinguish isolates of *Fusarium oxysporum* that specifically infect the weed *Orobanche ramose*. AFLP profiles have also been widely used for the phylogenetic analysis of *Fusarium oxysporum* complex (Baayen *et al*., 2000; Fourie *et al.,* 2011; Groenewald *et al.,* 2006).

2.8.6. DNA Sequencing

Morphological characteristics are not always enough to identify a pathogen. one of the most direct approaches to do that consists in the PCR amplification of a target gene with universal primers, followed by sequencing and comparison with the available publicly databases. In addition, new fungal species have been described by using

sequencing approaches. However, the use of sequence databases to identify organisms based on DNA similarity may have some pitfalls including erroneous and incomplete sequences, sequences associated with misidentified organisms, the inability to easily change or update data and problems associated with defining species boundaries, all of them leading to erroneous interpretation of search results. An effort for generating and archiving high quality data by the researcher's community should be the remedy of this drawback (Kang *et al*., 2010). Other limitation of sequencing as diagnostic tool is the need to sequence more than one locus for the robustness of the result and the impractical of this method in cases when rapid results are needed such as for the control or eradication of serious plant disease outbreaks. Nevertheless, the increase of sequencing capacity and the decrease of costs have allowed the accumulation of a high numbers of fungal sequences in publicly accessible sequence databases and sequences of selected genes have been widely used for the identification of specific pathogens.

2.8.6.1. Sequencing of tubulin gene

In molecular biology, housekeeping genes are typically constitutive genes that are required for the maintenance of basic cellular function and are expressed in all cells of an organism under normal and patho-physiological conditions. Although some housekeeping genes are expressed at relatively constant rates in most non-pathological situations, the expression of other housekeeping genes may vary depending on experimental conditions. Tubulin is one of those housekeeping genes and can refer either to the tubulin protein superfamily of globular proteins or one of the member proteins of that superfamily. α and β tubulins polymerize into microtubules, a major component of the eukaryotic cytoskeleton. Microtubules function in many essential cellular processes, including mitosis. It gives structural support, intracellular transport and DNA segregation. Microtubules are assembled from dimmers of α- and β-tubulin.

Because of its unique conserved sequences diversity analysis of fungi based upon tubulin gene is now a day's gaining popularity over conventional technique. On the other hand β-tubulin, the structural components of microtubules is the targets of benzimidazole fungicides which was

used to control many diseases of agricultural importance. Intron polymorphisms in the intron-rich genes of these proteins have been used in phylogeogrphic investigation of phytopathogenic fungi. In this context similar kind of work was previously reported by Maciel *et al*., 2010.

2.9. Defense strategy of plants

2.9.1. Defense enzymes

2.9.1.1. Chitinase (EC 3.2.1.14)

Plants represent the major component of biota and have the capability to synthesize their food through the process of photosynthesis. Physiological and environmental changes affect their health and make them vulnerable to variety of diseases thus directly or indirectly affect other components of ecosystem. A large number of environmental issues are linked with the eradication of plant diseases with chemical compounds. Most of these diseases are caused by fungal and insect pathogens. Chitin is the main structural component of these organisms and thus the enzyme responsible to hydrolyze chitin content are receiving attention in regard to their development as biopesticides or chemical defense proteins in transgenic plant and in microbial biocontrol agents.

Chitinase attack on chitin molecules which are the main structural component in fungal cell wall. In nature chitin is found to be in the form of complex with other biomolecules such as carbohydrates and protein (Sietsma and Wessels, 1979). The enzyme chitinase hydrolyzes the chitin polymer into N-acetyl glucosamine by either endo or exo cleavages of the 1-3 and 1-4 bond (Van Aalten et al., 2000). The enzyme is classified into several categories on the basis of their isolation, structural and functional characteristics (Sharma *et al*., 2011).

2.9.1.2. β-1,3-Glucanase

Plant β-1,3-glucanases are pathogenesis-related (PR) proteins, which belong to the PR-2 family of pathogenesis-related proteins and are believed to play an important role in plant defense responses to pathogen infection. In addition to plant, β-1,3-glucanases have been found in yeasts, actinomycetes, bacteria, fungi, insects. (Pan *et al*., 1989, Simmons 1994). β-1,3- glucanases are able to catalyze the cleavage of the β-1,3-glycosidic bonds in β-1,3-glucan (Simmons 1994). β-1,3 -glucal is a another major structural component of the cell walls of many

pathogenic fungi (Wessels 1981, Adams 2004). Synthesis of these enzymes can be induced by pathogens or other stimuli. β-1,3-glucal (called callose in plants) is unlike chitinases, the substrate for β-1,3-glucanases is widespread in plants and therefore these enzymes may have other physiological functions as well as in plant defense. It has been suspected that β-1,3- glucanases have direct effect in defending against fungi by hydrolyzing fungal cell walls, which consequently causes the lysis of fungal cells. In addition, β-1,3-glucanases was showed to have an indirect effect on plant defense by causing the formation of oligosaccharide elicitors, which elicit the production of other PR proteins or low molecular weight antifungal compounds, such as phytoalexins (Keen *et al*., 1983, Ham *et al.,* 1991, Klarzynski *et al.,* 2000).

2.9.1.3. Phenylalanine ammonia lyase (E.C.4.1.3.5)

PAL is the primary enzyme in the phenylpropanoid pathway, which leads to the conversion of 1-phenylalanine into trans-cinnamic acid with the elimination of ammonia. PAL has been demonstrated in metabolic activity of many higher plants and is the key enzyme in the synthesis of several defense-related secondary compounds like phenols and lignins (Hemm *et al*., 2004). The presence of phenolic compounds in plants and their synthesis in response to infection is associated with disease resistance. PAL is one of the most intensively studied enzymes in plant secondary metabolism because of its key role in phenylpropanoid biosynthesis (Whetten and Sederoff, 1995).

2.9.1.4. Peroxidase (E.C 1.14.18.1)

Peroxidase is a nuclear encoded, plastid copper-containing enzyme, which catalyzes the oxygen dependent oxidation of phenols to quinines. Because of conspicuous reaction products and induction by wounding and pathogen attack, peroxidase has frequently been suggested to participate in plant defense against pests and pathogens. There are few reports of the role of peroxidase in plant defense against pathogens. Over-expression of peroxidase in transgenic tomato plants enhanced their resistance to *Psedomonas syringae* (Li and Steffens, 2002).

2.9.2. Phenolic compounds

Plant phenolics are secondary metabolites that encompass several classes structurally diverse of natural

products biogenetically arising from the shikimate-phenylpropanoids-flavonoids pathways. Plants need phenolic compounds for pigmentation, growth , reproduction, resistance to pathogen and for many other functions. Therefore, they represent adaptive characters that have been subjected to natural selection during evolution. Phenolic compounds are plant secondary metabolites that constitute one of the most common and widespread groups of substances in plants. As stated by Harborne (1989), the term “phenolic” or “polyphenol” can be precisely defined chemically as a substance which possesses an aromatic ring bearing one (phenol) or more (polyphenol) hydroxyl substituents, including functional derivatives (esters, methyl ethers, glycosides etc.) : as a general rule, the terms phenolics and polyphenols refer to all secondary natural metabolites arising biogenetically from the shikimate-phenylpropanoids-flavonoids pathways, producing monomeris and polymeris phenols and polyphenols. Phenol itself is a natural product but most phenolics have two or more hydroxyl groups. Unless they are complete esterified, etherified or glycosylated, plat phenolics are normally soluble in polar organic solvents.

Plants need phenolic compounds for pigmentation, growth, reproduction, resistance to pathogens and for many other functions. These compounds from one of the main classes of secondary metabolites and several thousand (among then over 8,150 flavonoids) different compounds have been identified with a large range of structures; monomeric, dimeric and polymeric phenolics. Several classes of phenolics have been categorized on the basis of their basic skeleton: C6 (simple phenol, benzoquinones), C6-C3 (hydroxycinnamic acids, coumarins, phenylpropanes, chromones), C60C4 (naphthoquinones), C6-C1-C6 (xanthones), C6-C2-C6 (stilbenes, anthraquinones), (C6-C3-C6)2 (biflavonoids), (C6-C3)n (lugnins), (C6)n (catechol melanins), (C6-C3-C6) n (condensed tannins) (Harborne1980)

Plants encounter numerous pests and pathogens in the natural envioronment. An appropriate response to attack by such organisms can lead to tolerance or resistance mechanisms that enable the plant to survive. Resistance mechanisms refer to traits that inhibit or limit attach, while tolerance

strategies do not limit attack but reduce or offset consequences on the plant fitness by adjusting its physiology to buffer the effects of herbivory or diseases.

Most plants produce a broad range of secondary metabolites that are toxic to pathogens and herbivores, either as part their normal program of growth and development or in response to biotic stress. Preformed antibiotic compounds that occur constitutively in healthy plants are likely to represent inbuilt chemical barriers to herbivorous and fungal enemies and may protect plants against attack by a wide range of potential pests and pathogens. In contrast, induced defence compounds are synthesized in response to biotic stress as part of the plant defense response and are restricted to the damage tissue (Lattanzio *et al.,* 2006).

2.9.3. Systemic Acquired Resistance (SAR) and Induced Systemic Resistance (ISR)

Systemic acquired resistance (SAR) is a mechanism of induced defense which provides long-lasting protection against a broad spectrum of microorganisms. SAR required the signal molecule salicylic acid (SA) and is associated with accumulation of pathogenesis-related proteins, which are thought to contribute to resistance. Induced resistance is a physiological state of enhanced defensive capacity elevated by specific environmental stimuli, whereby the plant's innate defenses are potentiated against subsequent biotic challenges (Van Loon *et al*., 1998). Systemic acquired resistance (SAR) and induced systemic resistance (ISR) are two forms of induced resistance; in both SAR and ISR, plant defenses are preconditioned by prior infection or treatment that results in resistance (or tolerance) against subsequent challenge by a pathogen or parasite. This resistance is effective against a broad range of pathogens and parasites, including fungi, bacteria, viruses, nematodes, parasitic plants and even insect herbivores (Benhamou and Nicole, 1999; Hammaerschmidt and Kuc, 1995; Kessler and Baldwin, 2002; McDowell and Dangl, 2000; Sticher *et al.,* 1997; Van Loon *et al.,* 1998; Walling, 2000).

2.10. Management of plant health

Soil microbes offer largely unexplored potential to increase agricultural yields and productivity in a low-input manner. Soil biota provides a number of key ecological services to natural and agricultural ecosystems.

Increasingly, inoculation of soils with beneficial soil biota is being considered as a tool to enhance plant productivity and sustainability of agricultural ecosystems. In the development of sustainable crop production practices, the use of microbial inoculants as replacement for chemical fertilizers and pesticides is receiving attention (Chakraborty and Chakraborty, 2013).

Many of the microbes isolated and classifies as biocontrol agents (BCAs) can be considered facultative mutualists, because survival rarely depends on any specific host and disease suppression will vary depending on the prevailing environmental conditions. Further down the spectrum, commensalism is a symbiotic interaction between two living organisms, where one organism benefits and the other is neither harmed nor benefited. Most plant-associated microbes are assumed to be commensals with regards to the host plant, because their presence, individually or in total, rarely results in overtly positive or negative consequences to the plant. And, while their presence may present a variety of challenges to an infecting pathogen, an absence of measurable decrease in pathogen infection or disease severity is indicative of commensal interactions. Competitions within and between species results in decreased growth, activity and/or fecundity of the interacting organisms. Biocontrol can occur when non pathogens compete with pathogens for nutrients and around the host plant. Direct interactions benefit one population at the expense of another also affect our understanding of biological control. Significant biological control most generally arises from manipulating mutualisms between microbes and their plant hosts or from manipulating antagonisms between microbes and their plant hosts or from manipulating antagonisms between microbes and pathogens (Chakraborty *et al.,* 2012a). In most research to date, biocontrol agents are applied singly to combat a pathogen. Although the potential benefits in the application of a single biocontrol agent has been demonstrated in many studies, it may also partially account for the reported inconsistent performance, because a single biocontrol agent is not likely to be active in all kinds of soil environments and agricultural ecosystems (Raupach and Kloepper, 1998). This may have resulted in inadequate colonization, limits tolerance to changes in

environmental conditions and fluctuations in production of antifungal metabolites (Weller and Thomashow, 1994; Dowling and O'Gara, 1994). Several approaches have been used to overcome these problems, including combined application of two or more biocontrol strains to enhance the level and consistency in disease control (Pierson and Weller, 1994; Schisler *et al*., 1997; Raupach and Kloepper, 1998). Multiple strain mixture of microbial agents has been employed with some success against plant pathogens in previous studies. These include mixtures of fungi (Paulitz *et al.,* 1990; Budge *et al.,* 1995, Schisler *et al*., 1997), mixtures of bacteria (Pierson and Weller, 1994; Raupach and Kloepper, 1998), mixtures of yeasts (Janisiewicz, 1996), bacteria and fungi

(Duffy *et al.,* 1996; Leibinger *et al*., 1997), and bacteria and yeast (Janisiewicz and Bors, 1995). In addition to disease control, strain mixtures enhanced the plant growth interms of increased seedling emergence (Dunne *et al*., 1998), plant height (Raupach and Kloepper, 1998) and yield (Nanadakumar *et al*., 2001; Pierson and Weller, 1994; Duffy *et al*., 1996). Enhancing biocontrol activity by using mixtures of antagonist may have advantages: (i) it may broaden the spectrum of activity, (ii) it may enhance the efficacy and reliability of the biocontrol and more importantly (iii) it may allow the combination of various traits without employment of genetic engineering (Janisiewicz, 1996). Moreover the deigning of combination of strains and making use of multiple antifungal traits exhibited by then may prove to be advantageous by ensuring that at least one of the biocontrol mechanisms will be functional under the unpredictable field conditions faced by the released PGPR strains (Niranjan Raj *et al*., 2005).

2.10.1. Plant growth promoting rhizobacteria (PGPR)

Plant growth promoting rhizobacteria are the soil bacteria inhabiting around/ on the root surface and are directly or indirectly involved in promoting plant growth and development via production and secretion of various regulatory chemicals in the vicinity of rhizosphere. Generally, plant growth promoting rhizobacteria facilitate the plant growth directly by either assisting in resource acquisition (nitrogen, phosphorus and essential minerals) or modulating plant hormone levels, or indirectly by decreasing the inhibitory

effects of various pathogens on plant growth and development in the forms of biocontrol agents. Various studies have documented the increased health and productivity of different plant species by the application of plant growth promoting rhizobacteria under both normal and stressed conditions (Ahemad *et al.,* 2013). PGPR can enhance plant growth by a wide variety of mechanisms like phosphate solubilization, siderophore production, biological nitrogen fixation, rhizosphere engineering, production of 1-aminocyclopropane-1-carboxylate deaminase (ACC), quorum sensing (QS) signal interference and inhibition of biofilm formation, phytohormone production, exhibiting antifungal activity, production of volatile organic compounds (VOCs), induction of systemic resistance, promoting beneficial plant-microbe symbioses, interference with pathogen toxin production eta. (Bhattacharyya and Jha, 2012). The concept of PGPR has now been confined to the bacterial strains that can fulfill at least two of the three criteria such as aggressive colonization, plant growth stimulation and biocontrol (Vessey 2003). The bacterial genera such as *Agrobacterium, Arthrobacter, Azotobacter, Azospirillum, Bacillus, Burkholderia, Caulobacter, Chromobacterium, Erwinis, Flavobacterium, Micrococcus, Pseudomonas* and *Serratia* belongs to ePGPR (Gray and Smith 2005), i.e . The Extracellular Plant growth promoting rhizobacteria wherein these bacteria may reside in the rhizosphere, on the rhizoplane or in the spaces between the cells of the root cortex. On the other hand, the intracellular plant growth promoting rhizobacteria (iPGPR) isolates generally inside the specialized nodular structures of root cells. These include the endophytes (*Allorhizobium, Azorhizobium, Bradyrhizobium, Mesorhizobium* and *Rhizobium*) and Frankia species both of which can symbiotically fix atmospheric N_2 with the higher plants (Verma *et al.,* 2010).

The export oriented agricultural and horticultural crops depends on the export of residue free produce and has created a great potential and demand for the incorporation of biopesticides in crop protection. To ensure the sustained availability of biocontrol agent's mass production technique and formulation development protocols has to be standardized to increase the shelf life of the formulation. It facilitates the

industries to involve in commercial production of plant growth promoting rhizobacteria (PGPR). PGPR with wide scope for commercialization includes *Pseudomonas flurescens, P. putida, P. aeruginosa, Bacillus subtilis* and other *Bacillus* spp. The potential PGPR isolates are formulated using different organic and inorganic carriers either through solid or liquid fermentation technologies. They are delivered either through seed treatment, bio-priming, seedling dip, soil application, foliar spray, fruit spray, hive insert, sucker treatment and sett treatment. Application of PGPR formulations with strain mixtures perform better than individual strains for the management of pest and diseases of crop plants, in addition to plant growth promotion. Supplements of chitin in formulation increase the efficacy of antagonists (Nakkeeran *et al*., 2005).

Nandakumar *et al*., (2001) developed talc based strain mixture formulation of fluorescent pseudomonads. It was prepared by mixing equal volume of individual strains and blended with talc as per Vidhyasekaran and Muthamilan (1995). Talc based strain mixtures were effective against rice sheath blight and increased plant yield under field conditions than the applications of individual strains. Vidhyasekaran and Muthamilan (1995) stated that soil application of peat based formulation of *P. fluorescens* (pf1) at the rate of 2.5 kg of formulation mixed with 25 kg of well decomposed farm yard manure; in combination with seed treatment increased rhizosphere colonization of Pf1 and suppressed chickpea wilt caused by *Fusarium oxysporum* f. sp. *ciceris*. Bioformulations of saw dust, rice husk and tea waste of two different PGPR *Serratia marcescens* and *Bacillus megaterium* was applied to five different tea varieties TV-18, TV-23, TV-25, TV-26 and T/17/1/154 in experimental field as well as in nursery. Application of these bioformulations increase height, emergence of new leaves and branches in all five varieties though similar response was nor found in all the varieties (Chakraborty *et al*., 2012).

Seed and foliar application of talc based fluorescent pseudomonads reduced leaf spot and rust of groundnut under field conditions (Meena *et al*., 2002). Delivering of rhizobacteria through combined application of different delivery systems will increase the population load of rhizobacteria and thereby suppress the pathogenic propagules.

Considering the good impact of PGPR in terms of biofertilization, biocontrol, and bioremediation, all of which exert a positive influence on crop productivity and ecosystem functioning, encouragement should be given to its implementation in agriculture. Hoping for the betterment of technology in developing successful research and development, PGPR use will surely become a reality and will be instrumental to crucial processes that ensure the stability and productivity of agro-ecosystems, thus leading us towards an ideal agricultural system.

2.10.2. Plant growth promoting fungus

As ubiquitous and often predominant components of the mycoflora in native and agricultural soils throughout all climatic zones, *Trichoderma* species play an important role in ecosystem health (Klein and Eveleigh, 1998). *Trichoderma* spp., are free-living fungi in soil and rhizosphere. They release a variety of compounds that induce localized or systemic resistance responses in plants. *Trichoderma* strains have long been recognized as biological agents, for the control of plant disease besides for their ability to their role in plant growth. *Trichoderma* is used extensively for post-harvest disease control as has been used successfully against *Fusarium, Phytopthara, Scelerotium and* other pathogen. *Trichoderma* strains are known to induce resistance in plants through ethylene production, hypersensitive responses and other defence related reactions in plant cultivates. Introduction of endochitinase gene from *Trichoderma* into plants such as tobacco and potato plants have increased their resistance to fungal growth. Selected transgenic lines are highly tolerant to foliar pathogens such as *Alternaria alternata, A. solani*, *Botrytis cirerea* and *Rhizoctonia* spp. as well as to the soil-borne pathogen.

Trichoderma strains have the ability to degrade a wide range of insecticides: fungicides, weedicides etc. *Trichoderma* spp are under intensive research because of their abundant natural occurrence, biocontrol potential against fungal and nematode diseases as well as host defense inducing ability and capable of promoting growth of certain crops (Haraman and Kubicek, 1998). The annual requirement of *Trichoderma* has been estimated as 5,000 tones to cover 50 per cent area in India (Jeyarajan, 2006).These fungi not only protect plants by killing other fungi and certain nematodes but induce

resistance against plant pathogens, impart abiotic stress tolerance, improve plant growth and vigor, solubilise plant nutrients, and bioremediate heavy metals and environmental pollutants (Hermosa *et al.,* 2012, Lorito *et al.*, 2010, Mastouri *et al.,* 2012, Shoresh *et al.,* 2010). In addition, this genus comprises fungi that produce secondary metabolites of clinical significance and enzymes with widespread industrial application. As *Trichoderma* has had a major impact on human welfare, recent genome sequencing projects have targeted seven species: *Trichoderma reesei*, *Trichoderma virens*, *Trichoderma atroviride*, *Trichoderma harzianum*, *Trichoderma asperellum*, *Trichoderm alongibrachiatum*, and *Trichoderma citrinoviride.* The genome sequencing of *Trichoderma* species has stimulated the development of systems biological approaches, initiated and enhanced whole-genome expression studies, and provided unique data for phylogenetic and bioinformatic analyses toward understanding the roles of these opportunists in ecosystems.

2.10.3. Arbuscular mycorrhizal fungi

Biological control of plant pathogens is the key practice in sustainable agriculture because it is based on the management of a natural resource. Use of Arbuscular mycorrhizal (AM) fungi have reduced damage caused by soil-borne plant pathogens. However some AM isolates appear to be more effective. The degree of protection varies with the pathogen involved and can be modified by soil and other environmental conditions. This prophylactic ability of AM fungi could be exploited in cooperation with other rhizospheric microbial antagonists to improve plant growth and health. Further research is needed for a better understanding of both the eco-physiological parameters contributing to effectiveness and of the mechanisms involved. However the improvement of plant nutrition, compensation for pathogen damage and competition for photosynthates or colonization/ infection sites have been claimed to play a protective role in the AM symbiosis (Azcon and Barea 1997).

The role of VAM fungi in the improvement of crop plant is well recognized as reported by (Krishna and Bagyaraj, 1982; Katiyar *et al.,* 1994, Rao *et al.,* 1995). According to them VAM fungi are known to improve the nutrient status of the plants, increase growth and development protects plant against pathogen and gives fight to

drought and salinity. Colonization by native plant has been reported earlier by Maiti *et al*.(1995). Partial dependency of upland rice on native AMF for Phosphorous acquisition has also been reported by earlier worker such as (Saha *et al.,* 1999). The occurrence of VAM fungi at altering stages of growth of rice plants has been studied by (Dubey *et al*., 2008). In recent years, the application of artificially produced inoculum of VAM fungi has increased its significance in the field of agriculture, horticulture and forestry.