2.1. Wheat

2.1.1. Origin and History

Wheat is very important crop for mankind; it is the most widely cultivated crop in the world today. It plays a vital role in the human diet and has higher protein, fat and fibre content, compared to other grains. It is also rich in vitamins and minerals such as manganese, phosphorus, potassium, zinc, vitamin B₆, folate, thiamin and riboflavin. Therefore, for thousands of years, wheat has been one of the most prominent food sources for human and livestock. Wheat flour is used to make a wide variety of foods such as bread, biscuits, cookies, cakes, breakfast cereal, pasta, noodles (McMullen et al., 1997). Using fermentation of wheat, beer, other alcoholic beverages, and biofuels are produced.

Wheat is believed to have originated in southwestern Asia. It was first cultivated about 10000 years ago, as a part of the “Neolithic Revolution”. Some of the earliest remains of wheat have been found in Syria, Jordan, and Turkey (Curtis 2002; Shewry 2009). Primitive relatives of present day wheat have been discovered in the Fertile Crescent, which date back 9,000 years. Other archeological findings show that bread wheat was grown in the Nile Valley around 5,000 B.C. as well as in India, China, and even England at about the same time (Curtis 2002; Shewry 2009). Through historical inscriptions it has been affirmed that wheat is the earliest field crop used for human food processing. Triticum monococcum (Einkorn) and Triticum dicoccum (emmer) were the earliest found wheat varieties, grown 12,000 – 17,000 years ago in the Near East (Curtis 2002; Gustafson et al., 2009; Shewry 2009).

Wheat is an ancient crop in the Indian sub-continent. Artha-Veda written in 1500 to 500 BC refers to wheat and in Rig Veda existence of two types of wheat varieties has been mentioned. The earliest record of wheat cultivation in India comes from the excavations carried out in Mohenjo-Daro (now in Pakistan), which revealed that the sub species T. sphaerococcum of Triticum aestivum was cultivated by farmers during that period (about 2000 BC). Despite a long history of wheat...
cultivation in the Indian sub-continent, its yield/unit area had been very low. The wheat production was 10-11 million tons in 1965. Situations however, have changed considerably due to introduction of new Mexican dwarf high-yielding varieties, which require better field management, high input of fertilizers and irrigations and is responsible for ‘Green Revolution’ in the country in mid 1960s’ and 70s’. Since the initiation of the Green Revolution in the mid-sixties, India achieved remarkable increase in production and productivity of wheat. As a result of consistent efforts during the last 45 years, India recorded an all-time high wheat production of 95.91 million tons from 30.34 m ha area during 2013-2014 (DES 2014) and maintained its second position among wheat producing nations next only to China.

Wheat is grown in more than 70 countries on 5 continents (Dixon 2007) and cover more land area worldwide than any other commercially cultivated crop (Baenziger et al., 2009; IGC 2009; FAOSTAT 2011) with a global production of 690 million metric tons in 2008 (According to the FAO statistics, http://faostat.fao.org). China, India, United States, Russian Federation, Kazakhstan and Canada are the other top wheat producers in the world (Curtis 2002).

2.1.2. Classification and Types of wheat

Wheat is classified under the genus Triticum, tribe Triticinae, family Gramineae, order Poales, class Liliopsidia, division Magnoliophyta and Kingdom Plantae. There are three types of Triticum species based on ploidy level; diploid (14 chromosomes), tetraploid (28 chromosomes) and hexaploid (42 chromosomes) (Gooding and Davies 1997; Gustafson et al., 2009). Wheat has three different sets of chromosomes; A, B and D. Diploid wheat contains AA, tetraploid and hexaploid wheat contains AABB and AABBDD chromosomes, respectively (Curtis 2002; Gustafson et al., 2009; Shewry 2009). Wheat is mainly divided into two types based on the growing season, spring wheat and winter wheat. This classification is not solely based on the seeding time and harvesting time. Winter wheat sown primarily in the fall requires vernalization to flower, and is tolerant to freezing temperatures while spring wheat is sown mainly in the spring and summer months (Baenziger et al., 2009). Winter wheat usually has higher yield and lower protein content than spring wheat (Curtis 2002). There are 11 types of wheat in the world, (1) Hard red
spring wheat, (2) Hard white spring wheat, (3) Soft red spring wheat, (4) Soft white spring wheat, (5) Hard red winter wheat, (6) Hard white winter wheat, (7) Soft red winter wheat, (8) Soft white winter wheat, (9) Compactum (*T. compactum*), (10) Spelta (*T. spelta*), and (11) Durum wheat (*T. durum*). The two most important commercial wheat types are common or bread wheat (*Triticum aestivum* L. $2n = 6x = 42$) and durum wheat (*Triticum durum* L. $2n = 4x = 28$).

In India, three wheat species namely *Triticum aestivum* L. (Bread wheat or common wheat), *T. durum* L. (Kathia or Macaroni wheat) and *T. dicoccum* ($2n = 2x = 14$, Khapli or Emmer wheat) are commercially cultivated in different parts of the country, are of spring type but cultivated during winter season. Bread wheat accounts for 95% to total Indian wheat production and more than 90% of global wheat production and is grown on a substantial scale (over 100,000 ha) in more than 70 countries on 5 continents (Shewry 2009). The main products include a great variety of leavened, unleavened and steamed breads, noodles, cookies, cakes, and breakfast cereals. Durum wheat (*Triticum turgidum* L.) accounts for approximately 4% to total Indian wheat production and around 30 million tons, or 5%, of global wheat production. Durum wheat is used mostly to produce semolina, pasta, and cracked wheat products. Dicoccum wheat [*T. dicoccum* (Schrank.) Suhulb] commonly known as Khapli or Emmer wheat is traditionally cultivated in Karnataka, Southern Maharashtra, Sourashtra region of coastal Gujarat, parts of Tamil Nadu and Andhra Pradesh in an area of 0.15 million hectare with approximate production of 0.35 million tons. Looking to the adaptive importance of Emmer wheat species and economic importance in producing value added products of export quality, it suggested that older obsolete forms of wheat, such as *T. dicoccum*, can themselves be promoted once again as cultivars and hence play a broader and more significant role in improving quality than being used merely to correct shortcomings of otherwise well-adapted modern varieties.

### 2.2.1. *Fusarium* head blight (FHB)

*Fusarium* head blight (FHB) is without doubt among the most important diseases of wheat (Goswami and Kistler 2004). The effects of FHB go beyond yield and kernel quality reductions, as trichothecene mycotoxins produced during infection contaminate raw grain and processed wheat products (Edwards *et al.*, 2009), placing human and livestock health at risk. Wheat is susceptible to infection
from the flowering (anthesis) stage up through the soft dough stage of kernel development. Infection at these growth stages is favoured by prolonged wet weather and high humidity. All or portions of infected wheat heads prematurely whiten. Yield losses occur from failed kernel development or because infected kernels are shriveled, discolored, and light in test weight. Market price is severely reduced when grain has low test weight and contains damaged kernels (scabby kernels are classed as damaged in U.S. Grain Grades) and contains fungal toxins (mycotoxins). Food grade grain may be reduced to feed grade because of this disease, or the grain may have no food or feed value at all. The predominant toxin associated with FHB infections is deoxynivalenol (DON) (Gale 2003). This mycotoxin causes feed refusal or poor weight gain in animals and may cause immunological and teratogenic problems in humans (Desjardins 2006). The U.S. Food and Drug Administration (2010) has established guidelines for DON levels in human and animal feed, but many food and beverage industries have self-imposed even greater restrictions.

2.2.2. History and Epidemics

_Fusarium_ head blight was first described as wheat scab by W.G. Smith in 1884 in England (Stack 2003). He identified _Fusarium culmorum_ as a causal agent of wheat scab. Later, Attanasoff (1920) used the term “_Fusarium_ head blight” for this disease. Epidemics of FHB disease are sporadic (Fernando et al., 2000). Historically, several epidemics of the disease have occurred worldwide. Some important outbreaks have since been reported in the America (Moschini and Fortugno 1996; Gilbert and Tekauz 2000; McMullen 2003), Eurasia (Parry et al., 1995; McCormick 2003), Australia (Burgess et al., 1987; Akinsanmi et al., 2004), South Africa (Scott et al., 1988; Kriel and Pretorius 2008) and Asia (Zhang et al., 2013). Epidemics of FHB are strongly influenced by local and regional environment, host factors such as physiological state and genetic make-up, and pathogen factors including adaptation and virulence. In the U.S., the disease has become a persistent problem since the 19th century for wheat growers. In the U.S., FHB was first reported in 1890 in the state of Ohio. In 1917 an outbreak of FHB occurred in the U.S., mainly in Ohio, Indiana, and Illinois, causing losses of over 288,000 metric tons of wheat (Attanasoff 1920). One of the most severe epidemics occurred in 1919 and caused a loss of 2.18 million metric tons of wheat. FHB outbreaks continued during the 1920s in the U.S. During these years, wheat fields in Minnesota and
Missouri were affected seriously by FHB. In 1928, wheat was affected by FHB epidemics in Indiana causing 15-20% losses (Dickinson and Mains 1929). FHB continued to occur in North America in the 1940s, 1950s, 1960s and 1970s. Major economic losses occurred again in the 1980s. Two hundred seventy two million metric tons of wheat was lost in 1982 alone (Bai et al., 2002; Lee et al., 2002). FHB epidemics in the 1990s were very important in the history of FHB in the U.S. According to McMullen et al. (1997) scab epidemics in 1993 caused huge losses in the tri-state area of Minnesota, North Dakota and South Dakota, and in the Canadian prairie province of Manitoba. Losses were estimated to be $1 billion. In 1996, scab occurred in almost all states of the U.S. Johnson et al. (2003) estimated that FHB caused direct losses in wheat and barley totaling more than $1.3 billion in the U.S. during the period from 1991 to 1997 (Stack 2003). In subsequent years, losses resulting from FHB have continued to cause major economic problems in one or more wheat classes in most years, but some years are clearly more problematic than others. Nganje et al. (2004) conducted an economic analysis of losses attributable to FHB for the period 1993 to 2001, for nine states in the northern Great Plains and central United States and determined that the cumulative direct economic loss attributable to FHB in wheat and barley for the entire period was US $2.491 billion, with $1.074 billion (43.1%) of the total being lost between 1998 and 2001. Annual losses varied greatly: total direct production losses were 18.7, 15.1, and 12.6% in 1998, 1995, and 1993, respectively. Losses were much lower in 1996 (6.8%), 2000 (6.4%), and 2001 (7.7%). During 2007 and 2008, FHB was a minor problem across most of the United States (Focus, 2007; Deja vu 2008); however, serious disease outbreaks occurred both years in parts of Nebraska and Kansas. In 2009, FHB was epidemic in parts of several mid-south and southeastern states (USWBSI 2009; Van Sanford 2009). In South Africa, a double cropping system (with maize as a summer crop and wheat as a winter crop) in combination with conservation tillage has led to a growing FHB problem, especially in regions where irrigation is required (Kriel and Pretorius 2008).

In the developing world, FHB has been a problem in China in some provinces and in some years since 1936, when the first serious outbreak occurred (Chen et al., 2000). Since 1985, epidemic outbreaks have become more frequent and widespread, though some areas are more prone to epidemics than others. The
provinces suffering most from this devastating disease are located along the Yangtze River in eastern and central China (Wang 1997; Chen et al., 2000). From 1951 to 1990, wheat farmers were burdened with seven severe epidemics (exceeding 40% yield losses) and 14 moderate epidemics (10-20% yield losses). During the past ten years, epidemic outbreaks have become more frequent and severe in China. The recent epidemics in 2003, 2008, 2010 and 2012 made wheat a less attractive crop for growers, resulting in a sharp decrease of wheat growing acreage (Zhang et al., 2010; Zhang et al., 2013). The disease frequently occurs in wheat growing areas in the middle and lower valleys of the Yangtze River and the mountainous areas in southwest China (Zhang et al., 2010). During recent years, changed agronomy, trade and shipment of cereal commodities, and global climate change have aggravated the spread and severity of FHB northward in China. A reduced frequency of severe epidemics has subsequently been observed with the introduction of moderately resistant cultivars (Hongxiang et al., 2008). In Bangladesh, FHB is found sporadically but conditions favourable for disease make situation problematic (Duveiller 2004). Rice-wheat rotations are routine in many Asian countries including India, and while FHB is more endemic in the latter, rice crops still serve as a host for inoculum buildup that could lead to an outbreak.

In India, FHB was first reported by Roy (1974) on such varieties as Sonalika, Kalyan Sona, Safed Lerma and Lerma Rojo in Siang District of Arunachal Pradesh. Disease was also reported during Kharif 1984, on high yielding wheat varieties in Nilgiris (Brahma and Singh 1985) and in Punjab (Singh and Aujla 1994; Saharan et al., 2004). In Punjab, FHB sometime becomes an important constraint to wheat production (Singh and Aujla 1994). In April-May, 1990, a severe outbreak of this disease was observed in some affected areas in Amritsar and Gurdaspur districts of Punjab and variety HD 2329 was badly affected. During 2005, frequent rainfall during flowering favoured disease development and caused enormous losses in yield and quality in Gurdaspur district. During a survey conducted on March, 2005 in the North Western Plain Zone, moderate to high incidence of FHB was observed in the bread wheat cv. PBW 343 (10-50 % infected heads) and durum wheat cv. PDW 274 (>90 % infected heads) in the Gurdaspur district of Punjab (Saharan et al., 2007). If the soil-borne inocula were to increase, this would threaten the already dwindling
food supply of wheat and other grains. This concern also emphasizes the need to improve FHB-management strategies.

2.2.3. Economic importance

FHB can significantly reduce grain yields, in some cases up to 100% yield loss on susceptible cultivars when conditions are favourable for disease (Bai and Shaner 1994). Substantial yield loss can result from shriveled or “tombstone” kernels being removed by the combine. When tombstone kernels are not removed they can significantly reduce test weight (Bai and Shaner 1994), but currently the major concern regarding FHB arises from the ability of the majority of species to produce mycotoxins, which accumulate in the grain of wheat spikes that become infected during or post-anthesis (Del Ponte et al., 2007), although significant yield losses are more relevant in early infections (during anthesis to the early stages of kernel development) (Bushnell et al., 2003; Steffenson 2003). In the mid 1990s, Fusarium epidemics caused large losses in wheat production in the US and Canada. Economic damage was estimated at US $2500 million in wheat and US $400 million in barley in the US alone and US $220 million and US $300 million in Canada for wheat and barley respectively (Windels 2000). The epidemics in the US were primarily caused by F. graminearum, which has been shown to be a complex in its own right, with seven or even eight lineages with different geographic and phytopathological characteristics (O'Donnell et al., 2000). In China, FHB epidemics can cause great, direct yield losses. During severe epidemics, the percentage of scabbed spikes is usually 30% to 50%, but can exceed 60% to 70% in the most susceptible cultivars during the most severe epidemics (Zhang 1998; Shi and Wang 1999). The outbreak of FHB during 1996 resulted in a yield loss of about 78,000 metric tons (Li 1996) in Sichuan Province. In China, cereals are mostly grown by small-scale farming and FHB can have a dramatic impact on local communities. The kernels that develop from FHB-infected spikes are usually contaminated with trichotheccenes, deoxynivalenol (DON) being the most prevalent. Trichotheccene-contaminated grain is readily distinguished from its shriveled and discolored appearance. Several studies have shown that the percentage of Fusarium Damaged Kernels (FDK) serves as a reliable estimate of DON content (Miedaner et al., 2001; Mesterhazy 2002). This allows for rapid visual screening of FDK in order to prevent contaminated grain entering the food chain in unsafe quantities. Nevertheless, FDK
screening for toxin contamination should be used with caution since reduced physical damage is observed if infection occurs past the soft dough stages of kernel development (Pestka and Smolinski 2005; Del Ponte et al., 2007). FDK can therefore be used as a preliminary screen when sorting and grading grain, but quantitative trichothecene testing is advisable before the grain enters the food chain. Maximum FDK limits in place for different Canadian wheat classes and varieties are enforced by the Canadian Grain Commission at licensed grain elevators. The established DON limits are also being reviewed, and more rigid standards may be imposed in order to harmonize the Canadian standards with those of the European Union. DON tolerance in China, Hungary, Russia, Switzerland, and the United States is 1 ppm; and in Austria, Germany, and the Netherlands is 0.5 ppm (Pestka and Smolinski 2005). Standards are also in place for grain that is used in animal feed, since trichothecenes cause similar ailments in farm animals as in humans (Rotter et al., 1996). Ingestion of contaminated grain can cause severe intestinal irritation in mammals, resulting in feed refusal in livestock (Eriksen and Pettersson 2004), and can lead to a potentially fatal condition in humans and other mammals known as alimentary toxic aleukia (ATA). In addition to gastrointestinal irritation, symptoms of ATA can include ataxia, dyspnea, and subcutaneous hemorrhaging (Lutsky et al., 1978). Reports from all continents demonstrate the reemergence of this devastating disease and its high economic impact.

2.2.4. Causal organism

_Fusarium_ spp. are considered to be one of the most economically important fungi causing disease in most genera of cultivated plants (Parry et al., 1995). It has been reported that at least one _Fusarium_- associated disease is found on many plants (Leslie and Summerell 2006). Three of the world’s most important crops, wheat, rice and maize, are susceptible to a number of _Fusarium_ species. _Fusarium_ head blight in wheat, Ear rot in corn, and ‘Bakane’ disease in rice are the common diseases caused by _Fusarium_ spp. in cultivated lands (Parry et al., 1995). The group of _Fusarium_ spp. is well adapted to saprophytic growth and survival. In the field of plant pathology, this group of fungi is mostly known by their anamorph name.

_Fusarium_ spp. are believed to belong to an ancient fungal group that appears in the early stages in the evolution of Ascomycetes (Backhouse et al., 2001).
Fusarium spp. have been found worldwide including the areas from sub-arctic to above the Arctic Circle. Fusarium belongs to anamorphic Hypocreaceous Ascomycetes (Ascomycota: Hypocreales: Hypocreaceae) in the genera Gibberella and Nectria (Liddell 2003). Most of these fungi are saprophytes that can colonize the living host at any stage during the life cycle of the host.

Fusarium head blight (FHB) is a very devastating disease of cereal crops worldwide and many Fusarium species are associated with the disease (Parry et al., 1995; Liddell 2003; Qu et al., 2008), they fall into four biologically distinct sections; Discolor, Roseum, Gibbosum and Sporotrichiella (Liddell 2003). But, Fusarium graminearum Schwabe (teleomorph Gibberella zeae [Schwein] Petch) is the principal pathogen responsible for head blight in many countries including India (O’Donnell et al., 2000; Saharan et al., 2003; Zeller et al., 2004). Fusarium graminearum is classified under the section Discolor. The fungi belonging to section Discolor are found to produce vivid carmine-red mycelium on high carbon sources, and the classic banana shaped macroconidia. The production of microconidia is not found among the fungi belonging to this section (Booth 1971; Liddell 2003). Fusarium graminearum is divided into to two groups; Group 1 is responsible for causing crown and root diseases in wheat and generally do not produce perithecia in culture. Group 2 which readily produce perithecia in culture is associated with head blight (Bushnell et al., 2003; Liddell 2003). Later, Group 1 has been re-classified as a distinct species, Fusarium pseudograminearum (teleomorph: Gibberella coronicola (Aoki and O’Donnell 1999a, 1999b). In a recent study, F. pseudograminearum was also found associated with FHB in China (Xu et al., 2015). Other related species such as F. culmorum (Smith) Sacc., F. avenaceum (Fries) Sacc., F. acuminatum Ellis & Everhart, F. verticillioides (Sacc.) Nirenberg (syn. F. moniliforme J. Sheld), F. crookwellense Burgess, Nelson & Toussoun, F. equiseti (Corda) Sacc., F. sporotrichioides Sherb., F. oxysporum Schlect., F. poae (Peck) Wollenw and Microdochium nivale (Fries) Samuel & Hallett may contribute to the head blight complex but are generally less important than F. graminearum (Wiese 1987; Clear and Patrick 2000; Fernandez et al., 2001; Gilbert et al., 2001; Xue et al., 2002). F. sacchari is the cause of an important disease of sugar cane, pokkah boeng (Leslie and Summerell 2006), and has been reported to produce the mycotoxin beauvericin, which causes toxicosis in human and other animals (Moretti et al.,
2007). This species was also found associated with FHB in wheat in China (Wang et al., 2015).

In India, several Fusarium spp. were found associated with FHB like *F. graminearum*, *F. avenaceum*, *Microdochium nivale*, *F. compactum*, *F. verticillioides*, *F. subglutinans*, *F. oxysporum* and *F. pallidoroseum (semitectum)* (Roy 1974; Brahma and Singh 1985; Singh and Aujla 1994; Kaur et al., 1999; Mann and Nanda 1999; and Saharan et al., 2003) but *F. graminearum* is more dominant than others.

### 2.2.5. Pathogen distribution

*Fusarium* spp. are widely distributed across all geographic regions, including soils, plants and air. Representatives of *Fusarium* occur in all major geographic regions of the world (Burgess 1981). Individual species may have a cosmopolitan geographic distribution or be limited to one or a few crops, climates, or ecological zones (Nelson et al., 1983; Burgess and Summerell 1992; Burgess et al., 1994). *Fusarium* species recovered from both natural and agricultural ecosystems have distinct climatic preferences (Saremi et al., 1999; Backhouse et al., 2001). The climate, and even local variations in weather, can limit the range of species observed even if several are present, and influence their relative frequency of recovery (Vigier et al., 1997; Stewart et al., 1998; Rossi et al., 2002; de Wolf et al., 2003; Moschini et al., 2004). In broad terms there are species that prefer tropical climates, hot arid climates or temperate climates, and a fourth group that has a cosmopolitan range.

Incidence and severity of FHB and the composition of *Fusarium* species involved are reported to vary among geographical regions (Doohan et al., 1998; Xu et al., 2005) and years (large-scale variability) due to variation in climatic and weather conditions and cropping practices (Schaafsma and Hooker 2007; Klix et al., 2008; Xu et al., 2008).

Since the FHB was initially recorded on wheat, barley and other small grains, 17 different *Fusarium* species have been associated with the disease. *Fusarium graminearum* is the species that predominates internationally, followed by *F. culmorum* (W.G. Smith) Saccardo and *F. avenaceum* (Fries) Saccardo (*G. avenacea* Cook) (Parry et al., 1995; Ruckenbauer et al., 2001).
F. graminearum, is considered as a single, panmictic species worldwide, at a more refined level, F. graminearum is in itself a species complex of phylogenetically distinct lineages (O’Donnell et al., 2000, 2004, 2008, Starkey et al., 2007). F. graminearum taxonomy divided isolates of F. graminearum from around the world into nine distinct phylogenetic lineages, each with strong geographic associations (O’Donnell et al., 2004), with nine phylogenetic species proposed, based on concordance of single nucleotide polymorphisms in the mating type locus and seven other genes. Two further phylogenetic species were introduced subsequently (Starkey et al., 2007). All of these species appear to have reached an advanced state of genetic isolation because each was reciprocally monophyletic within six individual nuclear gene genealogies and the combined phylogeny of these genes (O’Donnell et al., 2004). These species include, Fusarium austroamericanum (lineage 1), distributed in South America; F. meridionale (lineage 2) frequently found in South and Central America, South Africa, New Caledonia, Nepal and Korea; F. boothii (lineage 3) distributed in South Africa, Mesoamerica, Nepal, Guatemala and Korea; F. mesoamericanum (lineage 4), is found in Central America and Pennsylvania; F. acacia-mearnsii (lineage 5), is found in Australia and South Africa; F. asiaticum (lineage 6) distributed in Asia (China, Nepal, and Japan), F. graminearum (lineage 7) found worldwide; F. cortaderiae (lineage 8) and F. brasiliicum (not given a lineage designation), found in South America and Oceania (O’Donnell et al., 2004; Starkey et al., 2007). This study was further expanded by Starkey et al. (2007) including two more genes, elongation factor-1α (EF-1α) and reductase (RED) totalling 13 genes and found two new Fusarium species. The two new Fusarium species were F. vorosii collected in Hungary and F. geralachii in upper Midwest of USA. O’Donnell et al. (2008) identified another new F. graminearum clade species from Ethiopia, F. aethiopicum (no lineage number). Yli-Mattila et al. (2009) also identified another new species from the Russian Far East and designated as F. ussuriianum. In addition, a putative new species within the Fg complex was identified in Nepal by Chandler et al. (2003) and later characterized by Desjardins and Proctor (2011) as the “Nepal lineage”.

A biogeographic hypothesis suggests that the basal most species within the F. graminearum complex may be endemic to the southern hemisphere while the derived species evolved within the northern hemisphere (Starkey et al., 2007).
According to the surveys to date, *F. austroamericanum*, *F. meridionale*, *F. cortaderiae*, and *F. brasilicum* appear to be endemic to South America (Starkey et al., 2007; Sampietro et al., 2011); *F. acaciae-mearnsii* to Australia or less likely Africa (O’Donnell et al., 2004); *F. asiaticum*, *F. vorosii* and *F. ussurianum* to Asia (O’Donnell et al., 2004); *F. aethiopicum* to Africa (O’Donnell et al., 2008); *F. boothii* and *F. mesoamericanum* to Central America (O’Donnell et al., 2004, 2008); *F. gerlachii* to the US (Starkey et al., 2007).

In the surveys conducted worldwide to date, *F. graminearum sensu stricto* (*F. graminearum s.s.*) is cosmopolitan in distribution and has been found in Asia, Africa, America, Europe, and Oceania, while another species, *F. asiaticum*, is widespread in Asia (O’Donnell et al., 2000, 2004; Laday et al., 2004, Ramirez et al., 2007; Lee et al., 2009; Desjardins and Proctor 2011). FHB was initially reported in 1936 in China, and since then FHB epidemics have become more severe and frequent in the middle and lower regions of the Yangtze River, and in Heilongjiang province in northeastern China (Chen et al., 2000). More recently FHB frequently occurs in areas along the Yangtze River, a region with conditions favorable for infection by the pathogen. *F. asiaticum* is the predominant species in this region (Gale et al., 2002; Zhang et al., 2007; Qu et al., 2008a, 2008b; Yang et al., 2008; Zhang et al., 2010). Based on the climate data of 30 years during 1970–1999, the geographical distribution patterns of *F. graminearum s.s.* and *F. asiaticum* in China were assayed and indicated that *F. graminearum s.s.* was mainly obtained from cooler regions where the annual average temperature was 15 °C or lower. In contrast, 95% of *F. asiaticum* isolates were collected from warmer regions (Zhang et al., 2007; Qu et al., 2008b; Wang et al., 2011). In Japan, *F. graminearum s.s.* and *F. asiaticum* are the predominant species, with *F. asiaticum* predominating in the southern regions as in China (Suga et al., 2008). A preliminary report of the *F. graminearum* clade isolated from maize in Korea indicated that *F. graminearum s.s.* was dominant, accounting for 74% of strains, while three additional species, *F. meridionale*, *F. boothii*, and *F. asiaticum*, were also present at low frequencies (Jeon et al., 2003). However, *F. asiaticum*, *F. meridionale* and *F. boothii* were the major causal agents of *Gibberella* ear rot of maize in Nepal, whereas *F. graminearum s.s.*, which dominates in maize elsewhere in Asia and worldwide, was not detected (Desjardins and Proctor 2011). Similarly, *F. meridionale* and *F. boothii* play a
substantial role in the infection and trichothecene contamination of maize in Argentina. *F. graminearum* s.s. was not represented among the isolates examined (Sampietro *et al*., 2011). In contrast, a previous survey of the *Fg* complex collected from wheat in the center of Argentina identified all 113 isolates as *F. graminearum* s.s. (Ramirez *et al*., 2007). In Korea, 80% of the isolates collected from rice belonged to *F. asiaticum*, which is dramatically different from the results on maize (Lee *et al*., 2009). Although the underlying factors for species distribution are unknown, the occurrence of *F. asiaticum* in Louisiana closely overlaps with rice-growing areas in Louisiana (Gale *et al*., 2011). These results suggest ecological factors may have had a significant effect on the distribution of these species (Ji 2007; Qu *et al*., 2008a, 2008b; Lee *et al*., 2009; Desjardins and Proctor 2011; Sampietro *et al*., 2011).

### 2.2.6. Morphology and Identification

The identification and system of classification of *Fusarium* species are very complex. For more than half a century, a problem commonly discussed in *Fusarium* species is their high morphological variability in culture (Burgess *et al*., 1994). Traditional diagnostic methods for detection and identification of *Fusarium* species are based only on morphological characteristics observed on selective media under specific incubation conditions (Burgess *et al*., 1994). These morphological characteristics are still the most important criteria to identify *Fusarium* into species (Leslie *et al*., 2001). A high degree of variability in morphology and physiological characteristics enable some species to distinct from each other. The morphological characteristics that are used to identify *Fusarium* spp. are described in reviews and *Fusarium* identification manuals (Nelson *et al*., 1994; Leslie and Summerell 2006).

Unfortunately, with *Fusarium* as with many microfungi, the morphological characters are limited in number, are probably subjected to selection, and their expression is sensitive to environmental conditions. To add further confusion these morphological characters usually are subtle, easily misinterpreted, and can vary in importance depending upon the species.

Some physical and physiological characters have been used as morphological characters to distinguish *Fusarium* species. Differences in the shape of the macroconidia are central to the identification of many *Fusarium* species, although
other characters also are used, and in some cases are critical to distinguish sister species. In many cases the morphology of this spore alone is sufficient to identify a culture to species. Macroconidia are identified as multiseptate, moon crest or banana-shaped spores produced inside a sporodochium. Normally there are three shapes of macroconidia i.e. straight or needle like, dorsiventral curvature, and dorsal curvature. The species determination within the *Fusarium* genus is based on the shapes of the end, apical and basal cells of the macroconidia. The apical cells basically have four shapes i.e blunt, papillate, hooked and tapering whereas basal cells also have four shapes i.e foot-shaped, elongated foot shaped, distinctly notched and barely notched (Leslie and Summerell 2006). Other spores, *e.g.*, microconidia and chlamydospores, also are important in morphological species definitions. Microconidia are not produced by all *Fusarium* species, so their presence alone is an important character. The microconidia themselves, the conidiogenous cell on which they are borne, and the arrangement of the microconidia on and around the conidiogenous cell all are important and potentially diagnostic characters. Microconidia are produced from aerial mycelia and not from sporodochia. They are of various shapes, i.e oval, reniform, obovoid, napiform, globose and fusiform (Leslie and Summerell 2006). Chlamydospores are also an important character in many *Fusarium* species descriptions. They are not well conserved evolutionarily, however, and species that produce chlamydospores may be very closely related to those that do not. Chlamydospores may be formed singly, doubly, in clumps and in chains. They often take a long time (6+ weeks) to produce, and may not be produced in large numbers. Chlamydospores may be found in the aerial mycelia or embedded in the agar, and the location often is important in species identification.

A number of secondary and physiological characters also are used in the identification of *Fusarium* species. The most prominent of these secondary characters is pigmentation. Culture conditions and media are critical for the production of comparable pigments. Growth rate is another commonly used secondary character. There can be some variation in this trait. Traditionally measurements are made on PDA plates inoculated with single spores and grown for three days at either 25° or 30°C. The colony diameter is measured. In most cases the differences are subtle and the differences generated are not always clear cut. There are some species that grow considerably slower (or faster) than others, however, and
such slow (fast) growing species and the conditions resulting in their slow (fast) growth are noted in the individual species descriptions. At present, growth rates, most commonly at 25° and 30°C, sometimes are used by some researchers to separate closely related species.

On the basis of fungal colony characteristics, spore size, spore shape and number of septa, six *Fusarium* spp. namely, *F. graminearum*, *F. oxysporum*, *F. verticillioides*, *F. equiseti*, *F. semitectum* and *F. solani* were found associated with the head scab disease. Saharan *et al.* (2002) observed significant differences in colour and radial mycelial growth of *Fusarium* species on Potato Dextrose Agar media after 3, 6 and 9 days of incubation. After 6 days of incubation, mycelial growth of *F. oxysporum* isolate collected from Katrain, Himachal Pradesh was significantly higher as compared to other *Fusarium* spp./isolates except Theog isolate of *F. oxysporum*.

O’Donnell *et al.* (2004) determined nine phylogenetically distinct species within *F. graminearum* (group 2) with minor differences in macroconidial morphology but morphologically indistinguishable. These minor differences in macroconidial morphology could be important to distinguish within species.

### 2.2.7. Pathogenic variation (Aggressiveness)

No evidence has been found for the occurrence of races within *Fusarium* pathogenic to wheat (Snijders and van Eeuwijk 1991) but the variation in pathogenicity of *Fusarium* spp. on wheat was reported previously (Stack and McMullen 1985; Wong *et al*., 1995; Stack *et al*., 1997). Several investigations showed that, *F. graminearum* and *F. culmorum* were the most aggressive among these species (Gilbert *et al*., 2000; Fernandez *et al*., 2001; Xue *et al*., 2002; Golinski *et al*., 2002; Gale 2003), but there is no evidence for pathogenic specialization within *F. graminearum* and *F. culmorum* (Parry *et al*., 1995; Mesterhazy *et al*., 1999). Significant quantitative variation for aggressiveness has also been observed within individual field populations of these species (Bai and Shaner 1994; Parry *et al*., 1995; Miedaner *et al*., 2001). Several studies reported variation in aggressiveness among *F. graminearum* isolates sampled from various parts of the world (Bai and Shaner 1996; Miedaner *et al*., 2001), within a country or state (Dusabenyagasani *et al*., 1999; Walker *et al*., 2001; Carter *et al*., 2002) and even
within populations from individual fields (Miedaner and Schilling 1996). The *F. graminearum* European isolates were highly variable in aggressiveness (Miedaner *et al*., 2001; Laday *et al*., 2004; Toth *et al*., 2005). Walker *et al.* (2001) observed significant differences for pathogenicity on wheat among the isolates from North Carolina. Most of the work regarding aggressiveness of *F. graminearum* isolates was conducted under greenhouse conditions. Alvarez *et al.* (2010) found that 18 isolates of *F. graminearum* obtained from 17 different places within the wheat production area of Argentina, presented variation in aggressiveness under greenhouse conditions. Goswami and Kistler (2005), meanwhile, evaluated the variation in aggressiveness and mycotoxin production of 31 isolates belonging to 8 species from the *F. graminearum* species complex and found the high level of variability for both characters to be specific to each of the strains tested and independent of the species considered. Significant differences for aggressiveness have been found among isolates in other studies (Walker *et al.*., 2001; Carter *et al*., 2002).

The role of trichothecenes in plant disease is not clear. Deoxynivalenol (DON) and other trichothecenes appear to play an important role in the aggressiveness of *F. graminearum* and *F. culmorum* (Mesterhazy 2002, Wang *et al*., 2006). DON is a strong protein inhibitor (Snijders 1994), and this may cause inhibition of enzymatic activity in susceptible hosts, leading to a rapid increase of the disease. A positive correlation between aggressiveness and DON production by *F. culmorum* and *F. graminearum* has been reported (Proctor *et al*., 1995; Gang *et al*., 1998). Muthomo *et al.* (2000) also reported a close correlation between aggressiveness and DON production in *F. culmorum* isolates. Proctor *et al.* (1995) have disrupted the *Tri5* gene which encodes trichodeine synthase and plays a crucial role in the synthesis of the trichothecene skeleton. This resulted in a significant decrease in aggressiveness, again indicating that DON is essential for disease development (Desjardins *et al*., 1996, 2000; Bai *et al*., 2000). Alexander *et al.* (1997) concluded that trichothecenes are not necessary for pathogenicity, but that they increase the extent of the disease. The regulation of DON production during the development of FHB is a complex phenomenon. The aggressiveness of *F. graminearum* and *F. culmorum* correlates with production of DON and/or trichothecenes, suggesting that production of these toxins is an important component
of aggressiveness (Mesterhazy 2002). *F. graminearum* spreading within the head seems to be the major component of aggressiveness (Cumagun and Miedaner 2003). According to Van der Plank (1968), aggressiveness of a pathogen is measured by the quantity of disease induced by a pathogenic isolate on a susceptible host. Aggressiveness is often separated into elementary quantitative traits of the pathogen life cycle and usually estimated through disease severity, which is a composite variable resulting from the integrated effect of infection efficiency and lesion size (Pariaud *et al.*, 2009).

In various cases analyzed, the observed damage changes according to the geographical situation of *Fusarium* isolate (Walker *et al.*, 2001; Gagkaeva and Yli-Mattila 2004; Laday *et al.*, 2004). Little is known about the mechanisms involved in the virulence degree of this phytopathogen in crops. However, it could result from qualitative and quantitative differences in the production of mycotoxins and enzymes that degrade the plant cell wall. There is evidence that trichothecenes may be factors that affect the progress of infections on both wheat and maize rather than pathogenicity factors controlling the ability for infection to occur (Harris *et al.*, 1999). A reduced secretion of cell wall degrading enzyme might retard both the growth of the fungus on the host surface and its infection process, thus giving the host more time for a defensive response (Jenczmionka and Schafer 2005). Numerous virulence factors have been identified and characterized to varying degrees for *F. graminearum* (Hou *et al.*, 2002; Jenczmionka *et al.*, 2003; Lu *et al.*, 2003; Dyer *et al.*, 2005; Jenczmionka and Schafer 2005; Seong *et al.*, 2005; Voigt *et al.*, 2005). Wanyoike *et al.* (2002) microscopically observed the degradation of wheat cell wall components (xylan, cellulose, pectin) after *F. graminearum* infection, postulating that the secretion of CWDE played a role during penetration and disease establishment. Also the same behavior was observed in *F. culmorum* (Kang and Buchenauer 2000a, 2000b).

**2.2.8. Genetic variation**

In view of the importance of diseases caused by members of the genus *Fusarium*, procedures for effective management of these pathogens are urgently needed. In this context, information on the population genetic diversity and dispersal of the *F. graminearum* (*Fg*) consequently of importance. Various genotypic and phenotypic approaches have been in use over the past few years in research
pertaining to the population genetic structure of the Fg complex (Bowden and Leslie 1994, 1999; O’Donnell et al., 2000; Carter et al., 2000, 2002; Miedaner et al., 2001; Gale et al., 2002; Ward et al., 2002). There are differing reports on the genotypic diversity among the isolates of F. graminearum (Wang et al., 1997).

Molecular markers such as sequence characterized amplified regions (SCAR), single strand conformational polymorphism (SSCP), randomly amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), restriction fragment length polymorphism (RFLP), sequence related amplified polymorphism (SRAP), single nucleotide polymorphism (SNP), and variable number of tandem repeat (VNTR) have been used extensively to study genetic variability of the Fusarium spp./isolates.

RAPD (random amplified polymorphic DNA) (William et al., 1990) based on the polymerase chain reaction (PCR) offer very promising, versatile and informative molecular tool to detect genetic variation and classify strains in populations of plant pathogens. RAPD has been successfully used to identify strains (Chioccheti et al., 1999; Guzman et al., 1999), to characterize races (Malvick and Grau 2001) and to analyze virulence variability related to genetic polymorphisms (Chen et al., 1995; Browning et al., 1999; Chakraborty et al., 1999; Chen et al. 1999) in phytopathogenic fungi. It has also been used for studying inter and intraspecific variability among population from different and from the same geographic regions (Walker et al.; 2001). The RAPD pattern analysis visualizes variations in the total DNA and thus suitable for differentiation of Fusarium isolates below species level (William et al., 1990; Grazal-Martin et al., 1993; Ouellet and Seifert 1993).

RAPD technique is a PCR procedure involving very short (10 or fewer bases) arbitrary primers at a low annealing temperature (30–38 °C). RAPD analysis detects two types of genetic variation: (i) in the length of DNA between the two primer binding sites; and (ii) in sequence variation at the priming regions. When the amplified products from such a reaction are analyzed on an electrophoresis gel, unique banding patterns are seen. These patterns may reflect the differences characteristic of certain species or strains. The RAPD method is simple, sensitive, rapid and has been used for distinguishing several pathogens (Narayanasamy 2008).
Members of the Fg complex were thought to represent the single species *F. graminearum* (teleomorph *G. zeae*) (O’Donnell *et al*., 2008). Thus multiple Fg complex were reported under the name *F. graminearum* in previous studies. Morphological variation within the Fg complex is insufficient for discerning species identity. However, by employing GCPSR, a MLGT assay, and different molecular marker technologies, 13 phylogenetically distinct species have been identified within the Fg complex (O’Donnell *et al*., 2000, 2008; Starkey *et al*., 2007; O’Donnell *et al*., 2008; Yli-Mattila *et al*., 2009; Wright *et al*., 2010). These findings indicate that diversity within the Fg species complex is much greater than previously believed (Sampietro *et al*., 2011).

A high level of genotypic diversity in Fg complex isolates in Canada, via RAPD markers, was also demonstrated by Dusabenyagasani *et al*. (1999) and 90.56% of the genetic variability was explained by within-region variation. RAPD analysis of 42 isolates, which originated from northwest Europe, the US and Nepal, identified three groups, two of which contained the isolates from Nepal, and a third contained the isolates from Europe and the US (Carter *et al*., 2002). The genetic variation within the species is very high and influences aggressiveness, toxin spectrum and abundances, host interaction, sexual and asexual reproduction, and environmental response (Desjardin 2006).

A considerable genetic resemblance was found by RAPD between 34 isolates from northeastern and northwestern China. The isolate grouping was not related to pathogenicity or to host cultivar (Liu *et al*., 2002). Similar findings were obtained in the US, where *F. graminearum* s.s. is the predominant FHB species (Zeller *et al*., 2004; Gale *et al*., 2007; Starkey *et al*., 2007). However, relatively low amount of genetic diversity were revealed using RAPD analysis of isolates in Canada (Ouellet and Seifert 1993) and Brazil (Busso *et al*., 2007). Bowden and Leslie (1996) created nit mutants and reported high levels of variation for vegetative compatibility among *F. graminearum* isolates collected in the Midwestern United States. By using PCR- based DNA fingerprints, low genetic diversity among isolates of *F. graminearum* was noted in Canada (Ouellet and Seifert 1993).

Walker *et al*. (2001) observed, by using DNA (RAPD) analysis, high levels of genotypic diversity among isolates from North Carolina, and significant differences among the isolates were found for mycotoxin production and
pathogenicity on wheat. Gagkaeva and Yli-Mattila (2004) examined the variability of Russia, China, Germany and Finland *F. graminearum* isolates, detecting higher levels of genetic diversity in Asian isolates than in European ones. They also determined a correlation between genetic clusters of IGS sequences and geographic origin. When *F. graminearum* isolates from the USA, Europe and Asia were compared using SCAR and RAPD analyses, groups were obtained with different biological properties, especially as regards their pathogenicity to wheat and maize, based on their ability to produce mycotoxins (Carter *et al*., 2002). Genetic characterization of European isolates associated with FHB revealed large variation within *Fusarium* species (Nicholson *et al*., 1993; Schilling 1996).

Little information is available about this topic in South America. *F. graminearum* isolates from Brazil showed great genetic similarity among them, based on DNA (RAPD) analysis (Busso *et al*., 2007). Ouellet and Seifert (1993) characterized *F. graminearum* isolates from Canada using RAPD and PCR-RFLP and detected a relatively low amount of genetic diversity. However, other analyses from Canadian isolates by RAPDs showed that all isolates were genetically different (Dusabenyagasani *et al*., 1999). The analysis of the DNA (RAPD) fingerprint revealed a great deal of heterogeneity among *F. graminearum* isolates collected from different regions of India (Saharan *et al*., 2007).

AFLP markers and SCAR (Sequence Characterized Amplified Region) were utilized by Qu *et al.* (2008a) to investigate the genetic diversity and geographic distribution of isolates throughout China. This study included all regions with a history of FHB. Both analyses were completely congruent. RFLP markers were also used to distinguish among phylogenetic lineages within the *F. graminearum* complex in an epidemic area in China, and it was established that all isolates belonged to lineage 6 (Gale *et al*., 2002). When the structure and reproductive behavior among populations of *F. graminearum* were studied in Australia, a high level of diversity was observed (Akinsanmi *et al*., 2008). These results demonstrated that AFLPs and MCGs (Mycelial Compatibility Groups) revealed genetic diversity at different levels (Akinsanmi *et al*., 2006, 2008). High genotypic diversity has been revealed within the *Fg* complex, even within a species (Gale *et al*., 2002; Zeller *et al*., 2003, 2004; Schmale *et al*., 2006; Burlakoti *et al*., 2008; Karugia *et al*., 2009) using molecular markers.
2.2.10. Symptoms

FHB is best known as a flowering disease, being the anthers reported as the primary infection site where spores of fungus may land and then grow into the kernels, glumes, or other head parts. The Fusarium pathogens have no specialized structures for penetration of host cell, like appressoria or haustoria. Instead, the fungus either enters the host through natural openings (Pritsch et al., 2000), or penetrates the epidermal cell walls directly with short infection hyphae (Wanyoike et al., 2002). *Fusarium* head blight symptoms are characterized by the appearance of water soaked brown-coloured spots at the base or the middle of the glume or on the rachis. Eventually this water soaked appearance and discoloration spread further from the point of invasion (Parry et al., 1995). When all conditions are highly favourable for FHB development, a growth of salmon pink to red coloured mycelia can be seen on the base of the spikelet and spreads through the entire head. The symptoms of FHB are often characterized by the presence of fungus, which may include pink sporodochia and/or purple black perithecia on glumes and seeds often are shrunken or shrivelled and called “tombstones” and *Fusarium* damaged kernels (FDK) (Parry et al., 1995; McMullen et al., 1997; Goswami and Kistler 2004). Ultimately the infected grains become discoloured, shrunken and chalky white in appearance with black perithecia giving the name “scab” (Parry et al., 1995). FHB results in yield loss and poor grain quality, low kernel weight, reduced seed germination, seedling blight, and poor stands (Parry et al., 1995; McMullen et al., 1997; Dexter and Nowicki 2003).

2.2.11. Disease cycle

The components that are necessary to develop and establish a disease are described in the disease triangle. The disease triangle is composed of: 1. Inoculum, 2. suitable host, 3. favourable environmental conditions (Shaner 2003). All diseases, including FHB depend on the above components of the disease triangle.

2.2.11a. Inoculum source

The sexual cycle of *F. graminearum* has recently been described in detail (Trail and Common 2000; Guenther and Trail 2005; Trail 2009). The fungus overwinters on crop debris on the soil surface. All of the associated fungi may produce abundant mycelium as well as conidia, and some of the species produce
chlamydosporles on and within the crop debris. In the spring, perithecia develop, from which ascospores are forcibly discharged as the primary inoculum (Bai and Shaner 2004; Trail et al., 2005). Fernando et al. (1997) also concluded that airborne ascospores were the primary inoculum sources and primary infection is the main cause of the disease whereas the secondary spread only has a minor role in disease development. But, conidia are the primary infective particle for species with mainly (or exclusively) asexual development. Inoculum propagules for FHB include both ascospores and conidia and are likely to be found at nearly any time during the adult stages of the cereal crop in the region when the environment falls within the wide range of favorable conditions (Trail et al., 2002; Doohan et al., 2003; Beyer et al., 2005; Brennan et al., 2005). Pathogen survival is enhanced within reduced tillage systems while tillage (residue burial) speeds decomposition and reduces pathogen reproduction and survival (Khonga and Sutton 1988; Pereyra et al., 2004). FHB is considered as a monocyclic disease; therefore, the level of primary inoculum that is available to infect the host plant will have a great effect on epidemic development (Sutton 1982; Bai and Shaner 1994). Environmental factors such as temperature, moisture and wind have a direct impact on inoculum production and release and dispersal of spores. The mechanism by which ascospores discharge has also been investigated over the last decade (Trail and Common 2000; Trail et al., 2005; Hallen et al., 2007; Hallen and Trail 2008). Ascospores are forcibly discharged through a small tear or slit, the ostiole, at the apex of the perithecium (Trail and Common 2000). Rainfall induces the ascus wall to rupture. As the relative humidity (RH) increases, the osmotic pressure rises within the perithecium due to an influx of mannitol and ions of potassium and chloride, leading to the build-up of turgor pressure and the forcible discharge of the ascospores (Trail et al., 2002). During warm, moist and windy environmental conditions, the ascospores disperse by air currents and deposit on a susceptible host plant and initiate infection (Fernando et al., 1997; Shaner 2003). The amount of inoculum is determined by the amount of crop residue and the degree of infection of the crop residue on the soil surface (Dill-Macky and Jones 2000). Asymptomatic inflorescences of wild grasses, copious perithecia and ascospores on the soil surface have been examined as minor sources of Fusarium inoculums. Fusarium damaged kernels (FDK) do not act as a source of inoculum, sowing FDK does not cause FHB (Gilbert and Fernando 2004).
2.2.11b. Host plant

The abundance of fungal niches both during and after the growing season coupled with a widely adapted group of pathogenic species makes the FHB disease system difficult to manage and to predict. However, the role of the host as a component of the disease system cannot be overlooked. Host factors of importance include genetic resistance to the pathogen and also physiological condition of the host as influenced by nutrition, hydration, and age. The host response to infection and disease development varies widely. Genetic resistance to FHB is generally expressed as a quantitative trait, presumably due to many minor genes and few major ones conferring the resistance and as such, there is wide variation in phenotypic reaction and environmental response. In Europe, Mesterhazy et al. (2003) found that winter wheat cultivars differed in yield loss, severity, FDK, and DON. FHB-resistant cultivars had less FDK, severity, DON, and yield loss compared with susceptible cultivars. DON, severity, FDK, and yield loss were significantly correlated. In a separate study, Mesterhazy et al. (1999) found that DON decreased with decreasing FHB severity. Severity was best correlated with yield loss and least correlated with DON. The most resistant cultivars had no or very low DON. Previous studies have shown that FHB-susceptible wheat cultivars generally accumulate more DON than resistant cultivars. Lehoczki-Krsjak et al. (2010) found that some winter wheat genotypes with medium FHB severity had low FDK and DON concentration. However, there were some genotypes with relatively low FHB severity but high levels of DON. Wegulo et al. (2011) demonstrated differences among a limited number of hard winter wheat cultivars in their reaction to FHB and DON accumulation. Bai et al. (2001) demonstrated significant differences among 116 wheat genotypes in their reaction to FHB. They showed that DON concentration was significantly correlated with FHB. Cowger et al. (2009) demonstrated that there were significant differences among eight soft red winter wheat cultivars in FHB incidence, FHB severity, FDK, and DON. So, choice of cultivar (host) is a very important control strategy against FHB.

2.2.11c. Environmental factors

Environmental factors such as temperature and moisture affect the inoculum production, dispersal and the duration of the vulnerability of the host plant to the infection. Each fungus in the FHB disease system has somewhat different biological
and environmental requirements which can, in part, explain why the frequencies of these species vary by location. For example, *F. graminearum* grows well over a wide range of temperatures up to 30°C and is associated with the warmer regions of the world, whereas *F. poae*, which is a more efficient pathogen at lower temperatures (i.e. 20 °C), is found more frequently in temperate climates. Most of the species can be found in much of the geographical area affected by FHB, but individual species usually dominate a specific region and *F. graminearum* dominates in a majority of regions. Optimum temperatures for head blight development range between 25 to 32 °C (Sutton 1982). Precipitation has a great impact on epidemic development, because favourable temperatures for FHB development are common during the flowering stage of cereal crops. Moisture has a major role in inoculum production, dispersal and initial infection during disease establishment than the temperature (Sutton 1982). *F. graminearum* reproduces over a very wide range of temperatures and moisture conditions. Relative to other species, *F. graminearum* is favoured in warmer, wetter conditions, in terms of conidial production and infection rates. However, cooler conditions favour perithecial development (Dufault *et al*., 2006) and ascospore release, with the latter being inhibited by prolonged high humidity (Sung and Cook 1981). Infection by *F. graminearum* also occurs across a broader range of temperatures than for the other species, and infection may occur more rapidly as well (Rossi *et al*., 2001). Paulitz (1996) examined the daily pattern of ascospore release by *Giberella zeae* and reported that, the discharge of inoculum is triggered by the combined effect of drop in air temperature and a rise in relative humidity. Although rainfall has a role in ascospore and perithecial formation, it does not trigger the release of ascospores (Paulitz 1996). But Fernando *et al*., 2000 found correlation between release of ascospores and rain fall events and the time of day. Fernando *et al*., (1997) reported that the dispersal of *F. graminearum* inoculum occurs downwind from inoculated wheat heads and disease incidence was higher in irrigated plots than the non-irrigated plots. Environmental factors such as temperature, pH, humidity, aeration and light affects the germination of ascospores. Beyer and Verreet (2005) reported that age of ascospores, relative humidity, temperature and pH were the key factors that determine the germination of ascospores. After release, ascospores germinated within 4 hrs at 20 °C and a relative humidity of 100% (Beyer and Verreet 2005). According to the study done by Gilbert *et al*., (2008) the germination of *G. zeae* ascospores were highest at 90% relative
humidity and lowest at 60 %. It is also reported that ascospores can germinate successfully even under extreme environmental conditions (Gilbert et al., 2008). Anderson (1948) reported that the germination of macroconidia occurred within 3 hr at 28-32 °C or within 6 hr at 20-32 °C. A period of 92-94% high relative humidity in combination with warm temperatures during the flowering stage of host plant makes an ideal environment for FHB development and establishment (Sutton 1982). Environmental conditions play a critical role in the production of spores, as well as their dispersal.

2.3. Management

*F. graminearum* is an ubiquitous fungus that is found on many hosts, making it very hard to control with a single strategy (Bai and Shaner 1994). Therefore, combining multiple control strategies is essential in effective control of FHB. Host resistance, fungicides, cultural practices and biological control are the common management strategies being used to control FHB.

2.3.1. Types of resistance against FHB

The resistance of wheat to FHB is a complex phenomenon. The forms, types or components of physiological resistance (Mesterhazy 1995, 2001) are: Type I is resistant to initial infection or penetration (Schroeder and Christensen 1963); Type II prevents the spread within the head followed by infection (Schroeder and Christensen 1963); Type III is resistant to kernel infection (Mesterhazy 1995; Mesterhazy et al., 1999); Type IV is present when yields are maintained despite the presence of the disease or tolerance to infection (Mesterhazy 1995; Mesterhazy et al., 1999); and Type V is the ability of hosts to degrade the produce toxins or resistance to DON accumulation (Miller et al., 1985; Feng 2007). Research on two other forms of resistance is in progress. Resistance to late blighting means low *Fusarium*-damaged kernel (FDK) values even when a long rainy period occurs at harvest. Such resistance contrasts with cultivars with low FHB values, but high FDK values at harvest. The resistance to head death above the infection point of the head means that transport of assimilates is still possible following infection of the rachis. As a result, seed size is near normal. In susceptible genotypes, the transport vessels cease function, mycelial masses inhibit transport and the grains shrivel even though they are free of infection. However, the relationship between these
parameters and their genetic background is not clear (Mesterhazy 2002). The best-known component is the resistance to pathogen spread; all genetic work refers to this form of resistance. Type I resistance is common in spring wheat from Brazil such as Frontana and Type II resistance is exhibited by Chinese resistant varieties such as Sumai 3 and its derivatives (Bai and Shaner 2004). Type I resistance is assessed by using spray inoculation and Type II by single floret inoculation techniques (Schroeder and Christensen 1963). Type II resistance is assessed under controlled environmental conditions and found to be more stable than Type I as it is less affected by non-genetic factors (Bai and Shaner 1994). The different mechanisms of resistance show quantitative inheritance. In these cases additional experiments are needed to understand the biology in agreement with the plant architecture and FHB resistance. Although FHB resistance has been well documented and resistant cultivars have been developed to reduce incidence and severity of FHB, there is a limited understanding of the molecular mechanisms involved in plant resistance against the infection and spread of *F. graminearum* (Zhou *et al*., 2005; Zhang *et al*., 2008).

### 2.3.2. Cultural control

Cultural control is an ecofriendly approach that can be used to reduce the risk of FHB epidemics. Numerous studies have been done to evaluate the effect of crop rotation on FHB development. Depending on the previous crop, the severity of FHB can be affected. Rotation of wheat with non-host crops reduces the amount of inoculum in the crop residues (Sutton 1982; Parry *et al*., 1995; Dill-Macky and Jones 2000). Research showed that when wheat was grown following maize, FHB infection increased by 15% compared to only 4% infection when wheat was sown following alfalfa or oats (Pirgozliev *et al*., 2003). In another study, it has been found that cultural practices such as tillage do not have significant effects on the disease severity and kernel infection (Miller *et al*., 1998). Miller *et al*., (1998) examined the effect of tillage on FHB disease incidence and suggested that the use of FHB resistant cultivars is more important in controlling FHB epidemics than tillage practices. Dill-Macky and Jones (2000) evaluated the effect of crop rotation and tillage on FHB of wheat and reported that, FHB severity and incidence was less when wheat was grown after soybean than after wheat or corn irrespective of the tillage practice. Dill-Macky and Jones (2000) also reported that conventional tillage
and no tillage systems contributed to FHB epidemics in the Upper Midwest. Schaafsma et al. (2005) reported that previous crop, field size and tillage affect the FHB index, DON accumulation and, Fusarium-damaged kernels in infected fields. Studies done by Schaafsma et al. (2001) also reported that tillage had no effect on DON levels in infected wheat grains. Guo et al. (2010) quantified the effects of cropping practices on F. graminearum inoculum levels and developed a Cropping Practice Index (CPI) model to express the relationship. Applications of nitrogen fertilizers, can, however, increase the incidence of FDK in wheat, barley and triticale (Martin et al., 1991) but, Teich and Hamilton (1985) reported that application of nitrogen fertilizers had no significant effect on the FHB disease incidence. Weed control is another cultural practice that can be adopted to reduce the FHB. Instead weed can act as an alternative source of FHB inoculum, control of weeds can reduce the availability of alternative FHB inoculum (Pirgozliev et al., 2003). Fields with higher weed densities had higher numbers of infected heads than the weed-free fields (Teich and Nelson 1984).

2.3.3. Chemical control

Chemical control is one of the main parts of an integrated FHB management approach. Fungicides are currently used at both flowering stage and before flowering stage to reduce quantitative yield loss and mycotoxin contamination (Mullenborn et al., 2008). The two main critical factors in use of fungicides to control FHB are the timing and rate of application. The best time to apply fungicides is the period after the emergence of the head. Because the systemic triazole fungicides do not move from leaves to head from the point of contact, early application can protect only the leaves not the heads (Mesterhazy 2003). Application of fungicides several weeks before wheat anthesis may be more harmful for non-toxigenic microorganisms and can promote subsequent spread of toxigenic Fusarium species in the field (Henriksen and Elen 2005). Fungicides should be applied from both sides of the plots as partial coverage reduces the control of FHB. The rate of application may vary with the type of fungicides applied and comes with the fungicide label. It has been found that the concentration of fungicides was highest in the glumes and gradually decreases when moving to lemma and the embryo (Mesterhazy 2003). Effective fungicides should be safe products with short pre-harvest interval and have high efficacy in reducing FHB and DON. Traditionally
should have optimum application rates and techniques and a reasonable price. To date, many fungicides with different active ingredients are being used to manage FHB. But several authors have provided conflicting evidence regarding the efficacy of fungicides in controlling FHB and mycotoxin contamination in the field (Paul et al., 2007). Several factors such as level of inoculum, cultivar resistance, climatic conditions, crop sensitivity and yield potential affect the success of fungicide application in controlling FHB (Mesterhazy 2003). In some studies, fungicides based on the triazole chemistry (Tebuconazole, Metconazole or Prothioconazole) are considered to be the most effective among all available registered fungicides (Edwards et al., 2001; Simpson et al., 2001; Pirgozliev et al., 2002; Mesterhazy et al., 2003), resulting in reductions of head blight severity and mycotoxin contamination by 50-80% and 5-90% respectively (Matthies and Buchenauer 2000). Triazole based fungicides inhibit the 14α demethylase, an enzyme that is essential for ergosterol biosynthesis (Klix et al., 2007). On the contrary, in another study, application of fungicides has resulted in an increased trichothecene accumulation (Gareis and Ceynowa 1994; Simpson et al., 2001). Gareis and Ceynowa (1994) observed that application of the fungicide, Matador to F. culmorum infected winter wheat, increased the NIV content in infected seeds. Application of the fungicide, Azoxystrobin also increased the DON content in infected grains (Simpson et al., 2001). Therefore, presence of conflicting evidence in the use of fungicides to control the development of FHB needs to be clarified. However application of fungicides is still the major control method used in many countries. For effective and ecofriendly control of the disease; it is always recommended that fungicides should be used in conjunction with other control strategies such as biological control and resistant cultivars. The combined effect of several strategies would provide a better control with higher yield and less infection.

2.3.4. Biological control

Various control measures have been practiced to manage FHB, including destruction of diseased plants, sanitary measures, use of disease-free tissue culture planting material, use of tolerant varieties and chemical control method. But promising options for controlling FHB are chemical measures and the development of resistant cultivars. Conflicting evidence regarding the efficacy of fungicides and residue concerns regarding the use of fungicides late in crop development lessen
their attractiveness. Advances in developing resistant cultivars using traditional breeding (Bai *et al*., 2000) and genetic engineering (Bushnell *et al*., 1998) are occurring, but unfortunately, the comparatively few, well-characterized sources of resistance are in backgrounds with poor agronomic and quality traits (Comeau *et al*., 2010) so, all wheat cultivars currently in production remain vulnerable to infection. Due to lack of safe and effective strategy for controlling the disease, biological control of FHB holds considerable promise and is attractive due to its potential for being environmentally benign, durable and compatible with other control measures (Schisler *et al*., 2002b). Research priorities for alternative management practices compatible with sustainable agriculture and the environment include the use of beneficial microorganisms or biocontrol agents. This has also been acknowledged by the Food and Agricultural organization of the United Nations (FAO) stating; “Governments, (...) should encourage and promote research on, and the development of, alternatives posing fewer risks (...)” (FAO 2014). Biological control agents are mentioned as one of these alternatives. Since the 1st of January 2014 Integrated Pest Management (IPM) should be applied in agriculture in Europe (European Commission 2014). IPM is a working approach in agriculture aiming towards reducing the use of pesticides as much as possible.

Schisler *et al*., (2002b) demonstrated the feasibility of using biocontrol agents to control FHB on wheat. The biological control of the pathogen may be achieved by aborting, curtailing or delaying the germination of the spores in the infection court of the head (Fernando *et al*., 2000). Antibiosis, competition, mycoparasitism, induced resistance and inhibition of mycotoxin synthesis are considered to be the major modes of action of biocontrol agents (Schisler *et al*., 2002a; Luz *et al*., 2003). The strategies for biological control of FHB include the control of the pathogen by disrupting the fungal life cycle using nonpathogenic microorganisms. Spikelet infection, colonization, ascospore production and dispersal are considered to be potential points for this biological intervention (Luz *et al*., 2003). Several fungi, bacteria and yeast have been reported to have antagonistic effects against *F. graminearum* (Luz *et al*., 2003). The antagonistic effects of these microorganisms have been demonstrated by *in vitro* antibiosis (Chan *et al*., 2003) and *in situ* disruption of spikelet infection leading to reduced disease severity (Schisler *et al*., 2002a; Nourozian *et al*., 2006), reduced systemic movement of pathogen within
infected spikes (Yuen et al., 2003), lower mycotoxin accumulation in grain (Dawson et al., 2004; Stockwell et al., 2000), and reduced pathogen survival and subsequent ascospore production in residues (Fernandez 1992; Bujold et al., 2001).

Biological control of FHB mainly includes treatment of crop residue with antagonists to reduce the pathogen inoculum or application of antagonists to wheat heads during anthesis to reduce fungal infection (Schisler et al., 2002b). According to Leplat et al. (2013) *F. graminearum* is a weak competitor within the soil fungal community. Various research groups have examined the use of a wide range of microorganisms against the development of FHB (Stockwell et al., 2000; Luz et al., 2003). Schisler et al. (2002a) isolated microbial strains from wheat anthers during anthesis and examined the feasibility of using those organisms in biological control of FHB. They could identify four strains that utilize tartaric acid and three that did not utilize tartaric acid as potential biocontrol agents from wheat anthers. These strains reduced the FHB disease severity up to 95% under green-house conditions and 56% under field conditions (Schisler et al., 2002a). In another study, Schisler et al. (2006) identified 31 choline metabolizing strains from wheat flower tissue; all of them reduced FHB disease severity by 25% in a greenhouse trial where 17 of them reduced the disease severity up to 50% on wheat. Khan et al. (2001) isolated seven novel antagonists from wheat anthers and examined the efficacy of those antagonists against three isolates of *G. zeae* on the wheat cultivar Norm. During the last decade, some fungal and bacterial species have emerged as the most powerful bioprotectants for the management of a wide variety of plant diseases by virtue of their broad-spectrum action. While diverse microbes may contribute to the biological control of plant pathogens, most research and development efforts have focused on isolates of four genera, *Bacillus*, *Trichoderma*, *Pseudomonas* and arbuscular mycorrhizal fungi (AMF). Continued studies of these genera will be needed to further advance our understanding of the nature of biological control microbes and to improve our ability to successfully integrate biological control strategies into FHB management systems.

### 2.3.4.1. Bacteria as a bioagents

Bacterial biocontrol agent may express different mechanisms against pathogens during their antagonistic activity; it weakens or destroys the pathogens by
parasitizing the pathogen directly, by producing antimicrobial compounds, by competing for space and nutrients, by producing enzymes that attack the cell components of the pathogens (Agrios 2005). The main property of antagonist bacterial strains is production of antifungal antibiotics (Hernandez et al., 2006; Intana et al., 2008; Yuan et al., 2011, 2012) which seem to play a major role in biological control of plant pathogens (Raupach and Kloepper 1998; McSpadden and Driks 2004; Hernandez et al., 2006, 2008). Various microorganisms are known as potential elicitors of induced systemic resistance (ISR) and exhibit significant reduction in the incidence or severity of various diseases on diverse hosts (Choudhary and Johri 2009). It is believed that plants have the ability to acquire enhanced level of resistance to pathogens after getting exposed to biotic stimuli provided by many PGPRs and this is known as rhizobacteria mediated ISR (Choudhary et al., 2007).

In recent years PGPRs (plant growth promoting rhizobacteria) such as fluorescent Pseudomonas and Bacillus spp. are the most extensively used biocontrol agents against several phytopathogens. It has been already reported that Bacillus and Pseudomonas species have ability to produce a large number of antifungal metabolites such as bacitracin, gramicidin S, polymyxin, tyrotricidin, bacilysin, chlotetaine, iturin A, mycobacillin, bacilomycin, mycosubtilin, fungistatin, subsporin, 2,4-diacyethylphloroglucinol (DAPG) and phenazine-1-carboxylic acid (PCA) (Zhang et al., 2009; Khan et al., 2011). Gram-positive bacteria offer a biological solution through formulations as they form heat- and desiccation-resistant spores (Emmert and Handelsman 1999). Among them, Bacillus spp. is recognized as a powerful tool. Species of Bacillus are able to synthesize more than 60 different types of antibiotics which also act as plant growth promoters (Girish et al., 2010 Nihorimbere et al., 2010). Bacillus spp. are known to suppress growth of several fungal pathogens such as Fusarium (Zhang and Tambong 2009; Khan et al., 2011). Species of Bacillus have also been known to produce compounds which promote plant growth directly or indirectly viz., hydrogen cyanide (HCN), siderophores, indole acetic acid (IAA), solubilize phosphorous and antifungal activity (Godinho et al., 2010; Wahyudi et al., 2011). Fernando et al. (2002) examined three bacterial strains of B. subtilis and were found to reduce FHB disease severity. Ramarathnam et al. (2007) reported that B. subtilis strain DFH08
significantly inhibited the radial mycelial growth of *F. graminearum* by 60% as compared to the control and reduced the disease severity in a green-house study. Nourozian et al. (2006) reported the use of *B. subtilis* strains 53 and 71, and *Pseudomonas fluorescence* biov1 strain 32 and *Streptomyces* sp. strain 3 as potential biological agents for control of FHB. The TrigoCor strain of *B. amyloliquefaciens* provides consistent control against *Fusarium* head blight of wheat in controlled settings (Crane et al., 2013). An another strain of *Bacillus amyloliquefaciens* (NJN-6), isolated from the rhizosphere soil of healthy banana plants, acts as an efficient antagonist against *F. oxysporum* f. sp. *cubense* by producing several antibiotics (Yuan et al., 2011, 2012). The Brazilian isolates of *Bacillus* and *Paenibacillus* are found to be the most effective biocontrol agents that can reduce the FHB disease severity in field by 50-67% (Luz et al., 2003).

Specific strains of *Pseudomonas* species have also shown efficacy in controlling a number of fungal diseases, including *Pythium* root and seed rot of many crops (Mazzola 1998; Ellis et al., 1999), *Fusarium* wilt in cotton and tomato (Gamliel and Katan 1992), banana wilt caused by *Fusarium oxysporum* (Sivamani and Gnanamanickam 1988), bean disease caused by *Sclerotium rolfsii* (Gamliel and Katan 1992), *Rhizoctonia solani* root infection in tomato (Siddiqui and Shaukat 2002) and late blight of tomato (Tran et al., 2007). Several reports indicated that *Pseudomonas* spp. (Rasmussen et al., 1991; van Peer et al., 1991; Maurhofer et al., 1994; Liu et al., 1995; Wei et al., 1996) have been used to induce resistance in plants. In wheat, seed treatment with several bacteria, including fluorescent pseudomonads, has shown promise for control of *Fusarium* seedling blight caused by *F. graminearum*, *F. culmorum*, and *Microdochium nivale* in glasshouse and field studies (Bello et al., 2002; Johansson et al., 2003). Moreover, the fact that soil amendment with either *Pseudomonas* sp. strain MKB 158 or *P. fluorescens* strain MKB 249 or their culture filtrates reduced both *Fusarium* seedling blight of wheat and barley caused by stem base inoculation of *F. culmorum* suggests that perhaps metabolite(s) from these bacteria activated a systemic defense against the pathogen. Pieterse et al. (1996) reported systemic resistance toward both the fungal root pathogen *F. oxysporum* f. sp. *raphani* and the bacterial leaf pathogen *P. syringae* pv. *tomato* in Arabidopsis induced by the soil applied biocontrol bacterium *P. fluorescens* strain WCS417r. *Pseudomonas fluorescens* WCS417r elicit an ISR in
2.3.4.2. *Trichoderma* as a bioagents

During the last decade, species of *Trichoderma* have emerged as the most powerful bio-protectants for the management of a number of plant diseases by virtue of their broad-spectrum action (Mukhopadhyay 2005). The mycoparasitic ability of *Trichoderma* species against some economically important aerial and soil borne plant pathogens (Elad 2000; Freeman *et al.*, 2004; Ashrafizadeh *et al.*, 2005; Dubey *et al.*, 2007) allows for the development of biocontrol strategies. *Trichoderma* spp. are common saprophytic fungi found in almost any soil and rhizosphere micro flora. It has successfully controlled a wide range of plant pathogens including *Rhizoctonia solani*, *Pythium aphanidermatium*, *Fusarium* spp., *Gaeumannomyces graminis* var.*tritici*, *Sclerotium rolfsii*, *Phytophthora cactorum*, *Botrytis cinerea* and *Alternaria* spp. (Kucuk and Kivanc 2003). Root colonization by *Trichoderma* also enhances root growth and development, crop productivity, resistance to abiotic stresses and uptake of nutrients (Harman *et al.*, 2004). Altomare *et al.* (1999) and also Sivan and Harman (1991) reported solubilization of phosphates and micronutrients by *T. harzianum* and also mentioned that *Trichoderma* is capable of colonizing and growing along the whole root system and, can store solubilized phosphate gradually providing it to the plant throughout the life of the plant. Possible mechanisms of antagonism employed by *Trichoderma* spp. includes nutrient and niche competitions, antibiosis by producing volatile and non-volatile antibiotics and mycoparasitism (Dennis and Webster 1971b, c; Harman and Hadar 1983; Schirmbock *et al.*, 1994) that is inhibitory against a range of phytopathogenic fungi. Another proposed mechanism for biocontrol activity in *Trichoderma* sp. is stimulation of host defense responses (Howell *et al.*, 2000). Induced resistance has been reported with *T. harzianum* on bean (De Meyer *et al.*, 1998) an unidentified *Trichoderma* sp. on cucumber (Koike *et al.*, 2001) and *Gliocladium virens* on cotton.
(Hanson 2000). Similar results were recorded by several researchers (Sabatini et al., 2002; Perello et al., 2003; Muthomi et al., 2007) and found that *Trichoderma* spp. has been very effective in reducing the severity of foliar disease in wheat plants when compared to untreated plants. This concept is supported by the work of Yedidia et al. (1999) who demonstrated that inoculating roots of 7-day-old cucumber seedlings in an aseptic hydroponic system with *T. harzianum* (T-203) spores to a final concentration of $10^5$ per ml initiated plant defense responses in both the roots and leaves of treated plants. The plant response was marked by an increase in peroxidase activity (often associated with the production of fungitoxic compounds), an increase in chitinase activity, and the deposition of callose-enriched wall appositions on the inner surface of cell walls. Increased enzyme activities were observed in both roots and leaves. Later, Yedidia et al. (2000) showed that inoculation of cucumber roots with *T. harzianum* (T-203) induced an array of pathogenesis-related proteins, including a number of hydrolytic enzymes. Plants treated with a chemical inducer (2, 6-dichloroisonicotinic acid) of disease resistance displayed defense responses that were similar to those of plants inoculated with the biocontrol agent. Several reports have substantiated these observations (De Meyer et al., 1998; Hanson 2000; Koike et al., 2001). Some strains of *T. harzianum* establish robust and long lasting colonization of root surfaces penetrating into the epidermis (Harman 2000). Foliar spray of *Trichoderma* spp. (*T. harzianum* and *T. viride*) at the time of anthesis was found effective in controlling FHB in wheat (Panwar et al., 2014). Use of *Trichoderma* spp. in stubble management was investigated by Inch and Gilbert (2003). The latter were able to show a significant reduction in perithecial formation on autoclaved wheat straw pieces when *T. harzianum* was inoculated prior to, or coinnoculated with the pathogen. Though the mechanism has not been studied, it is speculated that mycoparasitism and (or) enzymatic activity may be involved. *T. harzianum* was identified as an effective biocontrol agent against *F. graminearum* on wheat residue (Fernandez 1992). However, in residue management, more work is needed to optimize the efficacy of the biocontrol agent, including the dose, formulation, and timing of application.

### 2.3.4.3. Arbuscular Mycorrhizal Fungi (AMF) as a bioagents

Arbuscular mycorrhizal (AM) fungi, which form symbiotic associations with a wide range of plant species, are another interesting group of microorganisms that
effectively reduce disease by a number of soil borne pathogens (Rosendahl 1985; Jalali and Jalali 1991; Linderman 1994). AM are indigenous to soil and plant rhizosphere and more suitable, environmentally acceptable alternative and potential tools for sustainable agriculture. There is no doubt that several mechanisms are at work, including reduced damage in mycorrhizal plants may be due to changes in root growth and morphology; histopathological changes in the host root; physiological and biochemical changes within the plant; changes in host nutrition; mycorrhizosphere effects which modify microbial populations; competition for colonization sites and photosynthates; activation of defense mechanisms by AM fungi (Siddiqui and Mahmood 1995). Despite this, still there is an ambiguity that the AMF has any direct involvement in the host’s defence signaling against phytopathogens. Although, there are some indirect functions which contribute to intensify the plant defence responses including augmentation of plant nutrition (Smith and Read 2008) and damage compensation. Moreover, it includes anatomical alterations in the root system (Wehner et al., 2010), microbial changes in the rhizosphere and enhancing the attenuated plant defence responses by altering the host’s signaling pathways (Pozo and Azcon-Aguilar 2007). This is accomplished primarily through modulation in Jasmonic acid (JA) and salicylic acid (SA) dependent pathways (Pozo and Azcon- Aguilar 2007). Furthermore, the AMF is likely to have role in induction of hydrolytic enzymes (Pozo et al., 1999), enhanced levels of Pathogenesis-related (PR) proteins, accrual of phytoalexins (Larose et al., 2002), callose deposition (Cordier et al., 1998) and reactive oxygen species generation (Salzer et al., 1999). Another common feature of the resistance responses induced by AMF is priming. Priming is a plausible strategy which includes preconditioning of plant tissues for a more effective activation of defenses (Conrath et al., 2006). For example, colonization of mycorrhizal fungi in tomato roots systemically protects the plant against Phytophthora parasitica infection without direct accumulation of PR proteins (Conrath et al., 2006). However, Conrath et al. (2006) found that upon pathogen attack, mycorrhized plants accumulate considerable amount of PR-1a, which confers SAR and basic β-1, 3 Glucanase (BGL) proteins than non-mycorrhized plants. AMF colonized rice was found to have induced lipid transfer protein (encoded from Ltp gene) (Blilou et al., 2000.) which is accountable for plant defence response for its antimicrobial property (Garcia-Olmedo 1995). Phenylalanine ammonia lyase (PAL) enzyme (encoded from Pal
gene), which leads to the production of phytoalexins and phenolic compounds, was also persuaded in the rice infected with AMF (Blilou et al., 2000). The interactions between different AM fungi and plant pathogens vary with the host plant and the cultural system. Moreover, the protective effect of AM inoculation may be both systemic and localized and there is evidence supporting both types of induced resistance (Linderman 1994). Several studies have proved that AM fungi single and in dual system protect the plant from several fungal plant pathogens in an efficient way. Caron et al. (1986) found that *Glomus intraradices* significantly reduced *Fusarium* root rot on tomato caused by *Fusarium oxysporum* and Al-Momany and Al-Raddad (1988) also found that *G. mosseae* significantly reduced *Fusarium* wilt on tomato. In another study, Ozgonen et al. (1999) and Bhagawati et al. (2000) found that *G. etunicatum* and *Glomus intraradices* reduced *F. oxysporum* disease severity in tomato. Sundaresan et al. (1993) suggested that prior inoculation of *G. fasciculatum* reduced colonization by pathogen *F. oxysporum* and severity of disease on cowpea.

Of the various mechanisms proposed for biocontrol of plant diseases, effective bioprotection is a cumulative result of all mechanisms working either separately or together. It is a fascinating subject, multidisciplinary in nature, and concerns scientists involved in plant health and plant protection. In order to apply AM fungi in sustainable agriculture, knowledge about the genetic diversity and structure of naturally occurring pathogen populations and factors such as fertilizer inputs, pesticide use, and soil management practices which influence AM fungi is essential (Allen 1992). In addition, efficient inoculants should be identified and employed as biofertilizers, bioprotectants, and biostimulants for sustainable agriculture.

Despite past achievements on the application of AM in plant protection, further research is needed for a better understanding of both the ecophysiological parameters contributing to effectiveness and of the mechanisms involved.

### 2.3.4.4. Mixture of microorganisms as a bioagents

The essential aim of biofertilizer technology is the development of inoculant composed of selected microorganisms to minimize the application of chemical fertilizers and maximize plants growth and nutrition. 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 have 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, limited tolerance to changes in environmental conditions and fluctuations in production of antifungal metabolites (Dowling and O’Gara 1994; Weller and Thomashow 1994). Several approaches have been used to overcome these problems, including combined application of two or more biocontrol agents to enhance the level and consistency in disease control (Oien et al., 1993; 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 (Oien et al., 1993; Pierson and Weller 1994; Raupach and Kloepper 1998; Nandakumar et al., 2001a, b), mixtures of yeasts (Janisiewicz 1996), bacteria and fungi (Janisiewicz 1988; Leibinger et al., 1997) and bacteria and yeast (Janisiewicz and Bors 1995). 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 (iii) it may allow the combination of various traits without employment of genetic engineering (Janisiewicz 1996).

Preliminary studies suggest that microbial antagonists of fungal pathogens, either fungi or PGPR do not antagonize AM fungi. Moreover, they can improve the development of the mycosymbiont and facilitate AM formation (Linderman 1994). This has been shown particularly for Trichoderma spp. (Calvet et al., 1993) and for Pseudomonas spp. producing 2, 4-diacetylphloroglucinol (Vidal et al., 1996). Therefore, the management of these interactions improving plant growth and health, in an integrated approach, should be one of the main objectives of sustainable agriculture (Barea and Jeffries 1995). Current interest in this topic has led to research on the manipulation of soil microorganisms, particularly with regard to improving the production, formulation and practical use of efficient microbial inoculants (Elliot and Lynch 1995).
Rhizospheric microorganisms like Arbuscular mycorrhizal fungi (AMF) and *Trichoderma* have the capacity of producing growth stimulating effect, in addition control of rhizospheric pathogens. Besides direct interaction with the plant pathogen, bioagents are also reported to induce systemic resistance in plants (Srivastava et al., 2010; Ojha and Chatterjee 2012).

Several reports have demonstrated that the interaction of AMF and *Trichoderma* may be beneficial for both plant growth and plant disease control (Linderman 1992; Tanwar et al., 2010; Mwangi et al., 2011). A synergistic effect of some saprophytic fungi on AMF spore germination and colonization has been confirmed (Calvet et al., 1993; McAllister et al., 1996). For example, it has been reported that some *Trichoderma* strains may influence AMF activity (Calvet et al., 1993; Brimner and Boland 2003; Martinez et al., 2004). Volatile and soluble exudates produced by saprophytic fungi are involved in these effects (McAllister et al., 1994). Boby and Bagyaraj (2003) found that *G. mosseae* reduced *Fusarium chlamydosporium* disease severity but best management was obtained when used with *T. viride*. In another study, Datnoff et al. (1995) reported a higher suppressive effect against *Fusarium* crown and root rot of tomato with the combination of *T. harzianum* and *G. intraradices* than with each biological agent applied alone. In another investigation, the antagonistic potential of *Glomus* spp. and *T. harzianum* against root wilt disease of sesame and their mutual beneficial effect on plant growth and productivity were recorded (Ziedan et al., 2011).

Nevertheless, the results of research on the interactions between soil saprophytic and AM fungi differ widely, even when the same species of saprophytic fungi are involved. For example, *T. harzianum* has been found to have antagonistic, neutral, and stimulating effects on AMF (Rousseau et al., 1996; Godeas et al., 1999). Even more, the beneficial effect attributable to these interactions under controlled experimental conditions may not be reflected in field experiments (Martinez et al., 2004).

Some rhizospheric bacteria are also known for their ability to produce plant growth promoting substances (Chabot et al., 1996), particularly when associated with mycorrhizal fungi (Babana and Antoun 2005). These microorganisms are known as Plant Growth Promoting Rhizobacteria (PGPR) colonizing or nesting near the crop roots, stimulate mycelial growth and their penetration into roots using
several mechanisms including: production of organic compounds and increase the production of roots exudates (Becard et al., 1992), production of active phytohormonal substances (Chabot et al., 1996) and solubilization of mineral phosphate.

Some bacteria have been shown to directly affect AM fungal germination and growth rate (Carpenter-Boggs et al., 1995) and thus the beneficial impact to the plant could be through the AM association. Other bacteria can directly influence the physiology of the plants, for example, by increasing root cell permeability. In addition to interacting directly to beneficially influence the mycorrhizal relationship and/or plant growth (Vivas et al., 2003), specific bacteria together with AM fungi may create a more indirect synergism that supports plant growth (Barea 1997), including nutrient acquisition (Barea et al., 2002), inhibition of plant pathogenic fungi. In addition to these effects of bacteria on AM fungi, the AM fungi themselves have also been shown to have an impact on the composition of bacterial communities (Artursson et al., 2005). Several reports have also demonstrated enhanced AM fungal colonization levels in roots in the presence of PGPR. For example, association of Pseudomonas putida with indigenous AM fungi resulted in a clear growth enhancement of clover plants (Meyer and Linderman 1986), suggesting that some PGPR may have properties that support both mycorrhizal establishment and function. In addition, Sanchez et al., (2004) showed that a fluorescent pseudomonad and an AM fungus (G. mosseae) had similar impacts on plant gene induction, supporting the hypothesis that some plant cell programmes may be shared during root colonization by these beneficial microorganisms. In a study at Mali, it has been reported that Pseudomonas sp. BR2, promotes wheat roots mycorrhization and phosphorus acquisition by wheat plants (Babana and Antoun 2005). Genetic variability between different microorganism species explains their great ability to adapt to different environments. P. fluorescence increased mycorrhizal colonization of tomato roots by G. mosseae (Gamalero et al., 2004). This result suggests that strain P. fluorescence behaves as a mycorrhizal helper bacterium (MHB) in L. esculentum. MHB have been reported for AM symbiosis (Toro et al., 1997; Singh and Kapoor 1998).

Specific interactions between AM fungi and PGPR occur most likely, and certain groups of bacteria have been shown to be established to a much higher extent
in the mycorrhizosphere as compared with other groups. This was shown by Andrade et al. (1997) who found that bacteria of the genera *Arthrobacter* and *Bacillus* were most frequent in the hyphosphere, the zone of soil surrounding individual AM fungal hyphae, whereas *Pseudomonas* spp. were most abundant in the rhizosphere of *Sorghum bicolor*. This study and others (Artursson et al., 2005) suggest that Gram-positive bacteria may be more commonly associated with AM fungi than Gram-negative bacteria, but this possibility needs to be more rigorously confirmed. It is noteworthy, however, that the bacterial groups most commonly reported to interact synergistically with AM fungi are mainly Gram-positive bacteria and γ–proteobacteria, supporting the hypothesis that some members of these phylogenetic groups are more integrally associated with AM fungi than others.

Research has shown compatibility of *Trichoderma* strains with bacterial antagonists. *T. viride* and *T. harzianum* were proved to be compatible with *P. fluorescens* and suppressed seedling disease of chilli and tomato significantly when they were applied together (Rini and Sulochana 2007; Chaube and Sharma 2002). Better efficacies were recorded for dual inoculation of *Trichoderma* and *Pseudomonas* in combination to *Fusarium* infected chickpea plants than the single inoculation of either one of them (Khan et al., 2004).

In another study, *P. fluorescens* strain Pf1 with *T. viride, B. subtilis* mixture enhanced protection of sheath blight to a level of equal or better than fungicide treatment and also intensive growth promotion by the bacteria increased grain yield (Karthikeyan et al., 2008).

### 2.3.4.5. Integrated Disease Management

Chemical fungicides have been the main weapons in controlling soil-borne plant pathogens and in increasing the yield in modern systems of crop production (Ulrike and Werner 1998). Although fungicides are used extensively for controlling these diseases, the results are temporary and do not protect the plants throughout the growing season. Moreover, fungicides may affect human health and the environment and become less effective due to the development of pathogen resistance in crop plants (Hwang 1994; Dunne et al., 1998; Andersen et al., 2003). With increasing public awareness for the environmental implications of the use of large fungicide
quantities in agricultural practices, alternative strategies for the control of plant disease are being sought (Ellis et al., 1999).

In recent years, low-input agricultural systems have gained increasing importance in many industrialized countries, for reduction of environmental degradation (Mader et al., 2002). Integrated farming systems with reduced inputs of fertilizers and pesticides have been developed.

Biological control using antagonistic microbes alone, or as supplements to minimize the use of chemical pesticides in a system of integrated plant disease management, has become more important in recent years (Hwang 1994; Mao et al., 1997). Commonly, the activity and efficacy of biocontrol agents are often profoundly affected by several factors such as sensitivity to environmental conditions in the soil and rhizosphere (Deacon and Berry 1993; Dunne et al., 1998), type and amount of inoculum applied, method and timing of application and age of the inoculum (Lewis and Papavizas 1987a, b), colonization ability of biocontrol agents in fields rich in infective propagules of pathogen (Deacon and Berry 1993) and narrow spectrum range of activity against one pathogens (Whipps 2001).

An integrated approach consisting of combinations of biological and chemical tools could provide a solution to the problems of individually applying fungicides and biocontrol agents. Many advantages are associated with an integrated system, for example when environmental conditions are temporarily unfavorable for activity of the biocontrol agents, an associated fungicide could provide an effective backup system and protection against specific pathogens (Lifshitz et al., 1985). In addition to that, there is an opportunity for additive or synergistic control by a combination of chemical and biological control strategies (Hwang 1994; Whipps 2001). According to Adejumo (2005) integration of chemical fungicides and biocontrol agents based on agro ecological zones would help farmers minimize their dependence on chemical control which would result to superior disease suppression thereby minimizing yield loss.

In several disease management strategies, the addition of resistance inducing chemical in combination with biocontrol agents has significantly enhanced disease control, compared to treatments with biocontrol agent alone (Frances et al., 2002; Buck 2004). Integrated use of biocontrol agent with chemical fungicide was
effective against *Fusarium* crown and root rot of tomato (Omar et al., 2006), late leaf spot of groundnut (Kishore et al., 2005), rhizoctonia root rot, take-all disease of spring wheat (Duffy 2000) and post-harvest diseases of fruits (Chand-Goyal and Spotts 1996) as compared to the individual components of disease management. Salman and Abuamsha (2012) in a study reported that the biologically active *P. fluorescens* isolate CW2 in combination with low rates of fungicides was very effective and increase the effectiveness and improve the consistency of biological control agents for combating root and soil-borne diseases. Integration of Captan + Metalaxyl with *Trichoderma harzianum* and *T. virens* also proved superior in controlling the wilt complex disease in Bell Pepper compared to their individual treatments (Rather et al., 2012)

One strategy of IPM is to develop fungal BCAs with tolerance to fungicides or to incorporate fungicide resistance into antagonists (Locke et al., 1985; Locke and Lumsden 1989; O’Neill et al., 1996). Combining resistant or tolerant fungal BCAs with fungicides can sometimes have twofold advantage in the treatment of seeds: a high level of seed protection is provided early on by the chemical component and then the biological component becomes active later in seedling development and can provide protection of root systems for improved plant health and function (Harman and Bjorkman 1998).