DOTTORATO DI RICERCA IN

Scienze e Tecnologie Vegetali, Microbiologiche e Genetiche

CICLO XXVII

COORDINATORE Prof. Paolo Capretti

New Sources of Resistance to Fungal Leaf and Head Blight Diseases of Wheat

Settore Scientifico Disciplinare AGR / 07

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Anni 2012 /2015

Dedication

This thesis is dedicated to:

Fatima my mother

Zuha my wife

Zahraa my daughter

Thank you for your love and inspiration...

ACKOWLEDGEMETS

I would like to thank the many people who were besides me throughout my PhD. studies. I take this opportunity to express my gratitude towards my supervisor Prof. Stefano Benedettelli and PhD. program coordinator Prof. Paolo Capretti, for the excellent guidance and support throughout my research. Sincere gratitude and regards are also to my CIMMYT based supervisor Dr. Pawan. K. Singh the head of wheat pathology lab and Dr. Xinyao He for their excellent guidance, advice and critical scientific comments during this study.

Special thanks are due to Prof. Giovanni Vannacci (University of Pisa), Prof. Renato D'Ovidio (University of Tuscia) and Dr. Gudio Marchi (University of Florence) and their teams for hosting me for the first year training.

Many thanks to CIMMYT's wheat pathology lab and field staff who was always willing to help me with my research including Maria Teresa, Monica Preciado, Susana Preciado, Cristofer Camargo, Javier Suegra and Fransisco Flores.

To my family, thank you for your love, confidence and encouragement.

Summary

Wheat (*Triticum aestivum* L.) is one of the world's most popular and cultivated crop and has been the major staple food of the major civilizations for 10,000 years. Unfortunately, this important crop is continuously threatened by different diseases that infect wheat heads, leaves in addition to other plant parts leading to yield and quality deterioration. Host-plant resistance is considered as the best approach and environmentally friendly method to reduce the devastating effects of many wheat diseases whenever sources of adequate resistance are found. However, breeding for disease resistance is largely influenced by the availability of resistant sources that possibly could be found in landraces, modern cultivars, breeding lines and wheat relatives. Multiple diseases could predominate and attack wheat in a particular area simultaneously the matter that raise many questions about continuing the adoption of breeding for individual disease resistance. Availability of multiple disease resistant genotypes is especially important in international breeding centers where hundreds of crosses are prepared annually, and the availability of parents having resistance for multiple diseases would potentially facilitate breeders task in combining multiple disease resistance in a single cross. The principal aim of this study was to identify novel sources of resistance to multiple wheat fungal pathogens including wheat head and leaf blight diseases. For this goal, wheat genotypes of different geographic origins were tested for different FHB resistance types and four leaf spotting diseases including Tan spot (TS), Stagonospora nodorum blotch (SNB), Septoria tritici blotch (STB), in addition to Spot blotch (SB), independently.

Key Words: Tan spot, Septoria tritici blotch, spot blotch, Stagonospora nodorum blotch, Fusarium head blight, DON, Multiple disease resistance

Publications

1. **Osman, M**., He, X., Singh, R. P., Duveiller, E., Lillemo, M., Pereyra, S. A., et al. (2015). Phenotypic and genotypic characterization of CIMMYT's 15th international Fusarium head blight screening nursery of wheat. Euphytica, 205, 521–537.
2. He, X., **Osman, M**., Helm, J., Capettini, F., & Singh, P. K. (2015). Evaluation of Canadian barley breeding lines for Fusarium head blight resistance. Canadian Journal of Plant Science, 95, 923–929.
3. **Osman, M**., He, X., Benedettelli, S., Ali, S., & Singh, P. K. (2015). Identification of new sources of resistance to fungal leaf and head blight diseases of wheat. European Journal of Plant Pathology. doi: 10.1007/s10658-015-0843-0
4. **Osman, M**., He, X., Benedettelli, S., & Singh, P. K. (2015). Trichothecene genotypes of Fusarium graminearum species complex and F. crookwllense isolates from Mexican cereals. Journal of Plant Pathology (Submitted).

Posters and Abstracts

1. **Osman, M**., He, X., Benedettelli, S., & Singh, P. K. (2015). Chemotype analysis of Mexican Fusarium graminearum sensu lato isolates. In 13th-european Fusarium seminar. Martina FrancaItaly.
2. He, X., **Osman, M**., Singh, P. K., Capettini, F., & Helm, J. (2014). Evaluation of Canadian barley germplasm for deoxynivalenol resistance at CIMMYT, Mexico. In Beijing mycotoxin conference. Beijing, China.
3. He, X., Singh, P. K., **Osman, M**., & Singh, R. (2014). Breeding for deoxinivalenol resistant wheat varieties at CIMMYT, Mexico. In Beijing mycotoxin conference. Beijing, China.

Abbreviations

15ADON	15-Acetyldeoxynivalenol
3ADON	3-Acetyldeoxynivalenol
AE	Anther Extrusion
AF	Aflatoxins
ANOVA	Analysis of Variance
AUDPC	Area Under Disease Progress Curve Standard
BSR	Broad Spectrum Resistance
CIMMYT	International Maize and Wheat improvement center
CTAB	Cetyltrimethyl Ammonium Bromide
DAI	Days After Inoculation
DNA	Deoxyribonucleic Acid
DON	Deoxynivalenol
DH	Days for Heading
ELISA	Enzyme-Linked Immunosorbent Assay
FDK	Fusarium Damaged Kernels
FHB	Fusarium Head Blight
FGSC	*Fusarium graminearum* Species Complex
FUS-X	Fusarenon-X
LTN	Leaf Tip Necrosis
MDR	Multiple Disease Resistance
NIV	Nivalenol
PCR	polymerase chain reaction
PH	Plant Hight
PTR	*Pyrenophora tritici-repentis*
PDA	Potato Dextrose Agar
PPM	Part Per Million
SB	Spot Blotch
Scar	Sequenced Characterized Amplified Region Marker
SNB	Stagnospora Nodorum Blotch

SSR	Simple Sequence Repeats
STB	Septoria Tritici Blotch
TS	Tan Spot
TRI	Trichodiene Synthase
QTL	Quantitative Trait Loci
ZEN	Zearalenone

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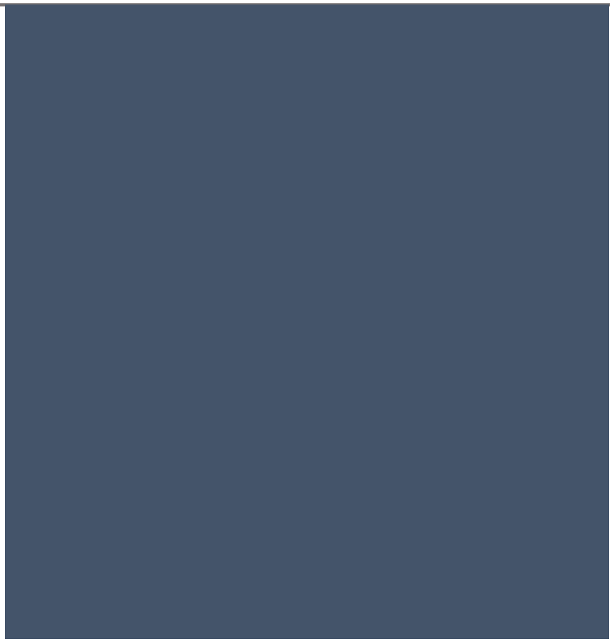


Chapter 1

General Introduction

Mohamed Osman

1.1. Wheat

Wheat (*Triticum aestivum* L.) is a valuable cereal crop that belongs to the grass family Gramineae (Poaceae) and has long been a staple food for nearly 40% of the world's population (Weise 1987) in many parts of the world (Curtis et al. 2002). It was domesticated, like many other cereal crops, in the Fertile Crescent during the period of agriculture development over 10,000 years ago and cultivated forms appeared (Salamini et al. 2002). Einkorn and emmer wheats, the ancestors of modern wheat, were harvested and cultivated in the so-called Fertile Crescent of south-western Iran, North Iraq, and south-eastern Turkey (Fig. 1.1), where wild wheat can still be found. The spread of bread wheat can be traced to the human migration to Asia and Europe, and then to the Americas (Zohary and Hopf 2000).

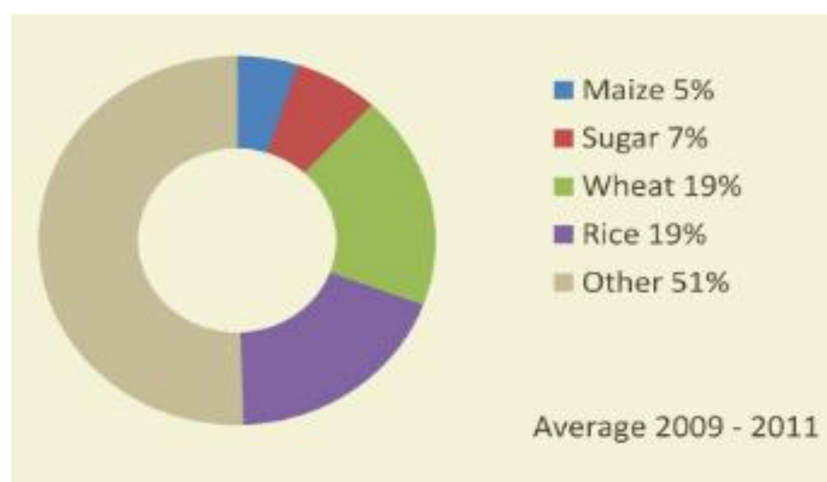
Fig. 1.1 The fertile crescent where wheat was domesticated 10,000 years ago

Wheat is one of the world's most popular crops that has played a principal role in supplying a hungry world and enhancing global food security. It is grown throughout the world under a broad range of conditions and soil fertility in extensive acreage covering larger land-area than any other crop (FAO 2013) with more than 220 million hectares in more than 70 countries on five continents planted annually and in many geographic

regions. Bread wheat covers about 90 percent of the world wheat area and makes up about 94% of production. Nowadays about 20% of the globally cultivated land is occupied by wheat. Global wheat utilization range from food (65%), feed (17%), and other uses including biofuels (12%). 95% of wheat consumption as food is from hexaploid wheat utilized for bread, cookies and pastries; the remaining 5% is from tetraploid durum that is usually used for pasta and other semolina products (Shewry 2009). Wheat production in 2011 (667.2 million T) grew by 10.2 million tonnes over 2010 (657.2 million T) (FAO 2011).

Wheat is an important source of carbohydrate, protein, vitamins and minerals and according to the Food and Agriculture Organization (FAO). It provides more than 20% and 18% of the world's protein and carbohydrate supply, respectively (FAO 2013), and according to Oleson, (1994) wheat is the most popular and staple crop that is extensively grown, traded and consumed worldwide. It is consumed in different ways by humans with minimal processing. Its flour is the basis of bread, biscuit and pastry products. Also, wheat is used widely in breakfast cereals, bulgur, macaroni and is also a commercial source of starch. People in developing countries consumed 70 kg/per capita/year on average in 2005–2009, as compared to 190 kg/per capita/year in developed countries.

Figure 1.2 The contribution of major world crops in total consumed calories



(http://www.fao.org/)

The ploidy changes that occurred during evolution can be obviously demonstrated in wheat. The domestication of wheat began with diploid wild relatives (2n=14) and then gradually shifted to the modern durum (4n=28) and common wheat (6n=42) (Cook and Veseth 1991). Based on the time of year in which wheat is sown two types of bread wheat, spring wheat and winter wheat, are grown. Winter wheat genotypes are planted in autumn and only grow preliminary before cold winter arrives wherein seedlings remain dormant. This period is usually referred to as vernalization period during low temperature, often combined with short day length that is essential to trigger flowering genes and initiate reproductive growth of winter wheat (Trevaskis et al. 2007). In the spring, wheat seedlings resume growth and grow rapidly until summer harvest. Spring wheat, on the other hand, is sown in the spring and harvested in mid-summer.

Since the 1960s, the total global demand for wheat has almost increased fourfold and due to the continuous rise in our population the demand for wheat is still growing. About two-third of the world demand for wheat arises from the developing countries. Nowadays, 71% of global wheat production is mainly used for food while less than 20% is utilized for feed. In order to meet global demand, it is assumed that annual wheat production need to grow from their current level of below 1% to at least 1.7% (http://www.wheatinitiative. org), and to feed world population in 2020; we need about 110 million tons of wheat.

The Green Revolution is considered as the most remarkable achievement of crop breeding and has a big contribution to increasing agricultural production in several regions worldwide. It took place during the 1960s and 1970s following the introduction of semi-dwarf high yielding varieties, accompanied with high production packages (mineral fertilizers, pesticides, irrigation, etc.) to significantly improve the cropping environment. Afterward, low-yielding wheat landraces in developing countries were replaced by semi-dwarf and high-yielding ones (Murphy 2007), predominately by the introduction of the Rht (reduced height) genes into modern wheat varieties. The development and distribution of these improvements was sponsored and coordinated by International plant-breeding institutes of the Consultative Group on International

Agricultural Research (CGIAR). The genetic diversity of wheat has been drastically narrowed down as a consequence of farmer selection for high production, intensive monoculture and modern plant breeding especially after the green revolution where few parental lines have been used consistently for breeding semi-dwarf, fertilizer responsive wheat varieties (Tanksley and McCouch 1997; Roussel et al. 2005).

1.2. Fusarium head blight

1.2.1. Importance

Wheat, barley, rice and maize the four most important small grain cereals along with other crops are continuously threatened by several *Fusarium* species that cause Fusarium head blight which is a devastating disease worldwide. Mycotoxin contamination of the grains following the colonization of the kernels as well as destroying starch granules, storage proteins and cell walls adversely affect the grain quality and lead to reduced seed germination (McMullen et al. 1997; Goswami and Kistler 2004). Outbreaks are associated with the wet and warm weather during anthesis and early dough stages and by cropping patterns in a given area. It has reached epidemic levels in several parts of the world including USA, Canada, Europe, China and South America (Bai and Shaner 1994; Goswami and Kistler 2004; Parry et al. 1995; Sutton 1982). Attributable to high yield losses that may reach 50%, FHB has become a major threat to the global food supply and safety, and is considered by the International Maize and Wheat Improvement Centre as a major limiting factor of worldwide wheat production and food safety. The United States Department of Agriculture called FHB the worst plant disease to hit wheat in the United States since the stem rust epidemics of the 1950s.

Mycotoxins such as zearalenone, HT-2 toxin, T-2 toxin, nivalenol, and DON and its acetylated forms (3-ADON and 15-ADON) are frequently formed in Fusarium-infected wheat and barley (Salas et al. 1999; Buerstmayr et al. 2012). DON is considered to be the most economically important toxin produced by *F. graminearum* (Culler et al. 2007)

and has been shown to be a virulence factor in FHB (Bai et al. 2002; Jansen et al. 2005). In the EU, legally enforceable thresholds in grain and food products allow a maximum DON content in unprocessed cereals other than durum wheat, oats and maize of 1.25 ppm, in bread and biscuits of 0.5 ppm and in baby food for infants and young children of 0.2 ppm (European commission 2006).

The 15-ADON chemotype was reported to be prevalent in the United States (Gale et al. 2007; Schmale et al. 2011), Mexico (He et al. 2013; Miller et al. 1991), UK (Jennings et al. 2004a&b), Central Europe (Talas et al. 2011), and Southern Russia (Yli-Mattila et al. 2009). However, a shift from 15-ADON to 3ADON genotypes has been detected in the last decade in many locations in North America including the US and Western Canada (Gale et al. 2007; Ward et al. 2008). A probable interpretation of this change is that the introduced 3-ADON isolates compete better than the local 15-ADON isolates in those areas (Guo et al. 2008); since *F. graminearum* 3 ADON isolates were reported to produce more trichothecenes and cause greater FHB severity on wheat compared with 15-ADON isolates (Puri and Zhong 2010; von der Ohe et al. 2010; Zhang et al. 2012). Therefore, the change in their relative frequencies is of high significance. The TRI genes, like other secondary pathway genes of fungi, are often arranged in clusters within the genome, encoding for enzymes involved in the trichothecene biosynthesis of the fungus (Keller and Hohn 1997; Pasquali and Migheli 2014). Since the variation in trichothecene production reflects the allelic polymorphisms of *TRI* genes (Amarasinghe et al. 2011; Ward et al. 2002), PCR assays were developed in the last two decades to characterize populations of toxigenic Fusaria rapidly in terms of their chemotypes, greatly facilitating studies on the diversity and mycotoxin potential of FHB pathogens worldwide (Chandler et al. 2003; Gale et al. 2007; Guo et al. 2008; Lee et al. 2001; Scoz et al. 2009; Starkey et al. 2007).

1.2.2. Host range

FHB can infect numerous commercial crop plants such as wheat, barley, maize, rye, rice and several other monocot plants (Shaner 2003; Tekle et al. 2012; Gilbert and Tekauz 2000; Desjardins et al. 2004; Schmale et al. 2005). Over the years, the host range of the FGSC has expanded from cereal to non-cereal crops such as dry bean, canola, potato agropyron, agrostis and bromus without causing disease symptoms (Goswami and Kistler 2004; Burkaloti et al. 2008).

1.2.3. Taxonomy of the pathogen

FHB of cereals is caused by several *Fusarium* species in humid and semi-humid farming areas, and the most frequent are *F. graminearum* sensu lato (referred to hereafter as *F. graminearum*) and the related species *F. culmorum* and *F. cerealis* (Buerstmayr et al. 2012; Mesterházy 1978; Miller et al. 1991; Pasquali and Migheli 2014; Xue et al. 2006). *F. graminearum* is the most important and the predominant causal pathogen of cereal FHB China (Zhang et al. 2012), Japan (Koizumi et al. 1991), Turkey (Yoruk and Albayrak 2012), North America, Brazil and many other parts of the world (Alvarez et al. 2009; Astolfi et al. 2011; Goswami and Kistler 2004; Ward et al. 2008). *F. culmorum* has been traditionally reported as a chief causal agent of FHB in Northern, Central and Western Europe (Hope et al. 2005; Wagacha and Muthomi 2007); but a shift is being noticed lately in Europe, and *F. graminearum* has been spreading northward in Europe (Osborne and Stein 2007; Waalwijk et al. 2003; Scherm et al. 2013). *F. cerealis* is another toxigenic FHB related species. It is more frequent in humid temperate areas (Burgess 1982) and has been isolated sporadically from infected spikes (Schmale et al. 2011; Sugiura et al. 1994).

Johann Heinrich Friedrich Link (1767-1851), a German mycologist, used the name *Fusarium* to refer to a group of fungi having fusiform spores in 1809 (Stack 2003; Booth 1971). The sexual stage of *F. graminearum*, the main causal agent of FHB, is a

homothallic ascomycete fungus (Goswami and Kistler 2004), but it also can outcross. It was named by Schwabe in 1838 to its present name. Although variation in aggressiveness in populations of *F. graminearum* has been observed, there is no evidence of the presence of physiological races in populations of this pathogen (van Eeuwijk et al. 1995). Many studies have shown that FHB resistance in wheat is horizontal, not species- nor strain-specific (Van Eeuwijk et al. 1995; Mesterhazy et al. 2005).

Teleomorphs are not known for all *Fusarium* species, and the teleomorph of the majority of *Fusarium* species belongs to the phylum Ascomycota, class Ascomycetes, order *Hypocreales*. The most common teleomorph belongs to the genus *Gibberella*, which is linked to the majority of the important pathogens including *Fusarium graminearum* (*Gibberella zeae*), *F. verticillioides* (*G. moniliformis*), in addition to other species (Kvas et al. 2009). While only a small number of *Fusarium* species have teleomorph in *Hemanectria* and *Albonectria* genera, all in the order *Hypocreales* and have *Fusarium* as their imperfect stage (Leslie and Summerell 2006; Rossman et al. 1999). Teleomorph of *F. graminearum*, *G. zeae*, is classified as follows (Goswami and Kistler 2004):

Kingdom Fungi

Phylum Ascomycota

Subphylum Pezizomycotina

Class Sordariomycetaidae

Subclass Hypocreomycetidae

Order Hypocreales

Family Nectriaceae

Genus *Gibberella*

The major pathogen of FHB, *F. graminearum*, resides in the section Discolor; which contains some of the world's most important cereal pathogens. The fungi belonging to

this section are characterized by the production of carmine-red mycelium on high carbon sources, banana shaped thick-walled, distinctly septated macroconidia and absence of microconidia (Liddell 2003; Leslie and Summerell 2006).

1.2.4. Life Cycle

Fusarium graminearum is a facultative parasite of wheat; its lifecycle includes a saprophytic stage in which it overwinters on crop residue, and a parasitic stage causing FHB of wheat (Miedaner et al. 2001; Sutton 1982). Following harvest, the remaining host debris (e.g. corn stalks and ears, diseased kernels left in the field and wheat straw) on which the fungus overwinters as saprophytic mycelia until the next spring, thereby completing the disease cycle (Bai and Shaner 2004, Goswami and Kistler 2004). During the saprophytic phase, the pathogen derives nutrients from decaying organic material. This makes infected crop debris the primary source of inoculum for FHB (Shaner 2003, Bai and Shaner 2004) and arguably the amount of primary inoculum is related to the mass of crop debris that is left in the field post harvest of an infected crop. Besides, Shaner (2003) reported that the more slowly host tissues decay, such as nodal tissues, the longer *F. graminearum* survives. For the same reason survives longer in maize residues that resist breakdown more than residues of other cereal crops.

The fungus survives between crops as mycelia, macroconidia, chlamydospores and perithecia on wheat residue (Gilbert and Fernando 2004; Guenther and Trail 2005; Bai and Shaner 1994). The multiple modes of survival and wide range of host species ensure its survival, proliferation, and dissemination in the environment. Warm, humid weather in spring is favorable for the development and maturation of conidia and perithecia that produce ascospores concurrently with the flowering of cereal crops during which wheat is most susceptible to FHB throughout anthesis and early grain development (Markell and Francl 2003).

The abundance of primary inoculum and weather conditions during and after anthesis determine the severity of the disease determine the occurrence of FHB epidemics (Bai

and Shaner 1994). When natural inoculum is abundant during warm and humid weather during flowering, the risk of an FHB epidemic is high (Sutton 1982). Both ascospore sand macroconidia were found during all collection periods throughout anthesis and early grain development with a few exceptions (Markell and Francl 2003). Therefore, it was concluded that the presence of sexual and asexual spores during the infection periods suggests they are both important inoculum sources. However, in another study it was found that the proportion of ascospores within single wheat heads varied from 40 to 90% and the average ratio was two ascospores for every macroconidium. Under natural conditions, ascospores are the primary inoculum for FHB produced by the sexual state *G. zeae* in fruit bodies called perithecia that develop on mycelia on the crop residue (Sutton 1982; Shaner 2003; Trail 2009). They contribute to the local inoculum but may also travel for longer distances so that airborne inoculum produced outside the field can initiate disease (Fernando et al. 1997). Hence, ascospores may originate from multiple geographic locations and are transported over long distances directly to flowering spikes of cereal crops (Parry et al. 1995). They are discharged from perithecia in the evenings or nights when temperature decreases and relative humidity (RH) increases and are distributed by the wind (Paulitz1996). Macroconidia, the asexual spores, are produced by *F. graminearum* on the surface of the crop residue when damp and humid conditions are present. These are mainly splash-dispersed over short distances from debris (Gilbert and Fernando 2004, Champeil et al. 2004).

The spores that land in a floret during anthesis germinate and developing mycelium penetrates the ovary and establishes itself in the embryo and remains in the seed. Germination of macroconidia and ascospores occurs under similar conditions. The germination of *Fusarium* conidia is influenced and dependent on environmental conditions such as temperature, moisture and spore density (Colhoun et al. 1968). When optimal growth conditions are met, macroconidia germinate in as little as 2 hours (Beyer et al. 2004). However, in general, germination of macroconidia occurs 6-12 hours after inoculation (Pritsch et al. 2000, Kang and Buchenauer 1999).

Between 36-48 hours after germination of conidia, the hyphae penetrate tissue directly or occasionally enter via stomata. Growing mycelia contact the glume and penetrate through its stomata. Afterwards fungal hyphae penetrate the epicarp and spread through the seed coat, colonizing the different layers of the seed coat and the endosperm (Jansen et al. 2005). In areas experiencing moderate to severe epidemics, Francl et al. (1999) found that on average 20 colony forming units could be detected on wheat spikes daily, suggesting that multiple infections contribute to FHB epidemics.

1.2.5. Disease Symptoms

Initial FHB symptoms are characterized by small water-soaked tan or brown discoloration at the base of a floret. These lesions on the infected spikelets lead eventually to premature bleaching of an individual or several spikelets during anthesis and early dough stages when the healthy heads are still green (Fig. 1.2). To spread from the initial point of infection to the next spikelets in all directions, *Fusarium* needs to reach the xylem tissue of the rachis. At which point, it will potentially damage vascular transport, block the movement of water and nutrients through vascular tissues and cause the typical premature bleaching symptoms of wheat spikelets (Kang and Buchenauer 2002).

Fig. 1.2 FHB typical symptoms, bleaching of central spikelets while the rest of the head still green.

(http://www.ag.ndsu.nodak.edu)

1.2.6. FHB resistance

1.2.6.1. Passive resistance

The term passive resistance is usually used to refer to resistance mechanisms associated with plant structure or morphology rather than plant reaction per se. For example, traits such as plant height, the presence of awns, head compactness, head morphology, peduncle length, time of flowering, span of flowering, the position and diversity of florets, the width and duration of flower opening, and anther extrusion are known to have a kind of relation with FHB incidence. (Miedaner 1997; Mesterhazy 1995; Rudd et al. 2001; Buerstmayr et al. 2009; Gilsinger et al. 2005; Taylor 2004). Although all of these traits have the potential to influence FHB resistance of wheat, their impact remains less

important compared to active resistance (Rudd et al. 2001). Studies have shown that this type of resistance is not consistent across years or environments and can confound results when trying to evaluate active FHB resistance (Kolb et al. 2001; Parry et al. 1995). Therefore, it is necessary to recognize the passive resistance mechanisms present in the material.

The wheat head is composed of differing numbers of opposite spikelets that are positioned along the rachis. Each spikelet encloses multiple perfect florets, and each floret is surrounded by the palea and lemma. Flowering time, duration and the width of opening also interfere with host resistance. The coincidence of wheat flowering with environmental conditions favorable for spore dispersal will more likely lead to higher infection with FHB. Bushnell et al. (2003) reported disease escape of wheat cultivars that open for flowering in the middle of the day avoiding water splash of dew droplets. On the other hand, genotypes with a shorter flowering period have a lower chance to be infected, and this is known as disease escape. The degree to which a flower open is also thought to affect disease incidence because genotypes with narrow opening florets were found to be less prone to FHB infection than genotypes with wider opening florets. This could be attributed either to one or both of the following facts:

- The fact that more time and space will be provided in wider opening florets in which *Fusarium* spores can access the floret and initiate infection (Gilsinger et al. 2005) limiting the probability of spores landing inside the floret.
- Due to the higher percentage of retained anthers within the florets of narrow opening genotypes leading to less FHB incidence.

Since anthers are required for the initial FHB infection (Dickson et al. 1921, Pugh et al. 1933), the lack of anther extrusion in these cultivars may have prevented FHB infection. However, Skinnes et al. (2008) demonstrated that even low anther extrusion is correlated with susceptibility to FHB infection.

Waxy glumes may serve as a barrier to the fungal infection and help to exclude moisture, and tight glumes may limit access of airborne inoculum to wheat flower organs. Although the degree of flower opening is considered to be a heritable trait, environmental conditions at anthesis, such as moisture stress, light intensity, and air movement, can greatly influence it.

1.2.6.2. Active resistance

FHB resistance in wheat is complex; it reacts to *Fusarium* infection by inducing various defense mechanisms (Walter et al. 2010). To date, five types of resistance have been discerned (Schroeder and Christensen 1963; Wang and Miller 1988; Mesterhazy 1995).

Type I

FHB type I resistance was detected for the first time by Schroeder and Christensen (1963) who described it as wheat resistance to initial infection or what is also known as the invasion. Sometimes it is also referred to Type I resistance as delay in initial infection.

Type I resistance is considered the host's first barrier to infection by *Fusarium* species and is essential for FHB-resistance breeding since it is necessary to estimate field performance of wheat genotypes. Although the significance of this component of FHB resistance is increasingly realized; the evaluation process of type 1 resistance is challenging.

This type of resistance is suggested to be best monitored under low disease pressure. It can be assessed in naturally infected or spawn inoculated field experiments that mimic naturally occurring infections. However, spray inoculation, which reduces the risk of disease escape due to plant height and late maturity, is more commonly used and it comprises both Type I and Type II. Type I resistance is estimated by spraying a spore suspension over flowering spikes and counting the diseased spikelets. If a genotype has

a high level of type I resistance, fewer heads will show disease (Bai and Shaner 2004, Buerstmayr et al. 2009).

Type I resistance is evaluated by different methods, often by estimating the disease incidence or percentage of symptomatic spikes of the total number of spikes after spray inoculation or natural infection in field nurseries, with the former including both type I and type II resistance. (Bai and Shaner 2004, Dill-Macky 2003, Buerstmayr et al. 2009). Reduced disease incidence is a useful indicator of type I resistance and can be figured out in parallel trials using moderate concentration of inoculum, under moderately favorable environmental conditions since type I resistance can be easily overwhelmed (Schroeder and Christensen 1963). Spikes are evaluated for resistance a fixed number of days post inoculation, depending on the prevailing conditions and the disease reaction of the resistant and susceptible checks. The interval (25 to 35 days) between inoculation and evaluation is variable from year to year but within the same year a fixed number of days is accepted. For example, He et al. (2014) reported that 25 days is appropriate under Mexican conditions.

Another adopted measure of type I resistance is counting the number of symptomatic florets within head seven DAI before symptoms have time to spread to non-infected spikelets (Bushnell et al. 2003); because the number of infected spikelets after 21 DAI does not necessarily reflect the damage caused by initial infection. The resistance level corresponds to the percentage of blighted florets on the susceptible control.

Type I resistance can be figured out following evaluation by both point (type II) and spray (type I plus II) inoculation as a difference between point and spray inoculation disease indices (Mesterhazy et al. 2008).

Gosman et al. (2009) suggested a new methodology based on using non-DON-producing FHB related pathogen i.e. nivalenol (NIV)-producing isolates of *F. graminearum* that are known to spread very slowly to a limited extent within the wheat head to distinguish wheat genotypes with FHB type I resistance.

Despite many reports on its importance, there still concerns regarding using type I resistance. For example, the term “initial infection” for type I has not been precisely specified, and everybody had his own definition widely diverging in content and type I resistance can be overwhelmed when a large amount of inoculum is directly applied on wheat heads, making it difficult to notice differences in type I resistance between cultivars (Bai and Shaner 2004). Therefore, a moderate concentration of inoculum should be used for this purpose. Reports have further indicated that type I resistance is easily overcome under epidemic conditions, and after that type II resistance is needed to as the next defense component to reduce FHB losses (Mesterházy 1995). It may be passive involving morphological avoidance features of the wheat head that generate an unfavorable environment for infection establishment on host surface tissues (Mesterhazy 1995) and may be confused in the field with escape disease mechanisms. Estimation of type I resistance is not always accurate and can be confused with type II resistance in the field. Moreover, resistance to initial infection does not necessarily grant protection against DON accumulation. Last but not least, type I resistance is unstable in comparison with relatively stable type II and type III resistance due to a relatively lower heritability for type I resistance implying that it is more affected by nongenetic factors (Bai and Shaner 2004; Kolb et al. 2001). In other words, reproducibility of results among experiments designed to quantify type I resistance has been problematic. It should always be taken into consideration that if a genotype exhibits type I resistance, FHB incidence is low but does not necessarily grant resistance to FHB spread, in which case disease would be restricted to the area but not in severity. Hence, a combination of type I and type II resistance is more effective to deal with such cases, and genotypes with a combination of type I and type II resistance should receive greater attention for future targeted crosses.

Type II

Type II resistance was first suggested by Schroeder and Christensen (1963) who noticed that the resistant cultivars have a kind of prevention of hyphal spread inside the head. During head colonization intercourse, the fungus spread from spikelet to spikelet via the rachis. However, genotypes with type II resistance respond to fungal spread by inducing depositions of heterogeneous materials at the point of entry to form a "barrier" which potentially retards head colonization (Kang and Buchenaur 2000). Another study considered FHB type II resistance as a consequence of genotype's ability to detoxify DON because high type II is usually associated with low DON content that is involved in FHB-disease spread.

Type II resistance has been the most studied among other FHB resistance components because of its has high heritability (Bai and Shaner 2004; Mesterhazy 1995; Kolb et al. 2001). It is also more robust for evaluation and less affected by environmental factors than type I resistance since it is usually evaluated under controlled conditions (Bai and Shaner 1994).

The best-known genotypes with type II resistance belong to a related group of cultivars from Nanjing, China (Bai and Shaner 1996). This group of wheat genotypes include the Chinese cultivar Sumai 3, its derivatives along with other cultivars that exhibit high levels of FHB type II resistance and are widely used in international wheat breeding programs (Bai and Shaner 2004).

Type III

During the process of wheat head colonization by *F. graminearum*, trichothecene mycotoxins including DON, nivalenol and their acetylated forms 3-ADON and 15-ADON, T-2 toxins may be produced in high amounts in tissues of susceptible genotypes (Mesterházy et al. 2005). Wheat resistance to toxin accumulation (especially DON) is recognized as type III resistance (Mesterhazy 1995) and has gained particular

importance, due to concerns regarding food safety. Type III resistance is usually assessed as DON content of infected kernels using traditional chemical or immune assays. The difficulty with using DON content regularly as a tool for selecting resistant genotypes is attributed to the lack of test facilities and cost of analysis. Testing numerous lines in a breeding program for routine selection of low DON-accumulating genotypes each year is not possible. Therefore, breeders usually depend on selection based on other related characters such as FHB severity or percent scabby seed has been proposed (Bai et al. 2001, Mesterhazy 1999).

Regulation of DON content, however, depends on a number of factors including host genotype, pathogen population in addition to environmental conditions and their complicated interactions with the host and pathogen genotypes (Miedaner and Perkowski 1996; Mesterházy et al. 1999). Generally speaking, the more susceptible the wheat genotype is, the higher the DON concentration will accumulate (Bai et al. 2001). Mesterhazy et al. (2002) concluded that the effect of wheat genotype is more important in regulating DON accumulation than the impact of pathogen's genotype. Different mechanisms are hypothesized about type III resistance:

1- Prevention of DON synthesis by the pathogen (Miller et al. 1985).

2- DON is produced, but it is degraded in resistant wheat genotypes later on (Miller et al. 1985; Mesterhazy et al. 2002).

3- Resistant genotypes prevent DON movement into kernels (Bai and Shaner 2004).

Given the impact of mycotoxins on grain quality, type III resistance has recently drawn considerable attention from breeders.

Type IV

Mesterhazy (1995) suggested a new type of resistance named type IV resistance after realizing that genotypes with the same level of field resistance do not necessarily have

similar resistance to kernel infection. Type IV is considered as resistance mechanism that counteracts reductions in kernel number, weight or test weight, as well as the presence of tombstones (Rudd et al. 2001). It can also be defined as the ability of a genotype to restrict GVW loss despite *F. graminearum* infection and colonization. Grain filling process is interrupted as a consequence of blockage of water and nutrients movement within the infected head leading to small chalky shriveled kernels called Fusarium damaged kernels (FDK) which represent the damage level caused by FHB on wheat kernels. However, it was proposed that some wheat genotypes possess a mechanism enabling them to reduce kernel damage even if the chaff is displaying severe symptoms.

Type IV resistance could be attributed to the presence of specific metabolites that make kernel environment unfavorable for fungal growth and DON production, the resistance of distinct layers of the seed coat leading to a reduction in chaff-to-kernel movement of the fungus, as well as the limited movement of DON from the chaff to the kernel. All of these factors may result in less colonized kernels than expected based on chaff symptoms (Mesterhazy 1995; Mesterhazy et al. 2005).

Several approaches could be used to assess type IV resistance such as digital image analysis (Agostinelli et al. 2007), near infrared reflectance (Delwiche and Hareland 2004) and air separation (Agostinelli et al. 2007; 2008). Generally, resistance to kernel infection is measured by:

- Threshing infected spikes and observing the damaged grains (Mesterhazy 1995). This method is considered as an indirect measurement based on visual assessment of kernels that show symptoms of FHB colonization, including shriveled and reduced-size kernels that are usually called tombstones. Tested samples here are compared with reference samples (Jones and Mirocha 1999). Visual comparison of samples is a quick way of assessing FDK, but it is arguably too subjective.
- Manual separation of damaged and healthy kernels (Verges et al. 2006). On the other hand, manual separation is less subjective but it is very time-consuming.

Type III resistance is measured post-harvest by counting FDK and measuring test weight as an indication for healthy seeds (Rudd et al. 2001).

- Percent GVW loss, a direct measurement of type IV resistance, yielded similar results to those obtained using percentage FDK. The protocol includes calculation and comparison of a GVW loss in inoculated versus non- inoculated field trials. Type IV resistance is also evaluated sometimes on the basis of fungal biomass (ergosterol or DNA content).

Resistance to kernel infection (Type IV resistance) is a logical concept, but practically it poses problems. To correctly evaluate Type IV resistance, the following points should be considered:

- Every tested kernel should be exposed to infection.
- It is often difficult to evaluate type IV resistance without using genetic stocks that have a similar level of type I and type II resistances.
- Point inoculation can not be used to assess type IV resistance because genotypes with FHB type II resistance will also show high type IV resistance but, in this case, type II resistance which prevents or delays fungal movement within the head is behind the healthy grains and type IV has not the chance to express itself. Therefore, spray inoculation and spawn inoculation might be more suitable.
- There are more basic concerns about this type of resistance: Does type IV resistance refer to fungus ability to colonize a kernel or to the degree to which the fungus damage the grain?

However, estimation of FHB resistance using type IV resistance as a parameter still has many advantages i.e. FDK measurement appears to be more efficient than chaff symptom evaluation for FHB assessment because type IV resistance is related to kernel health and it directly measures FHB damage in grain. Additionally, grain coming from different fields can be evaluated in the same place and sampling randomization can be simply done by mixing the grain. It is also noteworthy that timing is not a concern since the results will not be affected by the time in which the evaluation was done.

Based on the mentioned reasons, it was concluded that FDK measurement appears to be more efficient than chaff symptom evaluation for FHB assessment without risking significant loss of information when using percent FDK as the disease index.

Type V

Type V resistance was also proposed by Mesterhazy et al. (1999) to refer to host's ability to resist yield loss despite infection and disease symptoms or what is called tolerance. In other words, type V resistance is behind the variation in yield loss among genotypes that have similar FHB symptoms. Type IV resistance or yield tolerance can be estimated by assessing grain yield of naturally or artificially inoculated plots to compare it with the yield of healthy plots (Mesterhazy et al. 1999). Although the comparison on a single infected/uninfected head is reported (Rudd et al. 2001), it may not be as representative as data obtained from whole plot.

1.2.7. FHB inoculation techniques and disease evaluation

To accurately evaluate the resistance of different wheat genotypes, favorable conditions for artificial epidemic establishment should be created. FHB reaches epidemic levels when conditions were favorable. These conditions had to be created by artificial means. Based on the targeted resistance component, the number of lines under evaluation, and available resources, different inoculation techniques that introduce inoculum into or on the wheat spike at 50% anthesis have been implemented.

The method used for disease establishment should minimize environmental and experimental variability. Appropriate inoculation techniques should mimic the natural infection process as closely as possible. It is also worthy to note during the production of inoculum, the use of a mixture of isolates that represent the local population is desirable to avoid the misinterpretation of a cultivar's resistance with the use of single strain. Despite not being any host-pathogen specificity present among FHB causing *Fusarium*

species, varying pathogenicity among isolates can exist. Though a complex of pathogens causes FHB, there is no known host-pathogen specificity.

1.2.7.1. Natural infection

FHB resistance can be evaluated under natural infection in wheat growing areas where frequent FHB epidemics usually take place. Although natural infection can considerably reduce related to pathogen isolation, identification, inoculum production and inoculation, nobody can guarantee the epidemic occurrence in a particular region and/or season. For example, in China, the epidemic frequency was once every four years as reported by Liu and Wang (1991).

1.2.7.2. Single Floret Inoculation or Point inoculation:

Point inoculation is used to evaluate Type II resistance. The delivery of *F. graminearum* spores to the wheat spike at the proper time is the principal concern in screening protocols. Point inoculation is usually carried out under controlled conditions by inoculating a single central floret. However, this technique can also be used in the field (Gilchrist et al. 1996; Mesterhazy 1997).

To evaluate spread of FHB symptoms in the head, a determined quantity of spore suspension is placed into a central spikelet at mid-anthesis (Stack 1989; van Ginkel et al. 1996) using a Hypodermic syringe, micropipette, batting small tufts of cotton soaked in the inoculum, and colonized wheat or millet seeds have all been reported as inoculum delivery means (Rudd et al. 2001). When Hypodermic syringes or micropipette are used, the volume of inoculum used ranges from 5 to 10 µl per floret of a macroconidial spore suspension delivering 500-1000 conidia/floret (Wang and Miller 1988; Bai and Shaner 1996). Following inoculation, plants are incubated at 20-25°C and high air humidity for 1-3 days. Either bagging inoculated heads using inverted glassine bags over hand-misted heads or misted chambers are usually used to maintain high after inoculation. Initial

symptoms can be observed within three days after inoculation (DAI) in susceptible wheat genotypes. However, the disease spread to the adjacent, non-inoculated spikelets could not be seen before six DAI. One disease evaluation is perhaps sufficient 21 DAI although some programs record the progression of the infection over time by assessing the number or percentage of diseased spikelets several times to calculate the area under the disease progress curves.

The measurement of FHB spread within head provides one of the most reliable estimates of cultivar's resistance that is less influenced by variable environmental conditions as compared with other inoculation techniques. (Schroeder and Christensen 1963; Wang and Miller 1988; Bai and Shaner 1996). This inoculation method is not affected by plant height or growth stage and can be conducted under controlled environmental conditions, which are critical for conducting genetic and mapping studies. The inoculum can be quantitatively applied. This method could be quickly and reliably used for evaluating the resistance of hyphal spread of elite materials, advanced lines, and even for the genetic study of scab resistance. Single floret inoculation is labour-intensive, and this test quantifies only type II resistance.

1.2.7.3. Spray Inoculation

This method is commonly used in field research to evaluate large numbers of lines, but it is also employed in indoor experiments. Field plot-scale spray inoculation gave higher heritabilities than point inoculation for the percentage of infected spikelets in a study with 20 genotypes across seven environments. Mesterhazy (1995) concluded that spray inoculation is preferred to point inoculation for assessing wheat FHB resistance in the field. Since it ensures that each plant receives a comparable amount of inoculum and provides an adequate level of disease pressure for selection pressure to handle mechanisms of passive resistance such as the presence of awns, the degree to which florets are open, and retention or release of anthers. So that the chance that an entry escapes infection is minimized.

Simultaneous evaluation and selection of type I, type II and other types of resistance, which eventually reflects overall field resistance can be achieved using this method since it provides proper conditions for such selection (Chen et al. 2000). Spray inoculation, in contrast to single floret inoculation, is also suitable for mass screening, and single plots can be inoculated individually according to their date of flowering.

A spore suspension is sprayed on the ears at flowering to minimize variability due to differences in plant height as well as growth stage and ensures homogenous inoculation. Macroconidia are usually used as inoculum (Mesterhazy 1978; Snijders and Perkowski 1990). Concentrations of macroconidial suspension vary typically from $5x10^4$ to 10^5 living macroconidia/ml (Luzzardi 1984) and the amount of applied inoculum ranges between 50 and 200 ml/m2. Hand-held sprayers, backpack sprayers or tractor-mounted sprayers can be used to apply inoculum. Inoculations are applied on flowering wheat plants in individual plots when 50% of plants have reached anthesis by spraying the conidial suspension. Inoculated plots are often sprayed again 2 to 7 days later to catch late spikes that were not in anthesis during the first application of inoculum. Inoculation is repeated until the last line in the FHB nursery reaches flowering (Buerstmayr et al. 2012). To correctly use spray inoculation certain factors should be taken into consideration:

- Inoculations are carried out during the afternoon and early evening to avoid strong solar insolation.
- Mist-irrigation is used during the evening in most FHB nurseries to maintain an adequate level of moisture that is critical to promote disease development (Snijders and Perkowski 1990). Irrigation starts on the first day of inoculation, and completes within three weeks after the last inoculation, giving enough time to evaluate the whole genotypes.
- Standardization of inoculation dates to physiological stages of the host in FHB nurseries is important to minimize erratic selection for late maturity (Mesterhazy 1983).

- Even resistant material could be considerably damaged when inoculated with highly concentrated spore suspension under favorable conditions for scab.

1.2.7.4. Spawn inoculation

One of the largely used methods for mass screening in field nurseries is spawn inoculation. In this method inoculum is not applied directly on wheat heads at flowering as in spray inoculation; instead infested grain spawn is spread throughout the field on the soil surface between and among the plots 2-4 weeks before flowering, from which the inoculum spread out. The inoculation should be repeated more than two times to ensure constant disease pressure that is especially needed when the evaluated lines differ largely in days to heading (Paulitz 1996).

The grain spawn is produced in the lab using wheat, corn, barley or millet kernels but some programs simply use FHB-infected wheat or inoculated corn residues in the offseason. Moisture levels of the heads and near soil surface should be maintained by mist or sprinkler irrigation to promote perithecia formation by the time of wheat flowering for successful infection. Disease assessment is usually done 21-30 days after anthesis; therefore, notes should be taken including the exact date of anthesis for each plot individually.

This method probably is the closest to simulating natural epidemics and reasonably represents the true field performance of a particular genotype Rudd et al. (2001). Furthermore, using of Fusarium colonized grain as the primary source of inoculum is convenient because it is relatively easy, inexpensive, only requires low labour input and can be used to screen large numbers of wheat genotypes in the field.

However, many factors can considerably affect the amount of infection/disease pressure resulting from this inoculation method like plant height, moisture conditions and the degree to which florets open (Chen et al. 2000) and may lack accuracy in both uniformity and timing of infection. In other words, uniform infection of the field plots is dependent

on suitable environmental conditions being conducive for spore production and release. Therefore, many susceptible plants may escape infection (Bai and Shaner 1994) and lower disease levels in some genotypes may not be necessarily attributed to their resistance, but rather be a function of later maturity or lack of suitable conditions for infection at the crucial growth stage.

1.2.8. Breeding for FHB resistance

Several strategies have been utilized to combat FHB and increase food safety including crop rotation, deep cultivation, and stubble burning. However, these tactics have not always given satisfactory results. Moreover, affordable and efficient fungicide are not available and do not necessarily lead to reduced mycotoxin levels in the treated crop (Jones 2000; Mesterházy 2003). Continuous efforts are in progress to control FHB in order to attain sustainable wheat production are needed worldwide. Arguably the most effective, economical and environment-friendly means to combat this significant disease is breeding and deployment of FHB resistant cultivars and reduced mycotoxin accumulation that has become a high priority of many wheat breeding programs (Parry et al. 1995; Ruckenbauer et al. 2001; He et al. 2013). It is an important component in the integrated FHB control programs that has the potential to reduce the need for fungicide application considerably and increase food safety consequently.

The first report mentioning the differences in response among wheat genotypes to FHB was as early as 1891 by Arthur, who was the first to realize the importance of resistant wheat genotypes. This author also associated FHB infection with the period of anthesis wherein wheat is most susceptible to FHB (Arthur, 1891). Since then, considerable efforts have been devoted to identifying resistant sources that can be usefully exploited in breeding programs.

Valuable FHB resistant sources have been identified in both spring and winter wheat and used in breeding programs. Despite the fact that great efforts were made to find new sources of FHB resistance and breeding for FHB resistance, no immunity or complete

FHB resistance has been found among wheat genotypes so far. FHB resistant sources have been identified in common wheat and can be traced to only a few cultivars (Buerstmayr et al. 2009). These sources are mainly from three geographic origins.

Although small detectable isolate by genotype interactions were reported by Mesterhazy et al. (1999), this relationship was valid in one set of multiple year data and was only significant for the severity trait but not for kernel ratings nor DON levels. FHB resistance in wheat was also effective against several isolates in the *F. graminearum* and *F. culmorum*. In other words, wheat genotypes show similar reactions to *F. graminearum* and *F. culmorum*. Vice versa *Fusarium* species that cause head blight in wheat can also infect other cereals without showing specialization for any one host (Mesterházy 1981; van Eeuwijk et al. 1995). Therefore, no specialized races adapted to different wheat genotypes within *F. graminearum* or *F. culmorum* have been reported but differences in aggressiveness among isolates have been found (Bai and Shaner 1994 1996; Parry et al. 1995). Hence, FHB resistance in wheat is considered as horizontal, due to the lack of proof for a host by Fusarium interaction. (Mesterhazy et al. 2005; Mesterhazy et al. 1999). Furthermore, it is quite durable (Miedaner 1997), and the resistance genes in current resistant sources, such as Sumai 3, are not expected to be overcome by new *Fusarium* species in the near future. Many reasons combine to limit the development of resistant varieties, making the breeding for FHB resistance in wheat very challenging including:

- The polygenic nature of disease resistance (Ruckenbauer et al. 2001).
- The association of undesirable agronomic traits e.g. tall plant stature and lateness with resistance to fhb due to pleiotropy or tight linkage leading to difficulties in combining short plant height and earliness with fhb resistance.
- Despite the significant progress made in breeding for resistance to FHB, combining a high level of FHB resistance in adapted backgrounds remains a great challenge (Bai and Shaner 2004).

- Resistance is not merely a single trait, at least five types of resistance have been reported and usually accepted till now (Schroeder and Christensen 1963; Wang and Miller 1988; Mesterhazy 1995).
- The presence of various types of resistance necessitates different inoculation techniques to be differentiated. Arguably, lines having one type of resistance does not necessarily guarantee the presence of other types of resistance.
- Due to the nature of the causal pathogen which is not a single strain or species but rather a complex of different *Fusarium* species with different toxin profiles.
- Screening large numbers of wheat genotypes in field nurseries for FHB resistance by conventional phenotypic selection on mature plants is a tedious, costly and time-consuming task. Further, the selection process can not be completely substituted by marker-assisted selection (MAS).
- Disease expression in FHB nurseries is largely dependent on environmental conditions. Hence to correctly characterize FHB resistance potential of a particular genotype, screening over several environments is a most (Fuentes et al. 2005).
- Bread wheat has a very complex genetic structures with three homeologous genomes making the development of disease resistance lines and genetic analysis difficult (Gupta et al. 1999; Röder et al. 1998).

The first step in wheat breeding for FHB resistance is to generate genetic variation by crossing different sources and types of resistance and simultaneously selecting for desirable agronomic traits subsequently and for enhanced FHB. Before starting crosses preparation and choosing selection scheme by breeders in order to introgress FHB resistance into locally adapted genotypes, several considerations should be thought about:

- None of the known resistance sources is immune.
- Resistant sources are unadapted in areas outside of their origin.

- Resistance is quantitatively inherited and is estimated to be oligogenic to polygenic.
- Environmental conditions have serious effects on the identification of resistant genotypes and evaluation errors should be expected.
- A combination of different types of resistance is necessary to go effectively through heavy FHB epidemics.

Hence, the evaluation in more locations and/or years could reduce the environmental conditions related errors. Major QTL can only explain part of the variation in FHB resistance that emphasizes the need for pyramiding of different QTL to obtain a satisfactory level of resistance (Buerstmayr et al. 2009; Liu et al. 2012).

Combining FHB resistance genes from different sources to produce transgressive segregants with higher levels of resistance has been proved to be possible. Positive and negative transgressive individuals will arise from the associations of positive and negative alleles, respectively. Transgressive segregation indicates that both parents contribute loci for FHB resistance and provides evidence for allele dispersion. Therefor it is important to screen new genotypes for FHB resistance continuously in order to enhance chances of identifying novel FHB resistance QTL. This process is the key to enhancing genetic diversity in FHB resistant sources and breeding cultivars with transgressive resistance. However, it should be taken into consideration that transgressive segregation toward susceptibility is easier to obtain than toward resistance.

1.3. TAN SPOT

1.3.1. Importance

Tan spot is an important foliar wheat disease that occurs throughout the major wheat growing regions of the world (Hosford 1982: Krupinsky 1982: Weise 1987; Ciuffetti and Tuori 1999). It caused by *Pyrenophora tritici-repentis* pied.) Drechs. (ana. *Drechslera tritici-repentis* (Died.) Shoern.) which has the potential to produce different host-selective toxins (HST) that interact specifically with its host cell (Strelkov and Lamari 2003). *D. tritici-repentis* arguably has the broadest host range of all Drechslera species as reported by Shoemaker, (1962). In addition to causing tan spot, *Pyrenophora* and *Drechslera* were associated with brown spot on grass species in Canada and the United Sates (Hosford 1971; Krupinsky 1982), but the disease levels were lower relative to those on wheat (Krupinsky 1992). Yield reductions up to 50% and kernel weight reductions up to 13% have been reported (Evans et al. 1999; Bhathal et al. 2003). The most severe damage takes place when the flag leaves are infected. Due to the potential destruction it can cause and the rapid spread, tan spot is of great concern to the International Maize and Wheat Improvement Center (CIMMYT) in Mexico. The increased frequency and severity of tan spot could be attributable to recent trends in cultural practices toward conservative agriculture where the fungus can over-winter on kept residues and cultivation of few commercially valuable but PTR susceptible wheat varieties over large areas (Bockus 1998; Rees 1982; Carignano et al. 2008). Moreover, resistance to fungicides was recently discovered by Ficke et al. (2011) in Norwegian *PTR* populations.

1.3.2. Host Range

By infecting more than 33 grass species, *Pyrenophora trïtici-repentis* (*PTR*) has a broad host range of grass species of all *Pyrenophora* species (Hosford 1982; Krupinsky 1992; Ali and Francl 2003; Cox et al. 1992). However, bread and durum wheats are the most important hosts due to their economic importance. Whereas it is not pathogenic on oat

and barley, and weak on rye (Maraite et al. 1992; Weise 1987). The survival of this fungus on various grass species, most of which being perennials grown in wheat producing areas, leads to the conclusion that these hosts may play a significant role as a source of primary inoculum. Also, they could play an important role as a source of fungal genetic variation (De Wolf et al. 1998; Ali and Lamari 1997; Krupinsky 1987). Indeed, Strelkov and Lamari (2003) hypothesized that *P. tritici-repentis* may have evolved on grass species before moving to its wheat host. Additionally, *PTR* is also capable of surviving saprophytically on infected wheat stubble and crop residues (Ciuffetti et al. 2014: Krupinsky 1992).

1.3.3. Taxonomy

The causal pathogen of tan spot of wheat, *Pyrenophora tritici-repentis*, is a homothallic ascomycete This facultative necrotroph pathogen not only causes extensive tissue damage to the host in its parasitic phase but also survive on dead or dying host plant tissue in its saprotrophic phase. It is classified in

Kingdom Fungi

Division Eumycota

Subdivision Ascomycotina

Class Loculoascomycete

Order Pleosporales

Family Pleosporaceae

Genus *Pyrenophora* (ana. *Drechslera*)

The family *Pleosporaceae* includes 16 genera in addition to the genus *Pyrenophora* such as *Cochliobolus* and *Pleospora* (Alcorn 1988; Kirk et al. 2001). Although it clusters within the family, the genus *Pyrenophora* is phylogenetically distinct from other members of the Pleosporaceae (Kodsueb et al. 2006).

This fungus has been historically referred to by various names. Names for the teleomorph have included: *Helminthosporium tritici-repentis* (Died.) Died., *H. tritici-vulgaris* Nisikado, *Drechslera tritici-vulgaris* (Nisikado) Ito., *D. tritici-repentis* (Died.) Shoem., *Pleospora tritici-repentis* Died., *Pyrenophora trichostoma* (Fr.) Fckl., *Pyrenophora tritici-vulgaris* Dickson, and *Pyrenophora tritici-repentis* (Died.) Drechs. (De Wolf et al. 1998; Hosford 1982). Based on differences in conidial germination Shoemaker (1959) assigned the anamorphic genus *Helminthosporium* into three genera (*Drechslera, Bipolaris* and *Helminthosporium*), placing the tan spot fungus within the genus *Drechslera*. Later, Shoemaker (1961) concluded that the appropriate classification of the perfect state of the fungus was in the genus *Pyrenophora*, which was established by Fries (1849), not in the genus *Pleospora*.

1.3.4. Life cycle

PTR goes through both sexual and asexual stages in the course of its life cycle. It produces ascospores during the sexual stage and conidia in the asexual stage (Schilder and Bergstrom 1992). The sexual spores are produced in cylindrical asci within a black spherical pseudothecium (Pfender et al. 1988). The primary inoculum of *PTR* comes from ascospores over seasoning on crop debris that can infect wheat seedlings and produce lesions on the young leaves. The ascospore density in a given area considerably influences epidemic pressure in that area (Adee and Pfender 1989). This role is clearly demonstrated by the correlation between the amount of initial inoculum and the diseased leaf area. However, the impact of ascospores on disease development is not only related to the number of lesions induced by the ascospores, but also to the number of conidia initiated from such lesions. Schilder and Bergstrom (1992) found that fungal spread by ascospores is restricted to only short distances, in contrast to long-distance dispersed wind-borne conidia. Conidia are produced on infected tissues and serve as the repeating phase triggering polycyclic tan spot epidemics in the field (Shabeer and Bockus 1988; Wright and Sutton 1990; Ronis and Samaskiene 2006). *PTR* is a diurnal fungus that

requires a diurnal (light-dark) cycles to induce conidiophores and conidia production (Khan 1971). The highest number of conidia is produced using a 12/12 h light/dark cycle and no conidial development occurs when this pathogen is exposed to continuous light or darkness (Khan 1971; Francl 1998). The light period is crucial for conidiophore formation while a dark period is required for conidia production (Francl and Jordahl 1997: De Wolf et ai. 1998).

1.3.5. Symptoms

The name 'tan spot' comes from the tan colour of the necrotic lesions that appear on infected leaves. It is also referred to as yellow leaf spot i.e. in Australia (Rees and Platz 1979). This disease may appear either as necrotic or chlorotic areas on leaves depending on wheat genotype and the pathogen race (Ciuffetti and Tuori 1999). Necrotic lesions consist of tan colored, collapsed tissue, whereas chlorotic lesions exhibit a gradual yellow discoloration of extensive areas of the leaf. The first symptoms appear on leaves as oval- to irregular-shaped, yellow, tan, or brown lesions, each containing a small dark spot and surrounded by a yellow border. Individual lesions over time may coalesce to occupy parts or whole leaf surface. However, on resistant cultivars, lesions are typically tiny and dark brown to black in color. Under rainy, cloudy and humid cool weather conditions, the symptoms progress from the lower leaves to the upper leaves as the plant grows and matures. The disease progresses rapidly. *PTR* can also infect wheat kernels resulting in red smudge or black point symptoms on seeds. Seed infection can seriously impact grain grading as well as subsequent seedling infection (Fernandez et al. 2001).

Fig. 1.3 Tan spot on wheat, both necrosis and chlorosis symptoms can be noticed.

(www.forestryimages.org)

1.3.6. Resistance

Some researchers (Elias et al. 1989; Effertz et al. 2002) reported quantitative resistance while others (Lamari and Bernier 1991; Gamba and Lamari 1998) have found that TS resistance is qualitative, controlled by single major recessive genes. Lamari et al. (2003) proposed that a one- to- one relationship existed in the wheat x *P. tritici repentis* pathosystem. This relationship is a mirror image of the one described by the classical gene-for-gene model (Flor 1955) and it is known as inverse gene-for-gene model. The main difference comes from the fact that compatibility rather than incompatibility is the basis of specificity in TS of wheat where the presence of toxin-sensitive gene product leads to susceptibility. *PTR* has different races that differ in specific toxins production (PtrTox A, B, C, D) and symptoms (chloroses or necroses) on particular wheat genotypes. When the pathogen produces a toxin for which the host has the corresponding receptor a susceptible reaction develops. Because of the evidence for pathogen races, Lee and Gough (1984) considered that it is unlikely to ensure durable resistance to any widely grown variety employing a single gene. In other words, race-specific resistance does not

guarantee a permanent protection because of the expected selection pressure on the common races of the targeted pathogen, because while the frequency of some races goes down, other races become prevalent, to which the current wheat cultivar may not be resistant. Therefore, the most prevalent races should be considered when breeding for resistance to TS to effectively control this disease and *PTR* races should be continuously monitored to check the occurrence of new races which were not considered at the time of cultivar development (Castro et al. 2003).

1.4. STAGONOSPORA NODORUM BLOTCH

1.4.1. Importance

Parastagonospora nodorum is distributed worldwide and has become a major pathogen of wheat (*Triticum aestivum*) in many wheat-growing areas, including Europe, North America, and Australia as well as in developing countries. (Duczek et al. 1999; Halama 2002; Oliver et al. 2012). Many factors have contributed to the increased frequency of this disease including the widespread of semi-dwarf wheat genotypes, concentrated wheat production and growing of susceptible cultivars (King et al. 1983; Wicki et al. 1999). Other factors increased in importance worldwide include changes in cultural practices such as a higher frequency of wheat in the crop rotation, zero tillage, and increased nitrogen fertilizers particularly in areas prone to warm, humid, and wet conditions during the growing season. (Eyal et al. 1987; Eyal 1999). However, in Europe *Septoria tritici*, a pathogen with a similar life cycle to *P. nodorum*, replaced *P. nodorum* as the most important foliar disease of wheat (Eyal 1999; Hardwick et al. 2001). This destructive disease can infect both the leaves and the glumes. Flag leaf and head infection by SNB have a larger impact on grain yield and quality compared to infection of lower leaves.

Losses related to reduced grain yield, grain weight, and grain quality result from the reduction in photosynthesis. Besides, shriveled kernels are often wasted during harvest,

and they have low milling and baking quality (Eyal et al. 1987; McKendry et al. 1995). Due to its importance, this pathogen was the first species to be genome sequenced in the large fungal Dothideomycete class. Yield losses up to 50% have been reported, and the pathogen can infect both the leaves and glumes (King et al. 1983; Eyal et al. 1987; Bhathal et al. 2003). *P. nodorum*, unlike *Z. tritici*, is able to infect and form fruiting structures on the glumes and is classified as a necrotroph, feeding exclusively on dead plant tissue.

1.4.2. Host range

The pathogen is common in the main geographical regions where wheat is grown, including the USA, Australia and Europe (Francki 2013; Duczek et al. 1999; Halama 2002)). The principal hosts of *P. nodorum* are bread wheat (*Triticum aestivum*), durum wheat (*T. durum*) and triticale (× *Triticosecale* Wittmack ex A. Camus) (Solomon et al. 2006). It is also pathogenic on other cereals, and a range of wild grasses can host the pathogen. *P. nodorum* appears to be the less specialised pathogen compared to *Z. tritici* in that it can colonise a wider range of grass genera. This may imply that grasses would present an additional source of inoculum (Solomon et al. 2006) and could play a part in the epidemiology of the disease. However, the host range of *P. nodorum* has not been clearly defined though papers have mentioned alternative graminaceous hosts (Solomon et al. 2006). Besides, a lower symptom severity was observed when wheat was inoculated with a strain isolated from barley implying that this is different biotype even though barley biotypes can cause symptoms on wheat, they are not as pathogenic on wheat as isolates cultured from wheat (Newton and Caten 1991).

1.4.3. Taxonomy

SNB is caused by *Parastagonospora* (syn. *Leptosphaeria*) *nodorum* (Müll), Hedjar, Quaedvlieg, Verkley & Crous (Shoemaker and Babcock 1989; Cunfer and Ueng 1999;

Quaedvlieg et al. 2013). The anamorph synonym is *Stagonospora* (syn. *Septoria*) *nodorum* (Berk.) Castell, and Germano. *Parastagonospora nodorum* has been one of several fungal pathogens that were subjected to many changes in their nomenclature. When firstly described, the sexual stage of *P. nodorum* was assigned to the genus *Leptosphaeria*, which was reclassified later as *Phaeosphaeria* in the late 1960's (Cunfer and Ueng 1999). Similarly, the anamorphic stage was formerly classified as *Septoria* sp. in 1850 but recently was moved to the genus *Parastagonospora*. The many changes in fungus nomenclature, coupled with besides the use inclusion of the full Latin scientific name of the anamorph in the common name of the disease, has caused some confusion (Cunfer and Ueng 1999).

This pathogen is a haploid heterothallic fungal pathogen of wheat, and belongs to:

Kingdom Fungi

Phylum Ascomycota

Subphylum Euascomycota

Class Dothideomycetes,

Order Pleosporales,

Family Phaeosphaeriaceae

Genus Phaerosphaeria

It is commonly known by both the teleomorphic and anamorphic names.

1.4.4. Life cycle

Parastagnospora nodorum is a heterothallic filamentous ascomycete that has the potential to reproduce sexually and asexually. However, both of the mating types have to be present for the sexual cycle to occur (Halama and Lacoste 1991). When different mating types meet produces ascospores in pseudothecia that develop in the wheat

residue. The pseudothecium contains numerous asci, each containing eight ascospores. The ascospores are wind-borne over long distances (Bathgate and Loughman 2001).

Infection triggers when either ascospores or asexual pycnidiospores from infected seeds or wheat stubble land on the wheat tissue (Solomon et al. 2006). The pathogen penetrates the plant tissue directly through the cuticle and opportunistically through stomata (Solomon et al. 2006). Following primary infection of the leaves plant, the fungus forms the anamorphic stage asexual including pycnidiospores inside Asexual fruiting structures called pycnidia in infected tissues within a week, approximately. Pycnidiospores serve as the repeating phase of the fungus over the growing season and are splash-dispersed spread by rain splash or wind blown rain within the canopy after being released from the pycnidia (Eyal et al. 1987; Shah et al. 2001; Solomon et al. 2006).

The mixed reproduction system of *P. nodorum* allows both great diversity due to sexual recombination, and fast replication of successful genotypes during the asexual cycles. Studies have shown that no single *P. nodorum* genotype dominates in any environment (Blixt et al. 2008; Francki 2013; Stukenbrock et al. 2006). The genetic diversity is high, and it is likely that selection in different environments has given rise to high levels of variation in aggressiveness and pathogenicity (Ali and Adhikari 2008; Engle et al. 2006; Francki 2013).

1.4.5. Symptoms of SNB

Symptoms may occur on any aerial part of the plant, including leaves, stem, nodes, and glumes. Symptoms of SNB usually first appear on the lower leaves, then progress to the upper leaves. Initial symptoms of SNB are small chlorotic lesions at the infection point eventually turn reddish brown having an oval or lens shape often surrounded by chlorosis that later enlarge and merge into irregular light-gray patterns (Eyal et al. 1987; Solomon et al. 2006). The lesions can be noticed on the seedling leaves within three weeks of emergence (Pedersen and Hughes 1992). The yellow halo is indicative of the production of necrotrophic effectors, also known as host-selective toxins, which are secreted

preceding fungal growth in order to kill host cells (Solomon et al. 2006). Pycnidia, asexual fruiting bodies, of the *P. nodorum* are difficult to see pycnidia in the field, even with a hand lens. The most striking symptom is dried leaves. Eventually, the entire leaf collapses as the chlorosis expands and takes over the whole sheath to form large necrotic patches (Solomon et al. 2006).

SNB not only affects wheat leaves, but the fungus can also be pathogenic on wheat heads (McMullen and Adhikari 2009), generally at the end of the season. Wheat glumes become infected from the glume tip downward. The head lesions have a purplish brown to gray appearance (Menzies and Gilbert 2003) and have a dry appearance compared to the lesions found on the leaves. If the wheat head is severely infected, the kernels will become small, shriveled, and of poor quality.

1.4.6. Resistance

Similarly to TS, SNB also reacts to dominant host sensitivity genes in an inverse gene-for-gene model involving host-selective toxins as virulence factors. Friesen et al. (2006) found that SnToxA and PtrToxA, which are toxins related to the causal pathogens of SNB and TS respectively, are 99.7% similar. Interestingly, the wheat gene, Tsn1, has the same interaction with SnToxA and PtrToxA. Tsn1 expression leads to programmed cell death, which is driven by either SnToxA or PtrToxA (Friesen et al., 2008). It is estimated that wheat susceptibility to SNB and TS can be reduced as much as 68% by eliminating Tsn1 or Snn1 from a wheat genotype (Zhang et al., 2009). Additionally, several non-toxin related resistance factors have also been identified.

Fig. 1.4 Stagonospora nodorum blotch on wheat

(www.nysipm.cornell.edu/fieldcrops/)

1.5. SEPTORIA TRITICI BLOTCH

1.5.1. Importance

Septoria tritici blotch of wheat (STB) is one of the most devastating biotic stresses occurs throughout the world wherever wheat is grown. STB was first reported as a severe wheat disease in Europe in the 1970s and has since become widespread (Hardwick et al. 2001; Cowger et al. 2000). It is currently the major wheat disease in Europe and the Mediterranean region North Africa, South Africa, parts of South America Central and West Asia. (Camacho-Casas et al. 1995; De Ackermann et al. 1995; Shaner and Finney 1982; Eyal 1999; Bearchell et al. 2005) and is rated as one of the most devastating diseases of wheat in Morocco.

STB is a residue-borne disease, and STB related losses escalate dramatically when the flag leaf or the leaf immediately below it are severely infected. Produced kernels when the crop is affected by STB are often of bad quality, shrivelled and not suitable for milling purposes. STB can induce yield losses up to 50% under conducive environmental conditions including cool, rainy climates. In the UK, 52% of wheat leaf samples surveyed by the UK's Home Grown Cereals Authority (HGCA) in 2010 were infected by STB (www.cropmonitor.co.uk) and the primary target for the breeding and agrochemical industry (McDougall 2003; Russell 2005).

Depending on the year and region STB is considered of the top 4 wheat diseases along with rust, powdery mildew and Fusarium head blight. Severity varies across locations and seasons and is affected by environmental conditions to a large extent. The increasing adoption of conservation agriculture which supports the over-summering of *Z. tritici* besides many other pathogens (Eyal et al. 1987), the climate change and the continuous development of fungicide resistant pathogen strains have all contributed to exacerbating STB incidence and losses.

1.5.2. Host range

Common wheat, durum wheat and their graminaceous ancestors are economically important crops and can be infected by *Z. tritici*. Other species of the genera Agropyron, Brachypodium, Bromus, Dactylis, Festuca, Hordeum, Poa, and Secale, (Eyal 1999) can serve as alternative hosts. However, their potential to serve as sources of primary inoculum is not well known (Eyal 1999).

1.5.3. Taxonomy

Mycosphaerella is one of the biggest genera of plant pathogenic ascomycetes known to date under which more than 10,000 names have been reported and its associated anamorph genera (Crous 2009). However, in recent multi-gene phylogenic studies have suggested Mycosphaerella to be polyphyletic and need to be separated into genera based on their anamorphs (Crous et al. 2007). Presently, *Septoria* sensu lato represents a polyphyletic assembly of genera that cluster mostly in the *Mycosphaerellaceae* (a family incorporating many plant pathogenic coelomycetes). Quaedvlieg et al. (2011) suggested the introduction of single generic names for discrete monophyletic lineages notwithstanding if it is an 'anamorph' or 'teleomorph' for all unambiguous monophyletic phylogenetic lineages. The current taxonomy status of Z. *tritici* is as follows:

Kingdom Fungi

Phylum Ascomycota

Class Dothidiomycetes

Order Pleosporales

Family Mycosphaerellaceae

Lineage Zymoseptoria gen. nov.

1.5.4. Life cycle-STB

Zymoseptoria tritici is a heterothallic ascomycete that has a diallelic mating type locus characterized by two mat alleles at a single locus or what also some researchers refer to as idiomorphs due to the absence of notable sequence similarity (Turgeon 1998), constituting the bipolar mating system (Kema et al. 2000). This heterothallic system prevents self-fertilization and stands for the high genetic diversity in *Z. tritici* and its ability to evolve rapidly. *Z. tritici* has an asexual (Quaedvlieg et al. 2011) as well as a sexual lifecycle that is directed by its heterothallic bipolar mating system producing splash dispersed pycnidiospores and airborne ascospore.

Sexual reproduction onsets when two pathogen strains of opposite mating types get in contact leading to cellular interactions that result in a transient diploid phase and eventual genetic recombination (Coppin et al. 1997). Each reproductive cycle takes five to seven weeks and results in complex populations with genetic variation (McDonald et al. 1996). Giving the fungus the ability to adapt to severe environmental conditions such as resistance development to new fungicides (Gisi et al. 2002, Torriani et al. 2009). The sexual reproduction usually takes place on wheat residues, and even on growing wheat plants (Hunter et al. 1999; Kema etal. 1996b). *Z. tritici* survives through the summer as pycnidiospores in pycnidia and ascospores in pseudothecia on crop debris and initiates primary infections in the next season. Besides, there is some evidence that the fungus can survive in association with other grass hosts and wheat seed. McDonald et al. (1999) demonstrated that infected seed has been linked to the global distribution of *Z. tritici*.

The air-borne haploid ascospores are the principal source of inoculum and can spread to long distances leading to initiation of STB epidemics in wheat fields (Shaw and Royle 1989, Hunter et al. 1999). Primary infection takes place during fall before the onset of cold weather, and the pathogen overwinters as asymptomatic infections. On the contrary of previous speculations, ascospores are not only released from overwintering stubble or volunteer plants but also can be released from ascocarps produced on infected leaves

during the growing season (Hunter et al. 1999). This agrees with the conclusion that the pathogen can complete the sexual phase within five weeks (Kema et al. 1996).

Local secondary infections, primarily conidia, serve as the repeating stage of the fungus during the growing season and are important in the epidemiological development cycle of the pathogen in wheat. The principal source of inoculum of *Z. tritici* during spring and summer are the splash-dispersed pycnidiospores (Shaw and Royle 1993; Eriksen and Munk 2003). These spores are dispersed from the base and upwards in the crop canopy from residues or lower infected leaves to newly emerging leaves, usually by forceful rain splash and the amount of carried inoculum decreases considerably with height (Shaw and Royle 1993). However, conidia are known to be limited to hot-spots that are only a few meters in diameter in a field because of their limited dispersal ability.

Germination of ascospores and conidia of *Z. tritici* occurs within hours of a spore landing on a wheat leaf when conditions of high humidity and moderately high temperatures (10-25) °C coincide within 15-20 hours (Magboul et al. 1992). Hyphal growth pattern has disagreed between studies; While Duncan and Howard (2000) mentioned that germ tubes grew towards stomata, others reported that most germ tubes grew away from the stomata (Shetty et al. 2003). Infection initiates by hyphae penetrating the leaf surface indirectly through stomatal cavities without developing infection structures and irrespective of host resistance within 24 hours' post-inoculation. (Kema et al. 1996; Mehrabi et al. 2006). Albeit direct penetration has also been detected (Rohel et al. 2001). The pathogen colonises the sub-stomatal cavity of the leaf after penetration.

The asymptomatic phase from the initial infection to the formation of the first visible sporulating structures is designated as latency period wherein no external symptoms can be observed, only a moderate and non-damaging mycelial growth occurs in the intercellular spaces of the mesophyll tissues of the leaf (biotrophic phase). Under conditions of central Europe, the latency period ranges from 22 to 28 days depending on wheat genotype, temperature, and moisture (Lovell et al. 2004). At the end of the latency period, the pathogen development exacerbates switching from biotrophic to necrotrophic

phase and results in the decomposition of cell walls (Keon et al. 2007), leading to the development of characteristic necrotic leaf lesions and sporulation eventually. The resistance occurs once the pathogen has entered the host and its growth is restricted to sub-stomatal chambers in resistant genotypes. No discrepancies are seen in incompatible and compatible interactions during germination or penetration.

1.5.5. Symptoms

Septoria leaf blotch is also known as speckled leaf blotch. Symptoms usually appear on the lower leaves and culms of tillering plants, but may occasionally occur on the rachis and glumes. Lesions develop first on the tips of lower leaves as small chlorotic areas, which later enlarge into irregular-to-oval, longitudinal reddish-brown spots with a light brown center. Lesions often coalesce under favorable environmental conditions, causing entire leaves to die prematurely and could even be found on tips of glumes Under severe epidemics. Pycnidia develop as tiny black specks scattered within the lesions, their size varies among wheat genotypes and is affected by pycnidia density of in the infected tissue (Eyal and Brown 1976).

Fig 1.5 Typical advanced STB symptoms on a wheat leaf

(http://www.lwk.lu)

1.5.6. Resistance

Breeding for STB resistance has a key role in confronting losses caused by this destructive disease and wheat breeders consider resistance to STB to be an important target. However, the challenge in breeding is to obtain STB resistant cultivars that combine STB resistance, other major diseases resistance and good production with high quality in balanced proportions. According to Narvaez and Caldwell (1979) and Kema et al. (1996), STB resistance is complex wherein both quantitative and qualitative mechanisms are involved in the expression of STB resistance in the *Z. tritici*- wheat pathosystem. One of the most common defence mechanisms against pathogen attack is the hypersensitive response, the rapid and localised programmed cell death (PCD) at the site of infection (Hammond-Kosack and Jones 1996). Several specific interactions following a gene for gene relationship are known from analyses of panels of isolates inoculated onto multiple wheat cultivars to identify additional potential sources of resistance (Zhang et al., 2001; Brading et al. 2002). Nonetheless, only a few genes have been widely used commercially as components of STB resistance (Chartrain et al. 2005b). Thus planting wheat genotypes with single or a few resistance genes over large areas, which results in changes in virulence in populations of *Z. tritici* as a result of exerted pressure on pathogen populations (McDonald and Linde 2002). One clear example was observed in an STB-resistant genotype with the Stb4 gene for resistance became susceptible within five years of its release in California and Oregon (Goodwin 2007).

1.6. SPOT BLOTCH

1.6.1. Importance

SB is of increasing concern in South Asia, parts of China, North and Latin America (Chang and Wu 1998; Chand et al. 2003; Joshi et al. 2004; Pandey et al. 2005), but much less frequent in Europe (Kwasna, 1995). It is vital wherever warm, humid conditions

persist during growing season. Yield losses are variable ranging from low damage up to 85.0% (Raemakers, 1988). The average yield losses caused by SB in South Asia have been calculated to be about 20% (Saari 1998). Replacement of landraces by high-yielding, rust-resistant cultivars in developing countries has exacerbated the losses.

1.6.2. Host range

Bipolaris sorokiniana is a facultative seed borne pathogen that has broad host range (Misra 1973). Besides wheat, barley and triticale, rice has also been reported as a host for this pathogen (Misra 1973). Rye is less susceptible than wheat whereas oats are occasionally infected (Zillinsky, 1983). Duveiller and Gilchrist (1994) inferred that alternative hosts could serve as a green bridge for the survival of *B. sorokiniana* across seasons.

1.6.3. Taxonomy

The spot blotch causal pathogen was named *Bipolaris sorokiniana* (sacc) Shoem., and this name is presently widely adopted. In addition to spot blotch, it also causes common root rot. It is a hemibiotrophic pathogen and has worldwide distribution. The genus *Cochliobolus* is characterized by globose ascomata with a long cylindrical neck, obclavate cylindrical asci, and helically coiled ascospores. Two anamorphs are associated with the genus *Cochliobolus*, *Bipolaris* and *Curvularia* (Alexopoulos et al. 1996; Zhang et al. 2012). Shoemaker (1959) proposed the generic name *Bipolaris* for the *Helminthosporium* species with fusoid, straight, or curved conidia, germinating by one germ tube from each end (bipolar germination). *Bipolaris sorokiniana* has thick-walled, ovoid conidia (60- 120 µm ×12-20µm) with 5-9 cells. The sexual stage is placed in:

Kingdom Fungi

Phylum Ascomycota

Class Dothidiomycetes

Order Pleosporales

Family Pleosporaceae

Genus *Cochliobolus*

Species *C. sativus*

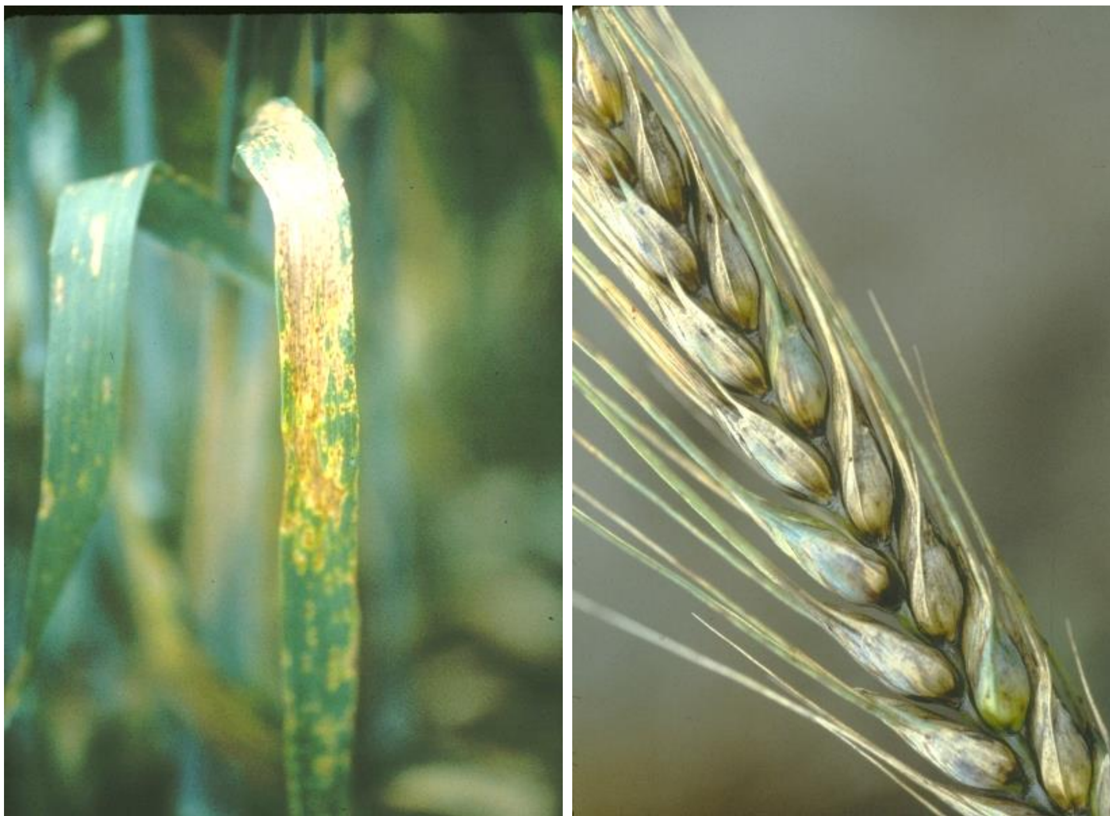
1.6.4. Life cycle

The main dispersal and survival propagules are conidia (Reis and Wunschr 1984). The soil, wherein conidia survive, is the most important source of primary of infection. During harvest, the soil gets infested with pathogen propagules spreading from the infected crop where thick black clouds of conidial dust can be seen in severely infested fields. The spores germinate in the soil and get transformed into mycelium and chlamydospores that can survive for several years (Ledingham et al. 1960). Besides, infected kernels could also be considered a major source of primary inoculum (Mehta 1993) since they will provide inoculum for the grown crop in new areas (Reis 1991). It was reported that even small amount of inoculum was sufficient to create an epidemic.

This fungus is a necrotrophic pathogen that cause leaf blotch, seed rot, crown rot, seedling blight, and black point on wheat grains (Mishra et al. 2001). Spot blotch pathogen may infect wheat seedlings right from first leaf stage, but the susceptibility of plants increases after flowering. In contrast to the perfect stage which does not occur in nature nor play a role in the epidemiology of the disease, asexual stage of this fungus is common (Raemaekers 1988). Nutrients available in the conidia of *C. sativus* are sufficient for germination and formation of appressoria, however, an external supply of nutrients, that leach out from the wheat leaf tissue and absorbed by the conidia, is necessary for successful penetration (Yadav 1981).

The secondary dissemination of the disease is through wind-borne spores. There are several cycles of conidia production during the cropping season that lead to secondary infections after spreading through wind and water drops (Duveillier et al. 2005) and initiate lesions on the leaves and stems later in the same season.

Fig 1.6 Disease symptoms caused by *Bipolaris sorokineana* on leaves and head



(http://wheatdoctor.org)

1.6.5. Symptoms

Early symptoms are characterized by small, brown to black, oval lesions ranging 3–4 mm long that extend very quickly on leaves of susceptible genotypes. Initial Symptoms may be confused with Septoria diseases when the fungus has not reached sporulation yet.

A yellow halo surrounding the lesions may be noticed on some genotypes. After sporulation under humid conditions, the lesion center becomes dark brown that can be wiped easily with fingers. Under severe epidemics lesions coalesce, and large leaf portions become blighted. In addition to the leaves, chocolate brown to black necrotic lesions could also be noticed on the crowns, stems and sometimes even on wheat heads. Stem infection can eventually lead to lodging. Moreover, severe seedling infections result in poor stand dwarfed plants, reduced tillering and white heads. Head infections appear as light-brown spots with dark brown margins on glumes resulting in shrivelled kernels (Kiesling 1985) and black point of kernels at the embryo end (Kumar et al. 2002).

1.6.6. Resistance

Like in TS, SNB and STB the most economical and efficient way to control SB is by growing resistant cultivars. In general, the degree of resistance in modern cultivars is still unsatisfactory (Mujeeb-Kazi 1998; Van Ginkel and Rajaram 1998; Joshi et al. 2004). Many studies have shown that SB resistance to be monogenic to polygenic trait. However, most results from different groups support the quantitative nature of SB resistance and doubt the presence of qualitative genes (Mehta 1993; Joshi and Chand 2002; Kumar et al. 2009). Nevertheless, SB resistance inheritance studies are limited compared with other diseases and nature of inheritance is still debatable. Several wheat genotypes with acceptable levels of SB resistance along with good grain yield have been reported (Sharma et al. 2004). New sources of resistance need to be explored and utilized to develop new varieties with higher levels of resistance than available in the current commercial cultivars. SB resistance improvement can be achieved by crossing adapted local varieties with good sources of SB resistance, including lines that harbor leaf tip necrosis (LTN) which is a morphological marker associated with resistance due to its strong linkage with the resistance gene Lr34 (Joshi et al. 2004). Some phenological traits other than LTN were shown to have a positive association with SB resistance as well like

leaf angle (Joshi and Chand 2002) and stay green trait (Joshi et al. 2007). Transgressive segregation for SB resistance was also reported (Joshi et al. 2002; Joshi and Chand 2003).

1.7. Multiple Disease resistance

When a wheat genotype has resistance to two or more diseases, it is referred to as a multiple disease resistant genotype (MDR), while having resistance to multiple major races of the same pathogen or multiple taxa is referred to as broad-spectrum resistance (BSR). There are various kinds of evidence suggesting the presence of MDR in wheat. The correlation between resistances of different diseases has been documented in wheat. Evidence that MDR genes exist in wheat also includes the detection of quantitative trait loci clusters for various diseases. Moreover, direct evidence for MDR is the observation of pleiotropic effects on multiple diseases shown with induced gene mutations.

Wheat is often infected simultaneously by multiple foliar diseases, but the components of the foliar disease complex differ from region to region despite the similar symptoms. For example, TS and SB co-present in South Asia, and it is often very hard to diagnose the pathogen based only on visual symptoms (Duveiller et al. 2005). Whereas STB, TS, and SNB co-exist in the United States and all of the four diseases, along with FHB, are common in Canadian prairies (Fernandez et al. 2014; Fernandez et al. 1999; Gilbert and Woods 2001; May et al. 2014). Accordingly, strategies based on multiple disease resistance will reduce risks related to the co-existence of different pathogens in certain environments. Moreover, it may facilitate further breeding efforts on combining resistance to blight diseases with resistance to other diseases (Gurung et al. 2012).

Although may need considerable time and a good understanding of the genetics of host resistance, breeding for multiple disease resistance is one of the best disease management strategies for wheat. Despite many advances made in wheat genomics and breeding, only a few published studies aimed at identifying multiple disease resistant wheat genotypes and even fewer are the studies tried to explore molecular events behind multiple disease resistance. The development of multiple disease resistance is crucial for the different

climatic conditions and cropping systems in CIMMYT partner countries. Such accessions are particularly valuable because it is then not necessary for the plant breeder to introduce resistance to each parasite separately. More importantly, cultivation of multiple disease resistance varieties will minimize yield losses and duration of resistance efficiency can be augmented when different resistant sources are available by appropriate application of rotation and other cultural measures. So that, chances of disease out breakage is reduced, and the duration of resistance can be increased (Singh et al., 2000).

1.8. Objectives

As a communication platform and a promoter of international cooperation, CIMMYT has developed extensive FHB collaborations with research organizations in both developed and developing countries (Duveiller et al. 2008) and worked on the incorporation of FHB resistance into high yielding, semi-dwarf and rust resistant CIMMYT wheat (He et al. 2000). CIMMYT established a series of FHB Screening Nurseries (FHBSN, previously known as Scab Resistance Screening Nursery, SRSN), which were distributed worldwide and are available to anyone attentive to wheat FHB resistance improvement. The current name FHBSN was adopted in 2010, and the 13th and 14th FHBSN nurseries were distributed in 2011 and 2012, respectively (He et al. 2013a; He et al. 2013b). To ensure the viability and virulence of the pathogen for both greenhouse and field screening activities, about 90 new Fusarium strains are collected annually by CIMMYT's wheat pathology laboratory as one of the routine tasks in late summer from naturally infected cereals in different farms in Mexico.

Availability of diverse germplasm with broad spectrum resistance to multiple diseases is important to the success of wheat improvement programs (Polák et al. 2002; Sharma et al. 2013; Singh et al. 2012b), for which the identification of new sources of resistance to multiple diseases is a prerequisite. The availability of broad-spectrum resistant sources is of great importance due to the fact that CIMMYT wheat breeding lines target different destinations across the globe with different biotic stresses. Despite its obvious importance, only a few studies have been conducted to identify sources of resistance to multiple pathogens of wheat and the resistance genes currently available for resistance breeding are still limited (Ali et al. 2007; Lamari et al. 2005; Singh et al. 2006). Therefore, the objectives of this thesis were to:

1. Identify the predominant cereal FHB causing pathogen in Mexico and characterize its chemotype and sub-chemotype composition using isolates newly collected in 2013 and those from previous years to see if temporal or spatial

variation exists, to better understand the chemo-diversity of FHB related species in Mexico.

2. Characterize the 15th FHBSN regarding field resistance, post-harvest indices of Fusarium damaged kernels (FDK) and DON, as well as phenological and morphological traits like PH, days to heading (DH), and AE.

3. Identify new sources of broad-spectrum resistance to TS, SNB, SB and STB in genotypes from different geographic origins. Most of these materials have been evaluated for their field resistance to FHB in previous studies (He et al. 2014; Osman et al. 2015); in the present study their type II FHB resistance was exclusively measured in greenhouse experiments to reinforce the previous studies.
   Together with the information for leaf spotting diseases, the resistant lines identified herein will contribute potentially in enhancing the genetic diversity and aid in developing wheat cultivars with durable resistance to these diseases.

References:

Agostinelli AM (2009) Phenotypic and genotypic selection for head scab resistance in wheat. University of Kentucky, Lexington, USA

Agostinelli A, Mundell N, Van Sanford D (2008) Percentage of Fusarium damaged kernels measured by air separation. In: Proceedings of the 2008 National Fusarium head blight forum. Indianapolis, pp 2–4

Agostinelli A, Clark A, Sanford D Van (2007) Air separation and digital photo analysis as novel methods to measure the percentage of Fusarium damaged kernels. Natl Fusarium head blight forum 161.

Akinsanmi O A, Mitter V, Simpfendorfer S, et al (2004) Identity and pathogenicity of *Fusarium* spp. isolated from wheat fields in Queensland and northern New South Wales. Aust J Agric Res 55:97-107

Alcorn JL (1988) The Taxonomy of "*Helminthosporium*" Species. Annu Rev Phytopathol 26:37–56.

Alexopoulos CJ, Mims CW, Blackwell M (1996) Introductory Mycology. John Willey and Sons, New York.

Ali S, Francl LJ (2003) Population race structure of *Pyrenophora tritici-repentis* prevalent on wheat and non-cereal grasses in the Great Plains. Plant Dis 87:418–422

Ali S, Adhikari TB (2008) Variation in aggressiveness of *Stagonospora* nodorum isolates in North Dakota. J Phytopathol 156:140–145

Arthur JC (1891) Wheat scab. Purdue University Agricultural Experiment Station Bulletin 36:129–138.

Alvarez CL, Azcarate MP, Pinto VF (2009) Toxigenic potential of *Fusarium graminearum* sensu stricto isolates from wheat in Argentina. Int J Food Microbiol 135:131–135

Amarasinghe C, Wang J-H, Liao Y-C, Fernando WGD (2011) Difference in *TRI13* gene sequences between the 3-Acetyldeoxynivalenol producing *Fusarium graminearum* Chemotypes from Canada and China. Int J Mol Sci 12:6164–6175

Bai G, Shaner G (1994) Scab of wheat: prospects for control. Plant Dis 78:760-766

Bai G, Shaner G (1996) Variation in *Fusarium graminearum* and cultivar resistance to wheat scab. Plant Dis 80:975-979

Bai GH, Plattner R, Desjardins a., et al (2001) Resistance to Fusarium head blight and deoxynivalenol accumulation in wheat. Plant Breed 120:1–6.

Bai GH, Desjardins AE, Plattner RD (2002) Deoxynivalenol-nonproducing *Fusarium graminearum* causes initial infection, but does not cause disease spread in wheat spikes. Mycopathologia 153:91–98.

Bai G, Shaner G (2004) Management and resistance in wheat and barley to Fusarium head blight. Annu Rev Phytopathol 42:135–161

Bathgate JA, Loughman R (2001) Ascospores are a source of inoculum of *Phaeosphaeria nodorum, P. avenaria f. sp. avenaria* and *Mycosphaerella graminicola* in Western Australia. Australas Plant Pathol 30:317-322

Bearchell SJ, Fraaije BA, Shaw MW, Fitt BDL (2005) Wheat archive links long-term fungal pathogen population dynamics to air pollution. Proc Natl Acad Sci U S A 102:5438–42

Beyer M, Röding S, Ludewig A, Verreet JA (2004) Germination and survival of *Fusarium graminearum* macroconidia as affected by environmental factors. J Phytopathol 152:92–97.

Bhathal JS, Loughman R, Speijers J (2003) Yield reduction in wheat in relation to leaf disease from yellow (tan) spot and Septoria nodorum blotch. Eur J Plant Pathol 109:435–443

Buerstmayr H, Ban T, Anderson JA (2009) QTL mapping and marker assisted selection for Fusarium head blight resistance in wheat: a review. Plant Breed 128:1–26.

Buerstmayr H, Adam G, Lemmens M (2012) Resistance to head blight caused by *Fusarium* spp. in wheat. In I. Sharma (Ed.), Disease resistance in wheat (pp. 236-276). Wallingford, UK: CABI.

Burgess LW, Nelson PE, Toussoun TA (1982) Characterization, geographic distribution and ecology of *Fusarium crookwellense* sp.Nov. Trans Br Mycol Soc 79:497–505.

Burlakoti RR, Ali S, Secor GA, Neate SM, McMullen MP, Adhikari TB (2008) Genetic relationships among populations of *Gibberella zeae* from barley, wheat, potato, and sugar beet in the upper Midwest of the United States. Phytopathology 98: 969-976

Bushnell W, Hazen B (2003) Histology and physiology of Fusarium head blight. In: Leonard K, Bushnell W (eds) Fusarium head blight of Wheat and Barley. APS Press, St Paul, Minnesota, USA, pp 44–83

Camacho-Casas, M.A., W.E. Kronstad, and A.L. Scharen. 1995. Sept oria tritici resistance and associations with agronomic traits in a wheat cross. Crop Sci. 35:971–976.

Champeil A, Fourbet J., Doré T, Rossignol L (2004) Influence of cropping system on Fusarium head blight and mycotoxin levels in winter wheat. Crop Prot 23:531–537

Chandler E a, Simpson DR, Thomsett M a, Nicholson P (2003) Development of PCR assays to Tri7 and Tri13 trichothecene biosynthetic genes, and characterisation of chemotypes of *Fusarium graminearum*, *Fusarium culmorum* and *Fusarium cerealis*. Physiol Mol Plant Pathol 62:355–367

Chen J, Griffey C, Pridgen T, Chappell M (2000) Assessment and rational utilization of scab resistance sources in the Virginia Wheat Breeding Program. In: Raupp W, Ma Z, Chen P, Liu D (eds) Proc Interna Symp on Wheat Improvement for Scab Resistance. Kansas State University, Manhattan, Kansas, pp 10–17

Ciuffetti LM, Manning VA, Pandelova I, et al (2014) Pyrenophora tritici-repentis: A Plant Pathogenic Fungus with Global Impact. In: Dean R, Lichens-Park A, Kole C (eds) Genomics of Plant-Associated Fungi: Monocot Pathogens. Springer Berlin Heidelberg, Berlin, Heidelberg, pp 1–39

Colhoun J, Taylor GS, Tomlinson R (1968) *Fusarium* diseases of cereals. Trans Br Mycol Soc 51:397–404.

Cook RJ, Veseth RJ (1991) Wheat health management. APS Press, St. Paul, MN.

Coppin E, Debuchy R, Arnaise S, Picard M (1997) Mating types and sexual development in filamentous ascomycetes. Microbiol Mol Biol Rev 61:411–428

Cowger C, Hoffer ME, Mundt CC (2000) Specific adaptation by *Mycosphaerella graminicola* to a resistant wheat cultivar. Plant Pathol 49:445–451

Cox TS, Raupp WJ, Wilson DL, et al (1992) Resistance to foliar diseases in a collection of Triticum tauschii germ plasm. Plant Dis 1061–1064.

Crous PW, Summerell BA, Carnegie AJ, et al (2009) Unravelling *Mycosphaerella*: do you believe in genera? Persoonia - Mol Phylogeny Evol Fungi 23:99–118

Crous PW, Braun U, Groenewald JZ (2007) Mycosphaerella is polyphyletic. Stud Mycol 58:1–32

Culler MD, Miller-Garvin JE, Dill-Macky R (2007) Effect of extended irrigation and host resistance on deoxynivalenol accumulation in *Fusarium*-infected Wheat. Plant Dis 91:1464–1472.

Cunfer BM, Ueng PP (1999) Taxonomy and identification of *Septoria* and *Stagonospora* species on small-grain cereals 1. Annu Rev Phytopathol 37:267–284.

De Ackermann, M.D., S. Stewart, and W. Ibanez. 1995. Pathogenic variability of Septoria tritici isolates from South America. p. 41–50 In S.L. Gilchrist et al. (ed.) Proc. Septoria tritici Workshop. CIMMYT, Mexico D.F.

De Wolf ED, Effertz RJ, Ali S, Francl LJ (1998) Vistas of tan spot research. Can J Plant Pathol 20:349–370

Desjardins AE, Jarosz AM, Plattner RD, Alexander NJ, Brown DW, Jurgenson JE (2004) Patterns of trichothecene production, genetic variability, and virulence to wheat of

Fusarium graminearum from smallholder farms in Nepal. J. Agric. Food Chem. 52:6341-6346

Dill-Macky R (2003) Inoculation methods and evaluation of Fusarium head blight resistance in wheat. In: Leonard K, Bushnell W (eds) Fusarium head blight of Wheat and Barley. APS Press, St Paul, Minnesota, USA, pp 184–210

Duveiller E, Kandel YR, Sharma RC, Shrestha SM (2005) Epidemiology of foliar blights (spot blotch and tan spot) of wheat in the plains bordering the Himalayas. Phytopathology 95:248–256

Duczek LJ, Sutherland KA, Reed SL, Bailey KL, Lanford GP (1999) Survival of leaf spot pathogens on crop residues of wheat and barley in Saskatchewan. Can J Plant Pathol 21:165–173

Engle JS, Madden L V, Lipps PE (2003) Evaluation of inoculation methods to determine resistance reactions of wheat to *Fusarium graminearum*. Plant Dis 87:1530–1535.

Engle JS, Madden LV, Lipps PE (2006) Distribution and Pathogenic Characterization of *Pyrenophora tritici-repentis* and *Stagonospora nodorum* in Ohio. Phytopathology 96:1355–62

European commission (2006) Commission Regulation (EC) No. 1881/2006 setting maximum levels of certain contaminants in foodstuffs. Off J Eur Union L364:5–24.

Eyal Z, Brown MB (1976) A quantitative method for estimating density of Septoria tritici pycnidia on wheat leaves. Phytopathology 66:11–14

Eyal Z (1999) The septoria tritici and stagonospora nodorum blotch diseases of wheat. Eur J Plant Pathol 105:629–641

Eriksen L, Munk L (2003) The Occurrence of *Mycosphaerella graminicola* and its anamorph *Septoria tritici* in winter wheat during the growing season. Eur J Plant Pathol 109:253–259

Fernando WG, Paulitz TC, Seaman WL, et al (1997) Head Blight Gradients Caused by *Gibberella zeae* from Area Sources of Inoculum in Wheat Field Plots. Phytopathology 87:414–421.

Francl L, Shaner G, Bergstrom GC, et al (1999) Daily inoculum levels of *Gibberella zeae* on wheat spikes. Plant Dis 83:662–666.

Francki MG (2013) Improving *Stagonospora nodorum* resistance in wheat: a review. Crop Science 53, 355–65

Gale LR, Ward TJ, Balmas V, Kistler HC (2007) Population subdivision of *Fusarium graminearum* sensu stricto in the Upper Midwestern United States. Phytopathology 97:1434–9.

Gilbert J, Tekauz A (2000) Review: recent developments in research on Fusarium head blight of wheat in Canada. Can. J. Plant Pathol. 22:1-8

Gilbert J, Fernando WGD (2004) Epidemiology and biological control of *Gibberella zeae/Fusarium graminearum*. Can J Plant Pathol 26:464–472

Gilchrist L, Rajaram S (1997) Fusarium scab screening program at CIMMYT. In: Dubin HJ, Gilchrist L, Reeves J, McNab A (eds) Fusarium head scab: Global status and future prospects. CIMMYT, Mexico, D.F., pp 7–12

Ginkel M Van, Van Der Schaar W, Yang Z, Rajaram S (1996) Inheritance of resistance to scab in two wheat cultivars from Brazil and China. Plant Dis 80:863–867

Gisi U, Sierotzki H, Cook A, McCaffery A (2002) Mechanisms influencing the evolution of resistance to Qo inhibitor fungicides. Pest Manag Sci 58:859–867

Gosman N, Srinivasachary, Steed A, et al (2010) Evaluation of type I Fusarium head blight resistance of wheat using non-deoxynivalenol-producing fungi. Plant Pathol 59:147–157

Goswami RS, Kistler HC (2004) Heading for disaster: *Fusarium graminearum* on cereal crops. Molecular Plant Pathology 5:515-525

Guenther JC, Trail F (2005) The development and differentiation of *Gibberella zeae* (anamorph: *Fusarium graminearum*) during colonization of wheat. Mycologia 97:229–237

Guo XW, Fernando WGD, Seow-Brock HY, et al (2008) Population structure, chemotype diversity, and potential chemotype shifting of *Fusarium graminearum* in wheat fields of Manitoba. Plant Dis 92:756–762

Gupta PK, Varshney RK, Sharma PC, Ramesh B (1999) Molecular markers and their applications in wheat breeding. Plant Breed 118:369–390

Halama P, Lacoste L (1991) Déterminisme de la reproduction sexuée de *Phaeosphaeria* (*Leptosphaeria*) *nodorum*, agent de la septoriose du blé. I. Hétérothallisme et rôle des microspores. Can J Bot 69:95–99

Halama P (2002) Mating relationships between isolates of Phaeosphaeria nodorum (anamorph Stagonospora nodorum) from geographical locations. Eur J Plant Pathol 108:593–596

Hardwick N V, Jones DR, Slough JE (2001) Factors affecting diseases of winter wheat in England and Wales 1989-98. Plant Pathol 50:453–462

He X, Singh PK, Duveiller E, et al (2013) Development and Characterization of International Maize and Wheat Improvement Center (CIMMYT) Germplasm for Fusarium Head Blight Resistance. In: Alconada Magliano TM, Chulze SN (eds) Fusarium Head Blight in Latin America. Springer Netherlands, Dordrecht, pp 241–262

Hope R, Aldred D, Magan N (2005) Comparison of environmental profiles for growth and deoxynivalenol production by *Fusarium culmorum* and *F. graminearum* on wheat grain. Lett Appl Microbiol 40:295–300

Hosford R (1982) Tan Spot of Wheat and Related Diseases Workshop, July 14-15 1981, North Dakota State University, Fargo, North Dakota.

Hunter T, Coker RR, Royle DJ (1999) The teleomorph stage, *Mycosphaerella graminicola*, in epidemics of septoria tritici blotch on winter wheat in the UK. Plant Pathol 48:51–57

Jansen C, von Wettstein D, Schäfer W, et al (2005) Infection patterns in barley and wheat spikes inoculated with wild-type and trichodiene synthase gene disrupted *Fusarium graminearum*. Proc Natl Acad Sci USA 102:16892–16897

Jennings P, Coates ME, Turner J a., et al (2004a) Determination of deoxynivalenol- and nivalenol-chemotypes of *Fusarium culmorum* isolates from England and Wales by PCR assay. Plant Pathol 53:182–190

Jennings P, Coates ME, Walsh K, et al (2004b) Determination of deoxynivalenol- and nivalenol-producing chemotypes of *Fusarium graminearum* isolated from wheat crops in England and Wales. Plant Pathol 53:643–652.

Jones RK, Mirocha CJ (1999) Quality parameters in small grains from minnesota affected by Fusarium head blight. Plant Dis 83:506–511

Jones RK (2000) Assessments of Fusarium head blight of wheat and barley in response to fungicide treatment. Plant Dis 84:1021–1030

Kang Z, Buchenauer H (1999) Immunocytochemical localization of fusarium toxins in infected wheat spikes by *Fusarium culmorum*. Physiol Mol Plant Pathol 55:275–288.

Kang Z, Buchenauer H (2002) Studies on the infection process of *Fusarium culmorum* in wheat spikes: Degradation of host cell wall components and localization of trichothecene toxins in infected tissue. In: Logrieco A, Bailey JA, Corazza L, Cooke BM (eds) Mycotoxins in Plant Disease. Springer Netherlands, Dordrecht, pp 653–660

Keller NP, Hohn TM (1997) Metabolic pathway gene clusters in filamentous fungi. Fungal Genet Biol 21:17–29

Kema GHJ, Verstappen ECP, Todorova M, Waalwijk C (1996) Successful crosses and molecular tetrad and progeny analyses demonstrate heterothallism in *Mycosphaerella graminicola*. Curr Genet 30:251–258

Kema GHJ, Yu D, Rijkenberg FHJ, et al (1996) Histology of pathogenesis of *Mycosphaerella graminicola* in wheat. Phytopathology 7:777–786

Kema GH, Verstappen EC, Waalwijk C (2000) Avirulence in the wheat septoria tritici leaf blotch fungus *Mycosphaerella graminicola* is controlled by a single locus. Mol Plant Microbe Interact 13:1375–1379

Keon J, Antoniw J, Carzaniga R, et al (2007) Transcriptional adaptation of *Mycosphaerella graminicola* to programmed cell death (PCD) of its susceptible wheat host. Mol Plant Microbe Interact 20:178–193

King J, Cook RJ, Melville SC (1983) A review of *Septoria* diseases of wheat and barley. Ann Appl Biol 103:345–373

Kirk PM, Cannon PF, David JC, Stalpers JA (2001) Ainsworth and Bisby's dictionary of the fungi. 9th ed. CABI Publishing

Kodsueb R, Dhanasekaran V, Aptroot A, et al (2006) The family Pleosporaceae: intergeneric relationships and phylogenetic perspectives based on sequence analyses of partial 28S rDNA. Mycologia 98:571–583

Koizumi S, Kato H, Yoshino R, et al (1991) Distribution of causal Fusaria of wheat and barley scab in Japan. Ann Phytopath Soc Japan 7:165–173

Kolb FL, Bai GH, Muehlbauer GJ, et al (2001) Host plant resistance genes for Fusarium head blight: Mapping and manipulation with molecular markers. Crop Sci 41:611–619

Krupinsky J (1987) Pathogenicity on wheat of *Pyrenophora tritici-repentis* isolated from *Bromus inermis*. Phytopathology 77:760–765

Krupinsky M (1992) Grass hosts of *Pyrenophora tritici-repentis*. Plant Dis 76:92–95

Kumar J, Schäfer P, Hückelhoven R, et al (2002) *Bipolaris sorokiniana*, a cereal pathogen of global concern: Cytological and molecular approaches towards better control. Mol Plant Pathol 3:185–195

Kvas M, Marasas WFO, Wingfield BD, et al (2009) Diversity and evolution of Fusarium species in the Gibberella fujikuroi complex. Fungal Divers 34:1–21

Ledingham RJ, Sallans BJ, Wenhardt A (1960) Influence of cultural practices on incidence of common rootrot in wheat in Saskatchewan. Can J Plant Sci 40:310–316

Leonard KJ, Bushnell WR (Eds.), Fusarium Head Blight of Wheat and Barley. American Phytopathological Society Press, St. Paul, MN (2003), pp. 84–119

Leslie JF, Summerell BA (2006) The Fusarium Laboratory Manual. Blackwell Publishing, Ames, IA

Liddell CM, Leonard KJ, Bushnell WR (2003) Systematics of Fusarium species and allies associated with Fusarium head blight. Fusarium head blight of wheat and barley 35-43

Luzzardi GC (1985) Wheat breeding for scab resistance. In Wheat breeding for more tropical environments p. 159-168. Mexico, DF, CIMMYT.

Liu ZZ, Wang ZY (1991) Improved scab resistance in China: sources of resistance and problems. In: International Conference on Wheat for the Nontraditional Warm Areas. CIMMYT, Foz do Iguacu, Brazil, pp 178–188

Liu S, Christopher MD, Griffey CA, et al (2012) Molecular Characterization of Resistance to Fusarium Head Blight in U.S. Soft Red Winter Wheat Breeding Line VA00W-38. Crop Sci 52:2283-2292

Lovell DJ, Hunter T, Powers S (2004) Effect of temperature on latent period of septoria leaf blotch on winter wheat under outdoor conditions. Plant Pathol 53:170–181

Magboul AM, Geng S, Gilchrist DG, Jackson LF (1992) Environmental influence on the infection of wheat by *Mycosphaerella graminicola*. Phytopathology 82:1407–1413

Maraite H, Berny J, Goffin A (1992) Epidemiology of tan spot in Belgium. In: Francl LJ, Krupinsky JM, McMullen M (eds) Advances in Tan Spot Research. North Dakota Agricultural Experiment Station, Fargo, USA, pp 73–79

Markell S, Francl L (2003) Fusarium head blight inoculum: species prevalence and *Gibberella zeae* spore type. Plant Dis 87:814–820

McDonald BA, Mundt CC, Chen R-S (1996) The role of selection on the genetic structure of pathogen populations: Evidence from field experiments with *Mycosphaerella graminicola* on wheat. Euphytica 92:73–80

Mckendry a. L, Henke GE, Finney PL (1989) Effects of septoria leaf blotch on soft red winter wheat milling and baking quality. Cereal Chem 72:142–146

McMullen M, Jones R, Gallenberg D (1997) Scab of wheat and barley: a re-emerging disease of devastating impact. Plant Dis. 81:1340-1348

McMullen M, Adhikari T (2009) Fungal leaf spot diseases of wheat: Tan spot, Stagonospora nodorum blotch and Septoria tritici blotch. NDSU. Ext. Serv. 1249:1–6

Mehrabi R, Van der Lee T, Waalwijk C, Gert HJK (2006) MgSlt2, a cellular integrity MAP kinase gene of the fungal wheat pathogen *Mycosphaerella graminicola*, is dispensable for penetration but essential for invasive growth. Mol Plant Microbe Interact 19:389–398

Mehta YR (1993) Spot blotch (*Bipolaris sorokiniana*). In: Mathur SB, Cunfer BM (eds.) Seed borne disease and seed health testing of wheat. Institute of Seed Pathology for Developing Countries. Copenhagen, pp 105–112

Menzies J, Gilbert J (2003) Diseases of wheat. Chapter 9 in: Bailey KL et al. (Eds.), Diseases of Field Crops in Canada. Canadian Phytopathological Society, Saskatoon, CA. pp. 94-128

Mesterházy A (1978) Comparative analysis of artificial inoculation methods with fusarium spp. on winter wheat varieties. Phytopathol 93:12–25

Mesterházy Á (1981) The role of aggressiveness of *Fusarium graminearum* isolates in the inoculation tests on wheat in seedling state. Acta Phytopathol Acad Sci Hungaricae 16:281–292

Mesterhazy A (1983) Breeding wheat for resistance to *Fusarium graminearum* and *Fusarium culmorum*. J plant Breed 91:295–311

Mesterházy A (1995) Types and components of resistance to Fusarium head blight of wheat. Plant Breed 114:377–386

Mesterhazy A (1997) Breeding for resistance to Fusarium head blight of wheat. In: Dubin HJ, Gilchrist L, Reeves J, McNab A (eds) Fusarium head scab: Global status and future prospects. CIMMYT, Mexico, D.F., pp 79–85

Mesterházy Á, Bartók T, Mirocha CG, Komoróczy R (1999) Nature of wheat resistance to Fusarium head blight and the role of deoxynivalenol for breeding. Plant Breed 118:97–110

Mesterhazy A (2002) Role of deoxynivalenol in aggressiveness of *Fusarium graminearum* and *F. culmorum* and in resistance to Fusarium head blight. Eur J Plant Pathol 108:675–684

Mesterhazy A (2003) Control of Fusarium head blight of wheat fungicides. In: Leonard K, Bushnell W (eds) Fusarium head blight of wheat and barley. APS Press, St. Paul, Minnesota, pp 363–380

Mesterházy Á, Bartók T, Kászonyi G, et al (2005) Common resistance to different *Fusarium* spp. causing Fusarium head blight in wheat. Eur J Plant Pathol 112:267–281

Mesterházy A, Tóth B, Bartók T, Varga M (2008) Breeding strategies against FHB in winter wheat and their relation to type I resistance. Cereal Res Commun 36:37–43

Miedaner T, Perkowski J (1996) Correlations among *Fusarium culmorum* head blight resistance, fungal colonization and mycotoxin contents in winter rye. Plant Breed 115:347–351

Miedaner T (1997) Breeding wheat and rye for resistance to Fusarium diseases. Plant Breed 116:201–220

Miedaner T, Schilling a. G, Geiger HH (2001) Molecular genetic diversity and variation for aggressiveness in populations of *Fusarium graminearum* and *Fusarium culmorum* sampled from wheat fields in different countries. J Phytopathol 149:641–648

Miller JD, Young JC, Sampson DR (1985) Deoxynivalenol and Fusarium head blight resistance in spring cereals. J Phytopathol 113:359–367

Mishra AN, Kaushal K, Varma PK, Pandey HN (2001) Incidence of head blight of wheat In Madhya Pradesh during 1997-98. Indian Phytopathol 54:476–478

Murphy, DJ (2007) People plants and genes: the story of crops and humanity. Oxford University Press, Oxford

Newton AC, Caten CE (1991) Characteristics of strains of *Septoria nodorum* adapted to wheat or to barley. Plant Pathol 40:546–553

Oleson, BT (1994) World wheat production, utilization and trade. In *Wheat* (pp. 1-11). Springer, US.

Oliver RP, Friesen TL, Faris JD, Solomon PS (2012) *Stagonospora nodorum* : From pathology to genomics and host resistance. Annu Rev Phytopathol 50:23–43

Osborne LE, Stein JM (2007) Epidemiology of Fusarium head blight on small-grain cereals. Int J Food Microbiol 119:103–108

Parry DW, Jenkinson P, McLeod L (1995) Fusarium ear blight (scab) in small-grain cereals–a review. Plant Pathology 44: 207–238

Pasquali M, Migheli Q (2014) Genetic approaches to chemotype determination in type B-trichothecene producing Fusaria. Int J Food Microbiol 189:164–182

Paulitz T (1996) Diurnal release of ascospores by *Gibberella zeae* in inoculated wheat plots. Plant Dis 80:674–678

Pedersen EA (1992) The effect of crop rotation on development of the septoria disease complex on spring wheat in Saskatchewan. Can J Plant Pathol 14:152–158

Pritsch C, Muehlbauer GJ, Bushnell WR, et al (2000) Fungal development and induction of defense response genes during early infection of wheat spikes by *Fusarium graminearum*. Mol Plant Microbe Interact 13:159–169.

Pugh GW, Johann H, Dickson JG (1933) Factors affecting infection of wheat heads by *Gibberella saubiuetii*. J Agric Res 46:771–797

Puri KD, Zhong S (2010) The 3ADON population of *Fusarium graminearum* found in North Dakota is more aggressive and produces a higher level of DON than the prevalent 15ADON population in spring wheat. Phytopathology 100:1007–14

Quaedvlieg W, Kema GHJ, Groenewald JZ, et al (2011) *Zymoseptoria* gen. nov.: A new genus to accommodate *Septoria*-like species occurring on graminicolous hosts. Persoonia Mol Phylogeny Evol Fungi 26:57–69

Quaedvlieg W, Verkley GJM, Shin HD et al (2013) Sizing up *Septoria*. Stud Mycol 75: 307–390

Raemaekers RH (1988) *Helminthosporium sativum*: Disease complex on wheat and source of resistance in Zambia. In: Klatt AR (Ed) Wheat production constraints in tropical environments. CIMMYT, Mexico, D.F., pp 175–186

Rasmusson DC, Kiesling RL (1985) The Diseases of Barley. In: Rasmusson D (ed) Barley Agronomy Monograph. pp 269–308

Reis EM (1990) Integrated disease management: The changing concepts of controlling head blight and spot blotch. In: Saunders D, Hettel G (eds) International Conference on Wheat for the Nontraditional Warm Areas. CIMMYT, Foz do Iguacu, Brazil, pp 165–177

Röder MS, Korzun V, Wendehake K, et al (1998) A microsatellite map of wheat. Genetics 149:2007–2023

Rohel E a, Payne AC, Fraaije BA, Hollomon DW (2001) Exploring infection of wheat and carbohydrate metabolism in *Mycosphaerella graminicola* transformants with differentially regulated green fluorescent protein expression. Mol Plant Microbe Interact 14:156–163

Rossman AY, Samuels GJ, Rogerson CT, Lowen R (1999) Genera of Bionectriaceae, Hypocreaceae and Nectriaceae (Hypocreales, Ascomycetes). Stud Mycol 1–248.

Roussel V, Leisova L, Exbrayat F, Stehno Z, Balfourier F (2005) SSR allelic diversity changes in 480 European bread wheat varieties released from 1840 to 2000. Theoretical and applied genetics 111:162-170

Ruckenbauer P, Buerstmayr H, Lemmens M (2001) Present strategies in resistance breeding against scab (*Fusarium* spp.). Euphytica 119:121–127

Rudd JCC, Horsley RDD, Mckendry ALL, Elias EMM (2001) Host Plant Resistance Genes for Fusarium Head Blight: Sources, Mechanisms, and Utility in Conventional Breeding Systems. Crop Sci 41:620–627

Salamini F, Ozkan H, Brandolini A, Schafer-Pregl R, Martin W (2002) Genetics and geography of wild cereal domestication in the Near East. Nat Rev Genet 3:429-441

Salas B, Steffenson BJ, Casper HH, et al (1999) *Fusarium* species pathogenic to barley and their associated mycotoxins. Plant Dis 83:667–674

Schroeder HW, Christensen JJ (1963) Factors affecting resistance of wheat to scab caused by *Gibberella zeae*. Phytopathology 53:831–838

Schmale DG, Shah DA, Bergstrom GC (2005) Spatial patterns of viable spore deposition of *Gibberella zeae* in wheat fields. Phytopathology 95:472-479

Schmale DG, Wood-Jones AK, Cowger C, et al (2011) Trichothecene genotypes of *Gibberella zeae* from winter wheat fields in the eastern USA. Plant Pathol 60:909–917

Scoz LB, Astolfi P, Reartes DS, et al (2009) Trichothecene mycotoxin genotypes of *Fusarium graminearum* sensu stricto and *Fusarium meridionale* in wheat from southern Brazil. Plant Pathol 58:344–351

Shaner G, Finney RE (1982) Resistance in soft red winter wheat to *Mycosphaerella graminicola*. Phytopathology 72:154–158.

Shaner G (2003) Epidemiology of Fusarium head blight of small grain cereals in North America. In: Leonard KJ, Bushnell WR (eds) Fusarium Head Blight of Wheat and Barley. American Phytopathological Society; St. Paul, MN, USA, pp 84–119.

Shah DA, Bergstrom GC, Ueng PP (2001) Foci of stagonospora nodorum blotch in winter wheat before canopy development. Phytopathology 91:642–647

Shaw MW, Royle DJ (1989) Airborne inoculum as a major source of *Septoria tritici* (*Mycosphaerella graminicola*) infections in winter wheat crops in the UK. Plant Pathol 38:35–43

Shaw MW, Royle DJ (1993) Factors determining the severity of epidemics of *Mycosphaerella graminicola* (*Septoria tritici*) on winter wheat in the UK. Plant Pathol 42:882–899.

Shetty NP, Kristensen BK, Newmana M a., et al (2003) Association of hydrogen peroxide with restriction of *Septoria tritici* in resistant wheat. Physiol Mol Plant Pathol 62:333–346

Shewry, PR (2009) Wheat. Journal of experimental botany 60: 1537-1553

Shoemaker RA (1959) Nomenclature of *Drechslera* and *Bipolaris*, grass parasites segregated from "*Helminthosporium*." Can J Bot 37:879–887

Shoemaker RA, Babcock CE (1989) *Phaeosphaeria*. Can J Bot 67:1500–1599

Shoemaker RA (1961) *Pyrenophora phaeocomes* (REB. EX FR.) FR. Can J Bot 39:901–908

Skinnes H, Tarkegne Y, Dieseth J, Bjørnstad Å (2008) Associations between anther extrusion and Fusarium Head Blight in European wheat. Cereal Res Commun 36:223–231

Snijders C, Perkowski J (1990) Effects of Head Blight Caused by *Fusarium culmorum* on Toxin Content and Weight of Wheat Kernels. Phytopathology 80:566–570

Solomon PS, Lowe RGT, Tan KC, et al (2006) *Stagonospora nodorum*: cause of stagonospora nodorum blotch of wheat. Mol Plant Pathol 7:147–156

Stack RW (1989) A comparison of the inoculum potential of ascospores and conidia of *Gibberella zeae*. Can J Plant Pathol 11:137–142

Starkey DE, Ward TJ, Aoki T, et al (2007) Global molecular surveillance reveals novel Fusarium head blight species and trichothecene toxin diversity. Fungal Genet Biol 44:1191–204

Strelkov SE, Lamari L (2003) Host–parasite interactions in tan spot [*Pyrenophora tritici-repentis*] of wheat. Can J Plant Pathol 25:339–349.

Stukenbrock EH, Banke S, McDonald BA (2006) Global migration patterns in the fungal wheat pathogen *Phaeosphaeria nodorum*. Mol Ecol 15:2895–2904

Sutton JC (1982) Epidemiology of wheat head blight and maize ear rot caused by *Fusarium graminearum*. Canadian Journal of Plant Pathology 4: 195-209

Sugiura Y, Saito H, Tanaka T, et al (1994) *Fusarium crookwellense*, a newly isolated fungus from wheat in Japan: Its mycotoxin production and pathogenicity to wheat and barley. Mycoscience 35:77–82

Talas F, Parzies HK, Miedaner T (2011) Diversity in genetic structure and chemotype composition of *Fusarium graminearum* sensu stricto populations causing wheat head blight in individual fields in Germany. Eur J Plant Pathol 131:39–48

Tanksley SD, McCouch R (1997) Seed banks and molecular maps: unlocking genetic potential from the wild. Science 277:1063-1066

Taylor M (2004) Incorporation of Fusarium head blight resistance into European winter wheat breeding programmes. In: Proceedings of the 2nd International Symposium on Fusarium Head Blight. Michigan State University, East Lansing, pp 195–196

Tekle S, Dill-Macky R, Skinnes H, Tronsmo A M, Bjørnstad Å (2012) Infection process of *Fusarium graminearum* in oats (*Avena sativa* L.). Eur. J. Plant Pathol. 132: 431-442

Torriani SFF, Linde CC, McDonald BA (2009) Sequence conservation in the mitochondrial cytochrome b gene and lack of G143A QoI resistance allele in a global sample of *Rhynchosporium secalis*. Australas Plant Pathol 38:202-207

Trevaskis B, Hemming MN, Dennis ES, Peacock WJ (2007) The molecular basis of vernalization-induced flowering in cereals. Trends Plant Sci. 12:352-57

Turgeon BG (1998) Application of mating type gene technology to problems in fungal biology. Annu Rev Phytopathol 36:115–137

Van Eeuwijk FA, Mesterhazy A, Kling CI, et al (1995) Assessing non-specificity of resistance in wheat to head blight caused by inoculation with European strains of *Fusarium culmorum*, *F. graminearum* and *F. nivale* using a multiplicative model for interaction. Theor Appl Genet 90:221–228

Verges VL, Van Sanford D, Brown-Guedira G (2006) Heritability estimates and response to selection for Fusarium head blight resistance in soft red winter wheat. Crop Sci 46:1587–1594

Von Der Ohe C, Gauthier V, Tamburic-ilincic L, et al (2010) A comparison of aggressiveness and deoxynivalenol production between Canadian *Fusarium graminearum* isolates with 3-acetyl and 15-acetyldeoxynivalenol chemotypes in field-grown spring wheat. Eur J Plant Pathol 127:407–417

Waalwijk C, Kastelein P, Vries I De, et al (2003) Major changes in *Fusarium* spp. in wheat in the Netherlands. Eur J Plant Pathol 109:743–754

Wagacha JM, Muthomi JW (2007) *Fusarium culmorum*: Infection process, mechanisms of mycotoxin production and their role in pathogenesis in wheat. Crop Prot 26:877–885.

Wang Y, Miller J (1988) Screening techniques and sources of resistance to Fusarium head blight. In: AR K (ed) Wheat production constraints in tropical environments. CIMMYT, Mexico, DF, pp 239–250

Walter S, Nicholson P, Doohan FM (2010) Action and reaction of host and pathogen during Fusarium head blight disease. New Phytol 185:54–66

Ward TJ, Bielawski JP, Kistler HC, et al (2002) Ancestral polymorphism and adaptive evolution in the trichothecene mycotoxin gene cluster of phytopathogenic *Fusarium*. Proc Natl Acad Sci 99:9278–9283

Ward TJ, Clear RM, Rooney AP, et al (2008) An adaptive evolutionary shift in Fusarium head blight pathogen populations is driving the rapid spread of more toxigenic *Fusarium graminearum* in North America. Fungal Genet Biol 45:473–84

Weise MV (1987) Compendium of wheat diseases, 2nd ed. APS Press, St. Paul.

Wicki W, Winzeler M, Schmid JE, et al (1999) Inheritance of resistance to leaf and glume blotch caused by *Septoria nodorum* Berk. in winter wheat. Theor Appl Genet 99:1265–1272

Yadav B (1981) Behaviour of *Cochliobolus sativus* during its infection of barley and wheat leaves. Aust J Bot 29:71-79

Yli-Mattila T, Gagkaeva T, Ward TJ, et al (2009) A novel Asian clade within the *Fusarium graminearum* species complex includes a newly discovered cereal head blight pathogen from the Russian Far East. Mycologia 101:841–852

Yoruk E, Albayrak G (2012) Chemotyping of *Fusarium graminearum* and *F. culmorum* isolates from Turkey by PCR assay. Mycopathologia 173:53-61

Zhang H, Van der Lee T, Waalwijk C, et al (2012) Population Analysis of the *Fusarium graminearum* Species Complex from Wheat in China Show a Shift to More Aggressive Isolates. PLoS One 7:e31722.

Zohary D, Hopf M (2000) Domestication of plants in the old world: the origin and spread of cultivated plants in West Asia, Europe, and the Nile Valley. Oxford University Press, Oxford.

Zhan J, Mundt CC, McDonald BA (2000) Estimation of rates of recombination and migration in populations of plant pathogens-A reply. Phytopathology 90:324–326.

Zhang Y, Crous PW, Schoch CL, Hyde KD (2012) Pleosporales. Fungal Divers 53:1–221.

Chapter 2

Chemotype analysis of Mexican Fusarium graminearum sensu lato isolates

Mohamed Osman

Chapter 2

Chemotype analysis of Mexican *Fusarium graminearum* sensu lato isolates

Abstract

Four of the world's most important crops, wheat, barley, rice and maize, in addition to other small grains are susceptible to *Fusarium graminearum* sensu lato (*F. graminearum* s.l.), the most important causal agent of Fusarium head blight (FHB). The major threat from this species complex comes from trichothecene mycotoxins such as deoxynivalenol (DON) and nivalenol (NIV) and their acetylated derivatives. Polymerase chain reaction (PCR) assays are being utilized to quickly characterize type B-trichothecene mycotoxin genotypes in populations of head blight causing Fusaria. In the current study, 388 isolates collected from different locations in 6 Mexican states between 1995 and 2013 were analyzed by chemotype and sub-chemotype specific markers. It was confirmed that the disease has been predominantly caused by *F. graminearum* s.l., while *F. cerealis* co-presented as FHB causal agent in Mexico. Both DON and NIV chemotypes were identified in isolates belonging to *F. graminearum* s.l., with the DON chemotype predominating. Furthermore, all DON isolates were shown to be the 15-ADON sub-chemotype and no 3-ADON sub-chemotype was identified. This was the first detailed study on the trichothecene genotypes of Mexican toxigenic *Fusarium* strains on large scale; wherein we report for the first time the occurrence of *F. graminearum* isolates belonging to NIV chemotype in Mexico.

Keywords: Fusarium head blight, *Fusarium cerealis*, Deoxynivalenol, Nivalenol, PCR

2.1. Introduction

Wheat, barley, rice and maize, the four most important crops globally along with other small grains crops, are threatened by a number of *Fusarium* species that cause Fusarium head blight (FHB, or Fusarium ear rot in the case of maize) which is a devastating disease worldwide (Hernandez et al., 2014; McMullen et al., 1997; Pritsch et al., 2000). In addition to direct yield losses, FHB infection leads to deterioration in grain quality (Bai and Shaner, 1994; Mardi et al., 2005) and contamination with mycotoxins such as nivalenol (NIV), deoxynivalenol (DON), and zearalenone (Buerstmayr et al., 2012; Reischer et al., 2004; Schisler et al., 2002).

Changes in agricultural practices, including more maize-wheat rotation and adoption of conservation agriculture, in addition to climate change, have contributed to an enhanced incidence and severity of FHB (He et al., 2013). FHB of cereals is caused by several *Fusarium* species in humid and semi-humid farming areas, and the most frequent are *F. graminearum* sensu lato (referred to hereafter as *F. graminearum*) and the related species *F. culmorum* and *F. cerealis* (Buerstmayr et al., 2012; Mesterházy, 1978; Miller et al., 1991; Pasquali and Migheli, 2014; Xue et al., 2006).

F. graminearum is the most important and the predominant causal pathogen of cereal FHB in Australia (Akinsanmi et al., 2004), China (Zhang et al., 2012), Japan (Koizumi et al., 1991), Turkey (Yoruk and Albayrak, 2012), North America, Brazil and many other parts of the world (Alvarez et al., 2009; Astolfi et al., 2011; Goswami and Kistler, 2004; Ward et al., 2008). *F. culmorum* has been traditionally reported as a chief causal agent of FHB in Northern, Central and Western Europe (Hope et al., 2005; Wagacha and Muthomi, 2007); but a shift is being noticed lately in Europe, and *F. graminearum* has been spreading northward in Europe (Osborne and Stein, 2007; Scherm et al., 2013; Waalwijk et al., 2003). *F. cerealis* (synonym *F. crookwellense*) is another toxigenic FHB related species. It is generally more frequent in temperate humid areas (Burgess, 1982) and has been isolated sporadically from infected spikes (Schmale et al., 2011; Sugiura et al., 1994).

Trichothecenes are toxic secondary metabolites, named after the compound 'trichothecin' from the fungus *Trichothecium roseum* (Bennett and Klich, 2003; Desjardins et al., 1993). These toxic compounds are divided, according to variations in the functional hydroxyl and acetoxy groups, into 4 types: A, B, C and D trichothecenes (Marin et al., 2013). Type A

trichothecenes include T-2, HT-2 and diacetoxyscirpenol (DAS), while type B trichothecenes include DON, NIV and their acetylated derivatives 3-ADON and 15-ADON, 4-acetyl NIV.

Fusarium graminearum and *F. culmorum* isolates are type B trichothecene producers (Ichinoe et al., 1983; Miller et al., 1991), to which *F. cerealis* also belongs. However, this species consists of a single entity, sexual reproduction of which is not known, which may explain why it exhibits low levels of variation (Malihipour et al., 2012; Miller et al., 1991) and produces only NIV (Gagkaeva, 2010; Sugiura et al., 1994; Xu and Nicholson, 2009). Accordingly based on their type B trichothecene production, there are DON-producing isolates (which are usually further subdivided into 3-ADON and 15-ADON chemotypes) and NIV-producing isolates (Desjardins and Plattner, 2003; Sugiura et al., 1994). Both DON and NIV have a carbonyl function at the C-8 position (Brown et al., 2003), and are considered to be the principal trichothecenes produced by *F. graminearum*. These two toxins have been reported to be the most commonly occurring mycotoxin contaminants detected in grain globally, and are associated with the incidence of FHB symptoms (Chandler et al., 2003; Placinta et al., 1999). Nevertheless DON is considered the most important toxin, though it is less toxic than other trichothecenes (Lattanzio et al., 2009; Pestka, 2007; Sobrova et al., 2010).

Fusarium populations in North America, South America and Europe are predominantly DON producers, whereas those in China, Japan, Korea, Australia and Nepal consist of much higher proportion of NIV producers in addition to DON producers (Chandler et al., 2003; Desjardins, 2006; Lee et al., 2004; Miller et al., 1991; Suga et al., 2008).

The 15-ADON sub-chemotype (referred to hereafter as 15-ADON) was reported to be prevalent in the United States (Gale et al., 2007; Schmale et al., 2011), Mexico (He et al., 2013; Miller et al., 1991), UK (Jennings et al., 2004), Central Europe (Talas et al., 2011), and Southern Russia (Yli-Mattila et al., 2009). However, a shift from 15-ADON to 3-ADON genotypes has been detected since the last decade in many locations in North America including US and Western Canada (Gale et al., 2007; Ward et al., 2008). A likely interpretation for this change is that the introduced 3-ADON isolates compete better than the local 15-ADON isolates in those areas (Guo et al., 2008); since *F. graminearum* 3-ADON isolates were reported to produce more trichothecenes and cause greater FHB severity on wheat compared with 15-ADON isolates (Puri and Zhong, 2010; von der Ohe

et al., 2010; Zhang et al., 2012), the change of their relative frequencies is of high significance.

The *TRI* genes, like other secondary pathway genes of fungi, are often arranged in clusters within the genome, encoding for enzymes involved in the trichothecene biosynthesis of the fungus (Keller and Hohn, 1997; Pasquali and Migheli, 2014). Since the variation in trichothecene production reflects the allelic polymorphisms of *TRI* genes (Amarasinghe et al., 2011; Ward et al., 2002), PCR assays were developed in the last two decades to rapidly characterize populations of toxigenic Fusaria in terms of their chemotypes and sub-chemotypes, greatly facilitating studies on the diversity and mycotoxin potential of FHB pathogens worldwide (Chandler et al., 2003; Gale et al., 2007; Guo et al., 2008; Lee et al., 2001; Scoz et al., 2009; Starkey et al., 2007).

In Mexico, routine maize -wheat rotations have resulted in inoculum build up since both of the crops are common hosts. Although many studies reported the chemo-diversity both in North and South America, only few scattered attempts aimed at characterizing Mexican FHB related *Fusarium* isolates. Miller et al. (1991) assessed the potential of 12 *F.graminearum* isolates of Mexico to produce trichothecenes by gas chromatography/mass spectromet and he found all of them to belong to the 15-ADON. Using a PCR based on the *Tri12* gene, Malihipour et al. (2012) included 7 isolates of *F.graminearum* and 8 *F.crookwellense* Mexican isolates. According to this study, all of *F.graminearum* isolates were of 15-ADON whilst the *F.crookwellense* isolates were NIV producers. Similarly, He et al. (2013) reported that all of the *F.graminearum* isolates collected in 2007 were 15-ADON producers using a multiplex PCR test.

As one of the routine tasks of CIMMYT's wheat pathology laboratory, about 90 new *Fusarium* strains are collected annually in late summer from naturally infected cereals in different farms in Mexico, to ensure the viability and virulence of the pathogen for both greenhouse and field screening activities. The objectives of this study were to identify the predominant cereal FHB causing pathogen in Mexico and to characterize its chemotype and sub-chemotype composition using isolates newly collected in 2013 and those from previous years to see if temporal or spatial variation exists, to better understand the chemo-diversity of FHB related species in Mexico.

2.2. Materials and methods

2.2.1. Sample collection

Two sets of *Fusarium* isolates were used in this study. The first set included a total of 104 isolates collected in 2013, and the second one involved 284 old isolates collected between 1995 and 2012, many of which were used for field and greenhouse screening activities of wheat pathology laboratory at CIMMYT, Mexico.

Isolates were collected annually from naturally *Fusarium* infected cereal crops (mostly wheat) about 4 weeks after flowering during August and September from different locations in six states of Mexico (Fig. 2.1 & 2.2). Infected kernels were visually inspected to separate out rough, wilted, pink to soft-grey or light-brown in color (tombstone) for fungal isolation (McMullen et al., 1997). Diseased kernels were surface-sterilized for 2 min by vortexing in a 5% sodium hypochlorite solution, rinsed in sterile distilled water for 1 min and dried in a laminar flow cabinet on sterile filter paper for an hour. Then, grains were incubated on Potato Dextrose Agar (PDA) at 25°C for 8 days. Fungal colonies grown from the damaged kernels were examined under microscope or stereoscope and isolates were tentatively identified based on colony characteristics and spore morphology on PDA according to Nelson et al., (1983). The selected isolates were subcultured on water agar and incubated for 18 to 24 h, and mono-spores were identified and transferred to synthetic nutrient agar (SNA) plates (1 g KH_2PO_4, 1 g KNO_3, 0.5 g $MgSO_47H_2O$, 0.5 g KCl, 0.2 g glucose, 0.2 g sucrose, and 20 g agar in 1-liter d.H_2o). For long term storage, isolates were stored on desiccated filter paper at 4°C as described by Correll et al., (1986).

2.2.2. DNA extraction and fungal characterization

All of the isolates were grown for 7 days in yeast extract-sucrose broth medium (2 g yeast extract, 6 g sucrose, 1000 ml d.H_2O) on a rotating shaker at 120 rpm. The resulting mycelial suspension was filtered through Whatman® Grade No. 4 filter paper, freeze dried for 48 h at -80°C, and lyophilized for 48 h. Mycelium was pulverized by vigorous shaking of the blocks for 3 min in a ball mill using stainless steel beads. DNA was extracted and purified according to the CTAB method recommended by the European Community Reference Laboratories for the isolation of maize DNA (European commission, 2007).

Subsequently the isolates were characterized by PCR-based markers listed in Table 2.2. *F. graminearum* isolates were identified with the species-specific marker Fg16NF/R with a PCR product of 280 bp. Then, DON and NIV producers were identified using the chemotype-specific marker ToxP1/P2 with their corresponding PCR products of 300 bp and 360 bp, respectively (Li et al., 2005). The sub-chemotyping of the DON producers was carried out in two concurrent experiments: 1) the multiplex PCR assay with Tri11-CON, Tri11-3ADON, Tri11-15ADON and Tri11-NIV for *Tri11* gene. *Tri11* gene encodes for a key enzyme in the trichothecene mycotoxin biosynthesis isotrichodermin C-15 hydroxylase, leading to T-2, DON, 3-ADON, 15- ADON, and NIV biosynthesis in *Fusarium* species. The *Tri11* based multiplex PCR primers, generating a 334 bp fragment from 3-ADON-producing strains, a 279 bp from 15-ADON producers and a 497 bp fragment from NIV producers. 2) a generic PCR assay using a single pair of primers Tri13P1 and Tri13P2 designed from the *Tri13* genes, which was reported to be the determinant for the DON-NIV switching in *Fusarium* (Wang et al., 2008) because it is not functional in DON-producing isolates due to the three deletion sites in this gene (Yoruk and Albayrak, 2012). This primer set detects a 583 bp fragment from 15-ADON-chemotypes, a 644 bp fragment from 3-ADON-chemotypes and an 859 bp fragment from NIV-producing strains. In addition, two primer sets Tri303F/Tri303R (with a 586 bp product amplified from 3-ADON producing strains) and Tri315F/Tri315R (with an 864 bp product amplified from 15-ADON producing strains) were used to further confirm results obtained above, through characterizing the *Tri3* gene which is essential for the production of C-15 acetylase (Jennings et al., 2004).

All PCR amplifications were performed in a 10 µl reaction volume containing 4.5 µl pre-mixed ReadyMix (Sigma-Aldrich, St. Louis, MO), 250 nM of each primer (except for Tri11 primers where 300 nM of Tri11-CON was used but 100 nM of the rest), and 50 ng template DNA. PCR-grade water was used instead of DNA in one reaction as a negative control. PCR was performed on a Mastercycler® (Eppendorf) following the cycling conditions listed in Table 2.2. The amplicons were separated by electrophoresis on a 2% agarose gel, stained with EnviroSafe® DNA/RNA stain and viewed under UV light.

Table 2.1 The number of studied isolates from each location and their hosts.

	State	City	Host	No. isolates
	State of Mexico	Batan	Wheat	219
		Toluca	Wheat, Corn, Triticale	45
		Boximo	Wheat	5
		Guanajuato	Wheat	1
		Juchitepec	Wheat	1
	Tlaxcala	Tlaxcala	Barley	2
	Jalisco	Jesús María	Wheat	1
		Tepatitlan	Wheat	24
	Puebla	Agua Fria	Wheat	2
	Michoacan	Patzcuaro	Wheat	48
	Oxaca	Oxaca	Wheat	40
Totale	6	11		388

Figure 2.1 Percentage of the used isolates based on sampling period: 2013, 2006-2012 and before 2006.

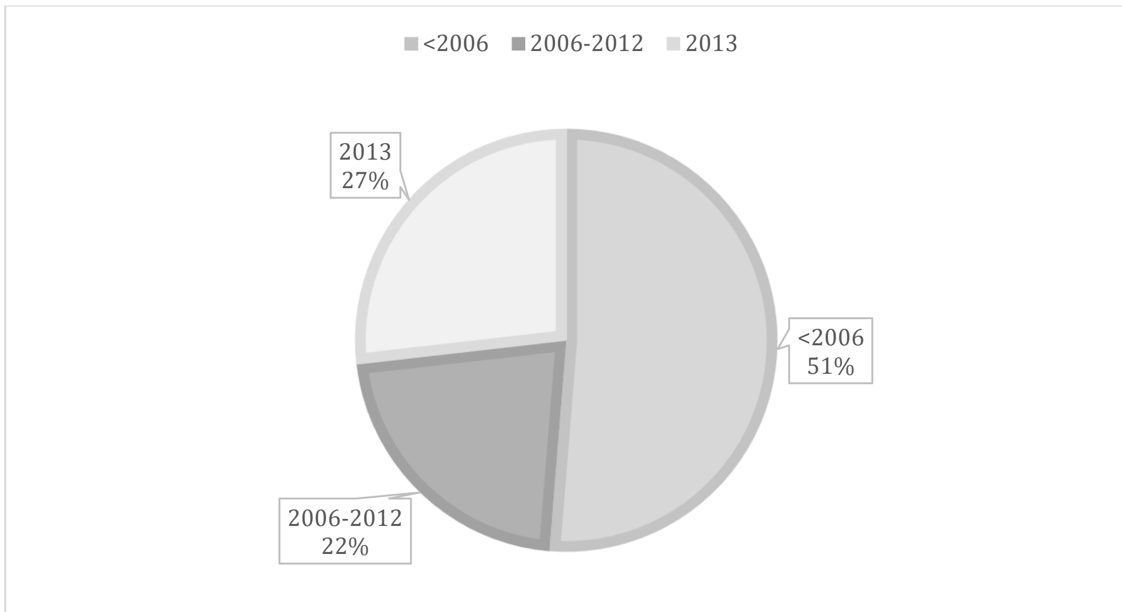


Figure 2.2. Sampling locations of *Fusarium* isolates used in this study

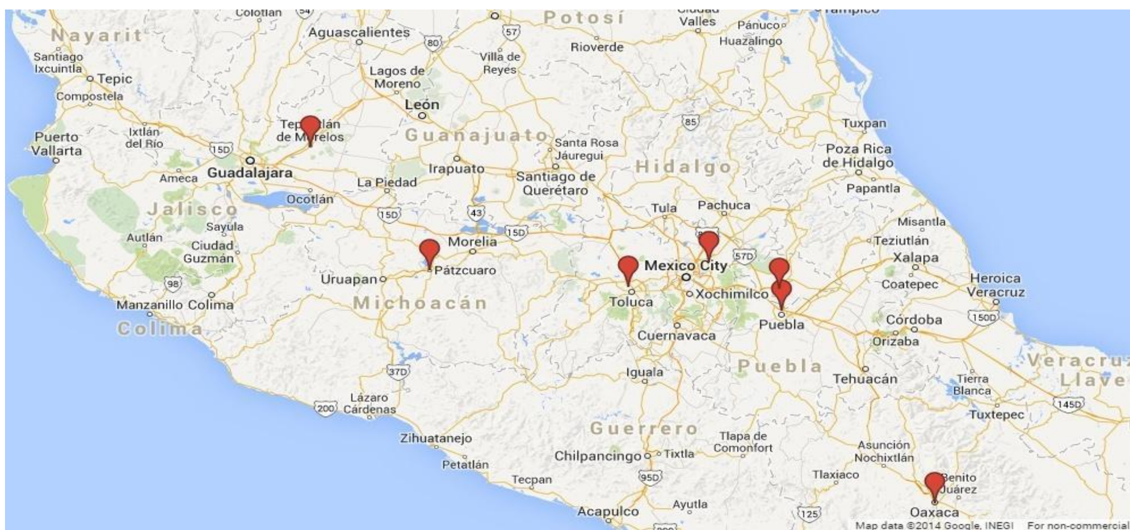


Table 2.2 Primers used in this study, their nucleotide sequences and product sizes

Primer	Primer sequence (5'–3')	Gene	Amplicon(bp)	Target	PCR program	References
Fg16NF	ACA GAT GAC AAG ATT CAG GCA CA	-	280	*F.graminearum*	95°:2'+(95°:30'',63:45'',72°:45'')$_{x35}$+72:10'	(Nicholson et al., 1998)
Fg16R	TTC TTT GAC ATC TGT TCA ACC CA					
Tri303F	GATGGCCGCAAGTGGA	*TRI 3*	583	3-ADON	94°:2'+(94°:30'',58:45'',72°:45'')$_{x35}$+72:10'	(Jennings, Coates, et al., 2004)
Tri30R	GCCGGACTGCCCTATTG					
Tri315F	CTCGCTGAAGTTGGACGTAA	*TRI 3*	863	15-ADON		(Jennings, Coates, et al., 2004)
Tri31R	GTCTATGCTCTCAACGGACAAC					
ToxP1	GCCGTGGGGRTAAAAGTCAAA	*Tri5–Tri6 intergenic*	360	NIV	95°:5'+(94°:60'',55:60'',72°:50'')$_{x30}$+72:6'	(Li et al., 2005)
ToxP2	TGACAAGTCCGGTCGCACTAGCA		300	DON		
Tri13P1	CTCSACCGCATCGAAGASTCTC	*TRI 13*	859	NIV	94°:4'+(94°:60'',58:40'',72°:40'')$_{x35}$+72:6'	(Wang et al., 2008)
Tri13P2	GAASGTCGCARGACCTTGTTTC		644	3-ADON		
			583	15-ADON		
Tri11-CON	GACTGCTCATGGAGACGCTG	*TRI 11*			94°:4'+(94°:30'',58:30'',72°:30'')$_{x25}$+72:5'	(Wang et al., 2012)
Tri11-3ADON	TCCTCATGCTCG GTGGACTCG		334	3-ADON		
Tri11-15ADON	TGGTCCAGT TGTCCGTATT		279	15-ADON		
Tri11-NIV	GTAGGTTCCATTGC TTGTTC		497	NIV		

2.3. RESULTS

2.3.1. Species identification

A total of 102 out of 109 isolates collected in 2013 were identified as *F. graminearum* using traditional identification techniques and confirmed by PCR analysis (Table S1.2). Two isolates were classified as *F. cerealis* based on morphological characters, whereas the rest of isolates that had different morphology (n=5) were excluded from further analysis. Of the 284 old isolates, 249 were previously verified to be *F. graminearum*, and 35 were classified as *F. cerealis.* Afterwards, both the two sets of isolates were combined for chemotyping analysis.

2.3.2. Chemotyping

Type B trichothene chemotypes were determined for a total of 388 monosporic isolates collected from 6 different Mexican states, of which 351 belong to *F. graminearum* and 37 isolates belong to *F. cerealis*.

The primers ToxP1/2, which were designed from the intergenic sequences between *Tri5* and *Tri6* genes, amplified a 300 bp fragment specific for DON producers from 347 *F. graminearum* isolates, but a 360 bp fragment indicative of NIV chemotype from 4 of *F. graminearum* isolates and all of the 37 *F. cerealis* isolates.

The primers Tri13 P1/P2 for the *Tri13* gene amplified a 583 bp fragment from all the 347 DON producing *F. graminearum* isolates, indicating that all of them were of the 15-ADON sub-chemotype. An 859 bp fragment for NIV producers was obtained from all the NIV isolates classified by the ToxP1/2 primer set. No 644 bp fragment for the 3-ADON chemotype was detected in any of the studied isolates.

To confirm our results and to check the congruity of the different PCR assays, the DON genotypes were tested with the Tri11, Tri315, Tri303 primer sets. Accordingly, a 279 bp fragment amplified by Tri11 and an 863 bp product by Tri315F/R (all specific to 15-ADON sub-chemotype) were produced from all the previously recognized 15-ADON genotypes,

whereas no amplicon was obtained with the Tri303 primer set for the 3-ADON sub-chemotype.

Apparently, the isolates carried chemotype-specific DNA sequences determining their chemotype characters as they were identified by different multiplex PCR assays (Table 1), i.e. none of the DON isolates based on Toxp primers showed to belong to NIV chemotype in Tri11 or Tri13 based assays and vice versa.

2.4. Discussion

The aims of this study were to identify the predominant cereal FHB causing pathogen and investigate its trichothecene chemotype composition in Mexico. Both morphological and molecular techniques confirmed that *F. graminearum* is the major species causing FHB in Mexico which is consistent with a previous study in Mexico (He et al., 2013) and with other studies in North America (Goswami and Kistler, 2004; Ward et al., 2008). Although a few previous reports studied the genetic variation of Mexican FHB related *Fusarium* isolates using chemical analysis (Miller et al., 1991) and molecular markers (He et al., 2013; Malihipour et al., 2012), this study provided the first detailed report on the incidence of type-B trichothecene genotypes of toxigenic FHB related *Fusarium* populations in Mexico. In the present study, more isolates were characterized than in the aforementioned studies, including 347 of *F. graminearum* and 37 of *F. cerealis*. Of the 347 *F. graminearum* isolates, 99% (347⁄351) of the isolates were of the DON chemotype based on the amplicon from the intergenic sequences between *Tri5* and *Tri6* genes, while 1% of the *F. graminearum* isolates (4⁄351) were of the NIV chemotype. All of the NIV isolates were recovered from wheat in different states and years (Table S1.2). Further characterization of *Tri13, Tri11* and *Tri3* genes has revealed that all of the DON chemotype isolates belong to the 15-ADON, while the 3-ADON was absent. As expected, all of the *F. cerealis* isolates showed PCR products specific to NIV chemotype, the same finding as reported previously (Gagkaeva, 2010; Sugiura et al., 1994; Xu and Nicholson, 2009).

According to our results, both NIV and DON chemotypes were detected in isolates obtained from different locations and/or years in the states of Mexico, Puebla, Jalisco and Michoacan, whereas only DON chemotype was found in the other locations. Generally,

differences in chemotypes distribution could be attributed to: hosts distribution, soil type, cultivars, used fungicides and cultural practices in addition to putative climate effects (Jennings et al., 2004; Pasquali and Migheli, 2014), but in our study a clear reason can not be attributed. It is noteworthy that although they share very similar chemical structures, DON is more phytotoxic to plants. While NIV is more toxic towards animals and human (Ryu et al., 1988). Furthermore, NIV producing isolates were found to be less sensitive to tebuconazole than 15ADON isolates (Umpierrez et al., 2013), thus high incidence of the NIV chemotype should be of considerable concern. Accordingly, the occurrence of the NIV genotypes of *F. graminearum* and *F. cerealis* in the states of Mexico, Puebla, Jalisco and Michoacan emphasizes the need for more intensive sampling in different locations across these states and the surrounding regions. Moreover, it is also important to regularly inspect toxin content in cereal products obtained from these states to ensure their suitability for human and animal consumption, knowing that trichothecenes are readily toxic and do not require any metabolic activation to elicit their toxicity (Shank, 1981).

Shifts in chemo-genotypes within *Fusarium* species have been observed in different reports. For example, Ward et al., (2008) detected a shift from 15- to 3-ADON producing isolates in North America, which have the potential to produce higher quantity of this mycotoxin and are reported to be more aggressive. In the Netherlands, a slight increase in NIV frequency, which is more toxic to humans and animals, was detected by Waalwijk et al., (2003). These shifts could be enhanced by the competitive abilities of the recently overwhelming chemotypes over the replaced ones, reflecting pathogen's advantage of adaptation to different hosts and environments and possibly to overcoming of improved host resistance (von der Ohe, 2010). In other words, continuous depending on limited sources of resistance and overuse of fungicides could lead the pathogen populations to shift to increased aggressiveness and toxin production. In order to investigate any shift in chemotypes of the FHB related species, we tested newly collected 2013 isolates as well as the old collection of *F. graminearum* and *F. cerealis* available in the CIMMYT's wheat pathology lab with different PCR assays based on different *Tri* genes, due to the fact that a NIV chemotype PCR assay based on only one gene may not be reliable in every case (Chandler et al., 2003; Desjardins et al., 2008). Although the incidence of *F. cerealis* species, which is notably a NIV producer, was reported previously; this study provides the first report on the occurrence of NIV chemotype of *F. graminearum* in Mexico, which was not reported previously probably due to the small number of sampled isolates. However,

due to the fact that both of the genes *Tri13* and *Tri7*; which are required for conversion of DON to NIV and acetylation of NIV, respectively; are nonfunctional in DON producers, thus it is unlikely for the NIV isolates to be resulted from DON populations by mutation and the hypothesis of being introduced into Mexico maybe more acceptable. Hence no substantial change in chemotype and sub-chemotype structure of Mexican populations of *F. graminearum* could be suggested so far, and the 15-ADON sub-chemotype always predominated across years and locations.

In this study *F. graminearum* was used to refer to *F. graminearum* species complex (FGSC) which comprises at least 15 phylogenetically distinct species (Zhang et al., 2012), of which only one (lineage 3 or *F. boothii*) is repoted in Mexico (Backhouse, 2014). The maize-wheat rotation and adoption of conservation agriculture in addition to the warm weather may favored *F. boothii* predominance in Mexico (Backhouse, 2014; Boutigny et al., 2011). Though the identification of *F. graminearum* isolates belonging to the NIV chemotype may increase the chance to find another species of the FGSC in Mexico; the exact lineage/species identification is beyond scope of the present study. Thus routine monitoring of chemotypes, determining the distribution of mycotoxins throughout wheat and maize fields in Mexico and phylogenetic analysis to determine the fine species or lineages of the *F. graminearum* species complex are needed to obtain more comprehensive results about diversity and stability of the distribution of trichothecene genotypes in Mexico and to detect possible future shifts in chemotypes. In order to achieve this goal, samples from different hosts, new locations especially in north of Mexico and/or different seasons will be helpful.

2.5. Acknowledgements

The author gratefully acknowledges the financial support for this project from The CGIAR Research Program WHEAT and The Bill and Melinda Gates Foundation, USA through the Durable Rust Resistance Wheat project.

2.6. References

Akinsanmi, O.A., Mitter, V., Simpfendorfer, S., Backhouse, D., Chakraborty, S., 2004. Identity and pathogenicity of Fusarium spp. isolated from wheat fields in Queensland and northern New South Wales. Australian Journal of Agricultural Research 55, 97–107.

Alvarez, C.L., Azcarate, M.P., Pinto, V.F., 2009. Toxigenic potential of *Fusarium graminearum* sensu stricto isolates from wheat in Argentina. International Journal of Food Microbiology 135, 131-135.

Amarasinghe, C., Wang, J.-H., Liao, Y.-C., Fernando, W.G.D., 2011. Difference in tri13 gene sequences between the 3-acetyldeoxynivalenol producing *Fusarium graminearum* chemotypes from Canada and China. International Journal of Molecular Sciences 12, 6164-6175.

Astolfi, P., dos Santos, J., Schneider, L., Gomes, L.B., Silva, C.N., Tessmann, D.J., Del Ponte, E.M., 2011. Molecular survey of trichothecene genotypes of *Fusarium graminearum* species complex from barley in Southern Brazil. International Journal of Food Microbiology 148, 197-201.

Bai, G., Shaner, G., 1994. Scab of wheat: Prospects for control. Plant Disease 78, 760-766.

Backhouse, D., 2014. Global distribution of *Fusarium graminearum, F. asiaticum* and *F. boothii* from wheat in relation to climate. European Journal of Plant Pathology 139, 161-173.

Bennett, J.W., Klich, M., 2003. Mycotoxins. Clinical Microbiology Reviews 16, 497-516.

Boutigny, A.L., Ward, T.J., Van Coller, G.J., Flett, B., Lamprecht, S.C., O'Donnell, K., Viljoen, A., 2011. Analysis of the *Fusarium graminearum* species complex from wheat, barley and maize in South Africa provides evidence of species-specific differences in host preference. Fungal Genetics and Biology 48, 914-920.

Brown, D.W., Proctor, R.H., Dyer, R.B., Plattner, R.D., 2003. Characterization of a *Fusarium* 2-gene cluster involved in trichothecene C-8 modification. J. Agric. Food Chem 51, 7936−7944.

Buerstmayr, H., Adam, G., Lemmens, M., 2012. Resistance to head blight caused by *Fusarium* spp. in wheat, in: Sharma, I. (Ed.), Disease resistance in wheat. CABI, Wallingford, Oxfordshire, UK; Cambridge, MA, pp. 236-276.

Burgess, L.W., 1982. Characterization, geographic distribution and ecology of *Fusarium crookwellense* sp.Nov. Transactions of the British Mycological Society 79, 497-505.

Chandler, E.A., Simpson, D.R., Thomsett, M.A., Nicholson, P., 2003. Development of PCR assays to *Tri7* and *Tri13* trichothecene biosynthetic genes, and characterisation of chemotypes of *Fusarium graminearum, Fusarium culmorum* and *Fusarium cerealis*. Physiological and Molecular Plant Pathology 62, 355-367.

Correll, J.C., Puhaiia, J.E., Schneider, R.W., 1986. Identiication of *Fusarium oxysporum f. sp. apii* on the basis of colony size, virulence, and vegetative compatibility. Phytopathology 76, 396-400.

Desjardins, A.E., Busman, M., Manandhar, G., Jarosz, A.M., Manandhar, H.K., Proctor, R.H., 2008. Gibberella ear rot of maize (*Zea mays*) in Nepal: distribution of the mycotoxins nivalenol and deoxynivalenol in naturally and experimentally infected maize. Journal of Agricultural and Food Chemistry 56, 5428-5436.

Desjardins, A.E., 2006. Fusarium mycotoxins: Chemistry, genetics, and biology. American Phytopathological Society (APS Press).

Desjardins, A.E., Hohn, T.M., McCormick, S.P., 1993. Trichothecene biosynthesis in *Fusarium* species: Chemistry, genetics, and significance. Microbiological Reviews 57, 595-604.

Desjardins, A.E., Plattner, R.D., 2003. Diverse traits for pathogen fitness in *Gibberella zeae*. Can. J. Plant Pathol, 21–27.

European commission, j.r.c., 2007. Report on the validation of a DNA extraction method from maize seeds. Community reference laboratory for GM food and feed (CRL-GMFF) biotechnology & GMOs unit, Ispra- Italy.

Gagkaeva, T.Y., 2010. Phytopathogenic fungus *Fusarium cerealis* in Russia. Microbiology 79, 553-560.

Gale, L.R., Ward, T.J., Balmas, V., Kistler, H.C., 2007. Population subdivision of *Fusarium graminearum* sensu stricto in the upper Midwestern United States. Phytopathology 97, 1434-1439.

Goswami, R.S., Kistler, H.C., 2004. Heading for disaster: *Fusarium graminearum* on cereal crops. Molecular Plant Pathology 5, 515-525.

Guo, X.W., Fernando, W.G.D., Seow-Brock, H.Y., 2008. Population structure, chemotype diversity, and potential chemotype shifting of *Fusarium graminearumin* wheat fields of Manitoba. Plant Disease 92, 756-762.

He, X., Singh, P.K., Duveiller, E., Dreisigacker, S., Singh, R.P., 2013. Development and characterization of international maize and wheat improvement center (CIMMYT) germplasm for Fusarium head blight resistance, in: Magliano, T.M.A., Chulze, S.N. (Eds.), Fusarium head blight in Latin America. Springer Science+Business Media Dordrecht, pp. 241-262.

Hernandez Nopsa, J.F., Wegulo, S.N., Panthi, A., Hallen-Adams, H.E., Harris, S.D., Baenziger, P.S., 2014. Characterization of Nebraska isolates of *Fusarium graminearum* causing head blight of wheat . Crop Science 54, 310.

Hope, R., Aldred, D., Magan, N., 2005. Comparison of environmental profiles for growth and deoxynivalenol production by *Fusarium culmorum* and *F. graminearum* on wheat grain. Letters in Applied Microbiology 40, 295-300.

Ichinoe, M., Kurata, H., Sugiura, Y., Ueno, Y., 1983. Chemotaxonomy of *Gibberella zeae* with special reference to production of trichothecenes and zearalenone. Applied and Environmental Microbiology 46, 1364-1369.

Jennings, P., Coates, M.E., Nicholson, P., 2004. Determination of deoxynivalenol and nivalenol chemotypes of *Fusarium culmorum* isolates from England and Wales by PCR assay. Plant Pathology 53, 182–190.

Jennings, P., M.E.Coates, K.Walsh, Nicholson, P., 2004. Determination of deoxynivalenol- and nivalenol-producing chemotypes of *Fusarium graminearum* isolated from wheat crops in England and Wales. Plant Pathology 53, 643 –652.

Keller, N.P., Hohn, T.M., 1997. Metabolic pathway gene clusters in filamentous fungi. Fungal Genetics and Biology 21, 17-29.

Koizumi, S., Kato, H., Yoshino, R., Hayashi, N., Ichinoe, M., 1991. Distribution of causal Fusaria of wheat and barley scab in Japan. Ann. Phytopath. Soc. Japan 7, 165-173.

Lattanzio, V.M.T., Pascale, M., Visconti, A., 2009. Current analytical methods for trichothecene mycotoxins in cereals. TrAC Trends in Analytical Chemistry 28, 758-768.

Lee, T., Oh, D.W., Kim, Y.H., Kim, H.S., Yun, S.H., Lee, Y.W., 2001. Identification of deoxynivalenol- and nivalenol-producing chemotypes of *Gibberella zeae* by using PCR. Applied and Environmental Microbiology 67, 2966-2972.

Lee, Y.W., Jeon, J.J., Kim, H., Jang, I.Y., Kim, H.S., Yun, S.H., Kim, J.G., 2004. Lineage composition and trichothecene production of *Gibberella zeae* population in Korea. Japanese association of mycotoxicology, Kagawa-Japan.

Li, H.P., Wu, A.B., Zhao, C.S., Scholten, O., Loffler, H., Liao, Y.C., 2005. Development of a generic PCR detection of deoxynivalenol- and nivalenol-chemotypes of *Fusarium graminearum*. FEMS Microbiology Letters 243, 505-511.

Malihipour, A., Gilbert, J., Piercey-Normore, M., Cloutier, S., 2012. Molecular phylogenetic analysis, trichothecene chemotype patterns, and variation in aggressiveness of *Fusarium* isolates causing head blight in wheat. Plant Disease 96, 1016-1025.

Mardi, M., Buerstmayr, H., Ghareyazie, B., Lemmens, M., Mohammadi, S.A., Nolz, R., Ruckenbauer, P., 2005. QTL analysis of resistance to Fusarium head blight in wheat using a 'Wangshuibai'-derived population. Plant Breeding 124, 329-333.

Marin, S., Ramos, A.J., Cano-Sancho, G., Sanchis, V., 2013. Mycotoxins: occurrence, toxicology, and exposure assessment. Food and Chemical Toxicology 60, 218-237.

McMullen, M., Jones, R., Gallenberg, D., 1997. Scab of wheat and barley: a re-emerging disease of devastating impact. Plant Disease 81, 1340-1348.

Mesterházy, A., 1978. Comparative analysis of artificial inoculation methods with *Fusarium* spp. on winter wheat varieties. Phytopathology 93, 12-25.

Miller, J.D., Greenhalgh, R., Wang, Y.Z., Lu, M., 1991. Trichothecene chemotypes of three *Fusarium* species. Mycologia 83, 121-130.

Nelson, P.E., Toussoun, T.A., Marasas, W.F.O., 1983. Fusarium species: an illustrated manual for identification. Pennsylvania State University Press.

Nicholson, P., Simpson, D.R., Weston, G., Rezanoor, H.N., Lees, A.K., Parry, D.W., Joyce, D., 1998. Detection and quantification of *Fusarium culmorum* and *Fusarium graminearum* in cereals using PCR assays. Physiological and Molecular Plant Pathology 53, 17-37.

Osborne, L.E., Stein, J.M., 2007. Epidemiology of Fusarium head blight on small-grain cereals. International Journal of Food Microbiology 119 103–108.

Pasquali, M., Migheli, Q., 2014. Genetic approaches to chemotype determination in type B-trichothecene producing Fusaria. International Journal of Food Microbiology 189C, 164-182.

Pestka, J.J., 2007. Deoxynivalenol: Toxicity, mechanisms and animal health risks. Animal Feed Science and Technology 137, 283-298.

Placinta, C.M., D'Mello, J.P.F., Macdonald, A.M.C., 1999. A review of worldwide contamination of cereal grains and animal feed with *Fusarium* mycotoxins. Animal Feed Science and Technology 78, 21-37.

Pritsch, C., Muehlbauer, G.J., Bushnell, W.R., Somers, D.A., Vanc, C.P., 2000. Fungal development and induction of defense response genes during early infection of wheat spikes by *Fusarium graminearum*. Molecular Plant-Microbe Interactions 13, 159-169.

Puri, K.D., Zhong, S., 2010. The 3ADON population of *Fusarium graminearum* found in North Dakota is more aggressive and produces a higher level of don than the prevalent 15ADON population in spring wheat. Phytopathology 100, 1007-1014.

Reischer, G.H., Lemmens, M., Farnleitner, A., Adler, A., Mach, R.L., 2004. Quantification of *Fusarium graminearum* in infected wheat by species specific real-time PCR applying a TaqMan probe. Journal of Microbiological Methods 59, 141-146.

Ryu, J.-C., Ohtsubo, K., Izumiyama., N., Nakamura, K., Tanaka, T., Hlsashi Yamamura, Ueno, Y., 1988. The acute and chronic toxicities of nivalenol in mice. Fundamental and applied toxicology 11, 38-47.

Scherm B, Balmas V, Spanu F, Pani G, Delogu G, Pasquali M, Migheli Q (2013) *Fusarium culmorum*: causal agent of foot and root rot and head blight on wheat. Mol Plant Pathol 14, 323-341.

Schisler, D.A., Khan, N.I., Boehm, M.J., Slininger, P.J., 2002. Greenhouse and field evaluation of biological control of Fusarium head blight on durum wheat. Plant Disease 86, 1350-1356.

Schmale, D.G., Wood-Jones, A.K., Cowger, C., Bergstrom, G.C., Arellano, C., 2011. Trichothecene genotypes of *Gibberella zeae* from winter wheat fields in the eastern USA. Plant Pathology 60, 909–917.

Scoz, L.B., Astolfi, P., Reartes, D.S., Schmale, D.G., Moraes, M.G., Ponte, E.M.D., 2009. Trichothecene mycotoxin genotypes of *Fusarium graminearum sensu stricto* and *Fusarium meridionale* in wheat from southern Brazil. Plant Pathology 58, 344-351.

Shank, R.C., 1981. Mycotoxins and N-nitroso compounds: Environmental risks. CRC Press, Boca Raton, Fla.

Sobrova, P., Adam, V., Vasatkova, A., Beklova, M., Zeman, L., Kizek, R., 2010. Deoxynivalenol and its toxicity. Interdiscip Toxicol 3, 94-99.

Starkey, D.E., Ward, T.J., Aoki, T., Gale, L.R., Kistler, H.C., Geiser, D.M., Suga, H., Toth, B., Varga, J., O'Donnell, K., 2007. Global molecular surveillance reveals novel Fusarium head blight species and trichothecene toxin diversity. Fungal Genetics and Biology 44, 1191-1204.

Suga, H., Karugia, G.W., Ward, T., Gale, L.R., Tomimura, K., Nakajima, T., Miyasaka, A., Koizumi, S., Kageyama, K., Hyakumachi, M., 2008. Molecular characterization of the *Fusarium graminearum* species complex in Japan. Phytopathology 98, 159-166.

Sugiura, Y., Saito, H., Tanaka, T., Ichinoe, M., Ueno, Y., 1994. *Fusarium crookwellense*, a newly isolated fungus from wheat in Japan: Its mycotoxin production and pathogenicity to wheat and barley. Mycoscience 35, 77-82.

Talas, F., Parzies, H.K., Miedaner, T., 2011. Diversity in genetic structure and chemotype composition of *Fusarium graminearum* sensu stricto populations causing wheat head blight in individual fields in Germany. European Journal of Plant Pathology 131, 39-48.

Umpierrez-Failache, M., Garmendia, G., Pereyra, S., Rodriguez-Haralambides, A., Ward, T.J., Vero, S., 2013. Regional differences in species composition and toxigenic potential among Fusarium head blight isolates from Uruguay indicate a risk of nivalenol contamination in new wheat production areas. International Journal of Food Microbiology 166, 135-140.

von der Ohe, C., 2010. Effects of non-adapted quantitative trait loci (QTL) for Fusarium head blight resistance on European winter wheat and *Fusarium* isolates. Hohenheim, Stuttgart-Hohenheim.

von der Ohe, C., Gauthier, V., Tamburic-Ilincic, L., Brule-Babel, A., Fernando, W.G.D., Clear, R., Ward, T.J., Miedaner, T., 2010. A comparison of aggressiveness and deoxynivalenol production between Canadian *Fusarium graminearum* isolates with 3-acetyl and 15-acetyldeoxynivalenol chemotypes in field-grown spring wheat. European Journal of Plant Pathology 127, 407–417.

Waalwijk, C., Kastelein, P., Vries, I.d., enyi, Z.K., 2003. Major changes in *Fusarium* spp. in wheat in the Netherlands. European Journal of Plant Pathology 109, 743–754.

Wagacha, J.M., Muthomi, J.W., 2007. *Fusarium culmorum*: Infection process, mechanisms of mycotoxin production and their role in pathogenesis in wheat. Crop Protection 26, 877-885.

Wang, J.-H., Li, H.-P., Qu, B., Zhang, J.-B., Huang, T., Chen, F.-F., Liao, Y.-C., 2008. Development of a generic pcr detection of 3-acetyldeoxy-nivalenol-, 15-acetyldeoxynivalenol- and nivalenol-chemotypes of *Fusarium graminearum* clade. Int. J. Mol. Sci 9, 2495-2504.

Wang, J.-H., Zhang, J.-B., Chen, F.-F., Li, H.-P., Ndoye, M., Liao, Y.-C., 2012. A multiplex PCR assay for genetic chemotyping of toxigenic *Fusarium graminearum* and wheat grains for 3-acetyldeoxynivalenol, 15-acetyldeoxynivalenol and nivalenol mycotoxin. Journal of Food, Agriculture & Environment 10, 505-511.

Ward, T.J., Bielawski, J.P., Kistler, H.C., Sullivan, E., O'Donnell, K., 2002. Ancestral polymorphism and adaptive evolution in the trichothecene mycotoxin gene cluster of phytopathogenic *Fusarium*. PNAS 99, 9278-9283.

Ward, T.J., Clear, R.M., Rooney, A.P., O'Donnell, K., Gaba, D., Patrick, S., Starkey, D.E., Gilbert, J., Geiser, D.M., Nowicki, T.W., 2008. An adaptive evolutionary shift in Fusarium head blight pathogen populations is driving the rapid spread of more toxigenic *Fusarium graminearum* in North America. Fungal Genetics and Biology 45, 473-484.

Xu, X., Nicholson, P., 2009. Community ecology of fungal pathogens causing wheat head blight. Annual Review of Phytopathology 47, 83-103.

Xue, A.G., Keh, M.H., Butler, G., Vigier, B.J., Babcock, C., 2006. Pathogenicity of *Fusarium* species causing head blight in barley. Phytoprotection 87, 55-61.

Yli-Mattila, T., Gagkaeva, T., Ward, T.J., Aoki, T., Kistler, H.C., O'Donnell, K., 2009. A novel Asian clade within the *Fusarium graminearum* species complex includes a newly discovered cereal head blight pathogen from the Russian Far East. Mycologia 101, 841-852.

Yoruk, E., Albayrak, G., 2012. Chemotyping of *Fusarium graminearum* and *F. culmorum* isolates from Turkey by PCR assay. Mycopathologia 173, 53–61.

Zhang, H., Van der Lee, T., Waalwijk, C., Chen, W., Xu, J., Xu, J., Zhang, Y., Feng, J., 2012. Population analysis of the Fusarium graminearum species complex from wheat in China show a shift to more aggressive isolates. Plos One 7, e31722.

Phenotypic and Genotypic Characterization of CIMMYT's 15th International Fusarium Head Blight Screening Nursery of Wheat

Mohamed Osman

Chapter 3

Phenotypic and Genotypic Characterization of CIMMYT's 15th International Fusarium Head Blight Screening Nursery of Wheat

Abstract

As an important cereal disease in humid and semi-humid areas, Fusarium head blight (FHB) has caused severe epidemics on wheat (*Triticum aestivum* L.) in different countries worldwide. By causing both yield loss and quality degradation, FHB presents a two-fold threat to farmers and consumers. Since the beginning of FHB research at the International Maize and Wheat Improvement Centre (CIMMYT) in the early 1980's, a large-scale FHB screening has been conducted to identify and incorporate new resistance genes into elite CIMMYT germplasm. Candidates of the 15th Fusarium head blight screening nursery (FHBSN) were derived from different CIMMYT wheat breeding programs and were tested for three years successively in El Batán, Mexico, before being included in the 15th FHBSN set. From 2010 to 2012, a set of 44 out of 2,794 lines were gradually selected depending on their FHB indices, pedigree information, and phenological traits like plant height and days to heading. The performance of these lines varied across years under different disease pressure, but they all showed high level of resistance compared to the susceptible checks. In 2013, the nursery was again evaluated in El Batán, as well as in artificially inoculated field trials in Norway, Uruguay, the Netherlands, and Japan (2014), and in naturally infected experiment in Toluca, Mexico, and Canada. Although not all lines demonstrated strong resistance across environments, promising lines with good FHB resistance can still be identified in each location. The genotypes were haplotyped with PCR-based markers for 10 loci on seven chromosomes associated with known FHB resistance, and the results suggested that 24 of the genotypes (55%) carried the 4BS QTL as in Wuhan 1, which was the most frequent QTL in this nursery, and the 7A QTL as in *T. dicoccoides* was noticed in five (11%) of the genotypes. The resistance QTLs on chromosomes 3B, 5A and 6B as in Sumai 3 and 3A as in *T. dicoccoides* were not detected in any of the genotypes denoting the uniqueness of these lines. Fifteen (34%) of the genotypes may not carry any of the 10 QTLs examined. The results provide valuable information that could be successfully utilized by breeders to select resistant parents for crosses since novel resistance sources were detected for better targeted crosses toward diversifying and/or pyramiding FHB resistance.

Keywords: FHB screening, Resistance, *Fusarium* spp., *Triticum aestivum* L.

3.1. Introduction

Fusarium head blight (FHB) is one of the most economically important diseases of small grains and continues to adversely impact crops. It is caused by numerous *Fusarium* species that infect florets at anthesis and produce similar symptoms. Yield and test weight reduction, contamination with the mycotoxin deoxynivalenol (DON), and additional costs on seed cleaning have caused high economic losses for farmers and the industry (McMullen et al. 2012). Economically important hosts of FHB include bread wheat (*Triticum aestivum* L.), durum wheat (*Triticum durum* Desf.), barley (*Hordeum vulgare* L.), and oats (*Avena sativa* L.).

FHB epidemics are monocyclic since spike infection generally takes place during anthesis and early stages of kernel development (Leonard and Bushnell 2003; Audenaert et al. 2009). At flowering, airborne ascospores and rain splashed conidia land on open florets and get access to the host easily (Leonard and Bushnell 2003). Frequent rainfall and high relative humidity from spike emergence through anthesis favour inoculum production on cereal debris and ensure disease development (Khonga and Sutton 1988; Fernando et al. 1997). Under favourable weather conditions, high amount of primary inoculum and growing susceptible cultivars give rise to epidemics.

Mycotoxins such as zearalenone, HT-2 toxin, T-2 toxin, nivalenol, and DON and its acetylated forms (3-ADON and 15-ADON) are frequently formed in Fusarium-infected wheat and barley (Salas et al. 1999; Buerstmayr et al. 2012). DON is considered to be the most economically important toxin produced by *F. graminearum* (Culler et al. 2007) and has been shown to be a virulence factor in FHB (Bai et al. 2002; Jansen et al. 2005). Recently, severe epidemics have occurred repeatedly and research on this disease has become very important in the Americas, East Asia and Europe. Attributable to high yield losses that may reach 50-60%, FHB has become a major threat to the global food supply and safety and is considered by the International Maize and Wheat Improvement Centre (CIMMYT) as a major limiting factor of worldwide wheat production (Dubin et al. 1997). In the EU, legally enforceable thresholds in grain and food products allow a maximum DON content in unprocessed cereals other than

durum wheat, oats and maize of 1.25 ppm, in bread and biscuits of 0.5 ppm and in baby food for infants and young children of 0.2 ppm (European commission, 2006).

Incorporating durable resistance to FHB in wheat is a challenging task for breeders since it is quantitatively inherited and is considerably affected by environment and pathogen populations (Miedaner et al. 2001; Buerstmayr et al. 2002). Wheat has different active and passive resistance mechanisms that act synergistically to combat fungal attacks. Mesterhazy et al. (1999) proposed five active resistance components, i.e. resistance to fungal invasion (Type I) and spread (Type II), resistance to toxin accumulation (Type III), resistance to kernel infection (Type IV) and resistance to yield reduction (Type V). Passive resistance involves different traits such as the cuticular wax which may decrease water availability and thus constrain fungal germination and penetration. The height, thickness and strength of a plant stem may indirectly affect its resistance to FHB, because the soil-borne spores can easily reach the heads of short or lodged plants. The results of a study conducted by Graham and Browne (2009) concluded that selection for anther extrusion (AE) among European wheat could improve FHB resistance, without negatively impacting on agronomic traits. Furthermore, it was shown that high AE led to low infection rate, contributing to Type I resistance (Skinnes et al. 2010). FHB severities were negatively correlated with both AE and plant height (PH) after spray and spawn inoculation as reported by Lu et al. (2013) and Kubo et al. (2013). The two dwarfing genes *Rht-B1b* and *Rht-D1b*, especially the latter, have been reported to be associated with increased FHB susceptibility (Hilton et al. 1999; Draeger et al. 2007; Holzapfel et al. 2008; Srinivasachary et al. 2009). According to Lu et al. (2011), two major resistance quantitative trait loci (QTL) are required to counteract the negative effect of *Rht-D1b*. As for *Rht-B1b*, it conferred Type II resistance in several studies (Srinivasachary et al. 2009; Lu et al. 2013), despite its possible effect on reducing Type I resistance.

The quantitative nature of the inheritance of FHB resistance is a subject to several resistance mechanisms that are not necessarily genetically linked (Miedaner 1997), its regular association with detrimental agronomic traits and the large effect of environment makes breeding for FHB resistance a very difficult task in addition to the concern about reproducibility of testing for FHB resistance (Bai and Shaner 2004), and disease evaluation process *per se* is also a tedious process. In this regard, molecular markers can be very helpful to supplement phenotyping and

classical breeding in selecting major resistance QTLs (Buerstmayr et al. 2009; Agostinelli et al. 2012) as well as to investigate novel resistance sources. Numerous QTL mapping studies have been performed since last decade, and resistance QTLs have been reported on all the 21 chromosomes (Liu et al. 2009).

As a communication platform and a promoter of international cooperation, CIMMYT has developed extensive FHB collaborations with research organizations in both developed and developing countries (Duveiller et al. 2008) and worked on the incorporation of FHB resistance into high yielding, semi-dwarf and rust resistant CIMMYT wheat (He et al. 2000). CIMMYT established a series of FHB Screening Nurseries (FHBSN, previously known as Scab Resistance Screening Nursery, SRSN), which were distributed worldwide and are available to anyone attentive to wheat FHB resistance improvement. The current name FHBSN was adopted in 2010, and the 13th and 14th FHBSN nurseries were distributed in 2011 and 2012, respectively (He et al. 2013a; He et al. 2013b). The aim of this study was to identify and characterize the 15th FHBSN regarding field resistance, post-harvest indices of Fusarium damaged kernels (FDK) and DON, as well as phenological and morphological traits like PH, days to heading (DH), and AE.

3.2. Materials and Methods

3.2.1. *Plant material and field trials*

Entries of the 15th FHBSN were selected from an initial set of 2,794 advanced breeding lines with known pedigrees which were developed at CIMMYT. Field experiments were conducted using the FHB sick plot established at El Batán (Table 3.1, with an average annual precipitation of 625 mm), CIMMYT, Mexico. The genotypes were sown and evaluated for FHB resistance in the summer season (May to September) from 2010 to 2013. In 2010, the experiment was done in 1 m double rows without replication, whereas from 2011 to 2013 it was done in two replications. Selection was made from 2010 to 2012 based on FHB index (Stack and McMullen 1994), PH and DH, as well as pedigree information to maintain a high genetic diversity of the nursery. A final selection of the 15th FHBSN was made in 2012 and the nursery was verified

again in 2013. Checks included three susceptible lines Gamenya, Ocoroni F 86 and Falcin/*Ae. squarrosa* (312)/3/THB/CEP7780//SHA4/Lira (referred to as Falcin# hereafter) and two resistant lines Sumai 3 and Heilo. The screening nursery was misted from flowering to early dough stage by a programmable misting system with DAN modular micro sprinklers (NaanDan Jain Irrigation Ltd.) arranged in 3 x 4 m spacing. The system operated automatically from 9am to 8pm, with 10 minutes of spraying per hour, to create a humid environment favourable for FHB development.

Table 3.1 Geographical information of the experimental stations used in this study

Name	State/Province	Country	Latitude	Longitude	Altitude (m)
El Batán	Mexico	Mexico	19.5°N	98.8°W	2,240
Toluca	Mexico	Mexico	19.2°N	99.5°W	2,585
Ås	Akershus	Norway	59.7°N	10.8°E	85
INIA La Estanzuela	Colonia	Uruguay	34.3°S	57.7°W	75
Dronten	Flevoland	Netherlands	52.5°N	5.7°E	-5
Minto	Manitoba	Canada	49.4°N	100.0°W	487
Kitami	Hokkaido	Japan	43.8°N	143.7°E	193

3.2.2. *Inoculation and phenotyping assays*

Annually about 70–90 *Fusarium* strains are collected in late summer from naturally infected wheat spikes from different farms in Mexico to ensure the viability and virulence of pathogen. The isolates were firstly verified with *F. graminearum sensu lato* specific primer set FG16N F/R (Nicholson et al. 1998) and then with the TOXP1/2 primer set for their chemotype classification (Li et al. 2005). For those DON-producing *F. graminearum* isolates, a rice medium assay was employed to determine DON productivity (He et al. 2013b). Briefly, the isolates were inoculated on 30 g autoclaved polished rice and incubated for 2 weeks, and then a subsample of 2 g was used to measure DON level. Subsequently, around ten strains with high DON production capacity were selected and evaluated in greenhouse for their aggressiveness on two resistant (Sumai 3 and Heilo) and three susceptible genotypes (SERI/CEP80120, BCN//DOY1/*Ae. squarrosa* (447), and Gamenya). Two *F. graminearum* strains with known aggressiveness that had been used for field inoculation in the previous year were used as control in greenhouse tests. The spikes were evaluated at 7, 14, and 21 days post inoculation (dpi) by counting symptomatic spikelets and rachis segments. Based on DON productivity and aggressiveness, four highest ranked isolates were selected and mixed with a control strain with known aggressiveness to generate the new inoculum for the year's field screening. Inoculum was produced in liquid mung bean [*Vigna radiata* (L.) Wilczek.] medium as mentioned in Buerstmayr et al. (2002). Inoculum concentration was adjusted to 50,000 conidia/ml (55,000 conidia/ml in 2013) for field application.

At anthesis, 10 spikes of each line were labelled in the morning and spray inoculated in the afternoon, using a precision CO_2 backpack sprayer with flat fan nozzles at a constant pressure of 40 psi and a rate of about 60 ml/m^2. The inoculation was repeated two days later. FHB symptoms were scored on the 10 tagged spikes at 25 dpi by counting the numbers of total and infected spikelets of each spike, and FHB index was calculated using the formula *FHB index* (%) = (*Severity* x *Incidence*)/100 (Stack and McMullen 1994), where 'Severity' stands for the averaged percentage of diseased spikelets, and 'Incidence' for the percentage of spikes which showed infection. Plots were sickle harvested at maturity, and spikes were threshed with a belt thresher set at low wind speed to retain scabby kernels. FDK was estimated only in 2013, from a random grain sample in a petri dish, with a scale of 0-9. For DON analysis, a sample of 20 g

grain of each accession was pulverized, and a 2 g sub-sample was tested using the Ridascreen Fast DON ELISA kit (R-Biopharm GmbH, Darmstadt, Germany) following the manufacturer's instruction. DON data was available from 2011 to 2013. AE was recorded only in 2013 based on a linear scale from 0 (no extrusion) to 9 (full extrusion) according to Skinnes et al. (2010).

3.2.3. *Field evaluation in international locations*

In addition to El Batán, six more locations were used for evaluation of the nursery, including Dronten in the Netherlands, Toluca in Mexico, Minto in Canada, Ås in Norway, INIA La Estanzuela in Uruguay (all in 2013), and Kitami in Japan (in 2014). See Table 1 for the detailed geographical information of these stations.

In the Netherlands, the nursery was sown in April 3 in 1 m triple row plots without replication. The inoculum consisted of a mixture of a *F. culmorum* and a *F. graminearum* strain (with a ratio of 7:3), and it was adjusted to approx. 25,000 conidia/ml for field application by a tractor mounted boom sprayer. The inoculation was repeated four times in the evening in June 25 and 28, and July 1 and 3, and disease notes were taken two times in July 19 and 30 with FHB severity estimated from 0 to 100%.

Toluca is a humid location with an average annual rainfall of 800 mm concentrated in the growing season from May to September. FHB infection occurs naturally in this location and it had been used as CIMMYT's main FHB screening site until 2005. The nursery was sown in Toluca in May 8 in 0.75 m double row plots without replication, and no artificial inoculation was done. Disease scoring was taken on Zadoks GS 80-85, with visual estimation of incidence and severity on the plot basis for calculating FHB index.

In Canada, the nursery was sown in June 4 in 6.7 m single row plots spaced 40 cm apart without replication, and no artificial inoculation was applied as there was severe natural infestation. Precipitation during the growing season was 339 mm. Visual FHB scoring was done in August 26, when most of the lines were at the stage of 25 days after anthesis. A composite estimation

based on % of heads infested with FHB and % of spikelets infested was adopted with a range from 0 to 100%.

In Norway, the nursery was planted on May 20, in hill plots of 0.40 x 0.45 m spacing with two replications. *F. graminearum* infected oat kernels were used as spawn inoculum and applied in the field at Zadoks GS 37-39. Mist irrigation was applied for 10 min at hourly intervals from 7 pm to 10 pm in the evening until the plants reached maturity. FHB evaluation was done at the beginning of maturity, when a bundle of 10-15 spikes was counted to determine disease severity. For more detailed information on FHB screening in this location, refer to Lu et al. (2013).

In Uruguay, the nursery was sown in July 17 in 1 m single row without replication. Maize kernels infected with a mixture of 10 *F. graminearum* isolates of known aggressiveness and representativeness were applied twice in the field as spawn inoculum, with the first application at Zadoks GS 45 of the early maturity genotypes and the second at three weeks later. Each time, 40 g/m^2 of inoculum was applied. Misting system worked from 2-3 weeks prior to flowering to milk grain stage, with two sprayings of 15 minutes in the morning and two in the afternoon. The nozzles used were NaanDanJain microsprinklers DANSPRINKLERS 03 (NaanDan Jain Irrigation Ltd.). Disease scoring was made at Zadoks GS 80-85 (late milky to soft dough stages), with disease incidence and severity estimated to calculate FHB index. In Japan, the nursery was planted in April 23, 2014, in single row plots spaced 40 cm apart without replication. Spawn inoculation was carried out at Zadoks GS 45, with oats seeds infested by a single isolate of *F. graminearum* of known aggressiveness. Approximately 5 g of spawn was spread for each row and the field was watered with a sprinkler system for 8 min per hour after heading to maintain high humidity. FHB severity was scored visually on 15 spikes at 21 dpi, using a linear scale of 0-8, in which 0 through 3 stands respectively for the infection of 0 through 3 spikelet(s), and 4 through 8 for the disease severity of 50%, 60%, 75%, 90%, and 100%, respectively.

3.2.4. *Statistical analysis*

The phenotypic data was analysed by the SAS program ver. 9.2. Analysis of variance (ANOVA) was carried out with the PROC GLM module. The data in the ANOVA table were used for calculating the heritability estimates, using the formula $h^2 = \sigma_g^2/(\sigma_g^2 + \sigma_e^2/r)$ for single year and $h^2 = \sigma_g^2/(\sigma_g^2 + \sigma_{g*y}^2/y + \sigma_e^2/ry)$ for multiple years; in which σ_g^2 stands for genetic variance, σ_{g*y}^2 for genotype-by-year interaction, σ_e^2 for error variance, y for the number of years, and r for the number of replications (Lu et al. 2013). In order to facilitate the identification of stably resistant lines across environments, a composite index was calculated, i.e. the sixth root of the product of FHB index (or severity) in El Batán (2013), Norway (2013), Uruguay (2013), the Netherlands (2013), Canada (2013), and Japan (2014). FHB parameters, PH, and AE were normalized with the PROC STANDARD function prior to the principal component analysis (PCA) using the PAST software ver. 3.01 (Hammer et al. 2001).

3.2.5. *Haplotyping*

To identify the genetic basis behind the FHB resistance in the 15th FHBSN entries, 17 PCR-based markers linked to 10 validated FHB resistance QTLs on seven chromosomes were chosen for haplotyping, to assess the 15th FHBSN entries for the possible presence of QTLs as in Wuhan 1, CJ 9306, Frontana, Sumai 3, and *T. diccocoides* (Table 3.2). Leaf tissue was harvested from the second leaf for DNA extraction, following the CTAB method recommended by the European Community Reference Laboratories for the isolation of maize DNA as cited in Brunner et al. (2009). The lines were genotyped at the GenServe Laboratories, Saskatoon, SK, Canada. The markers were fluorescently labelled (Schuelke 2000) and the PCR system and cycling program followed the endorsed protocols of each marker. All PCR reactions were performed in an Applied Biosystems Veriti 96 well thermal cycler. PCR products were analysed using an ABI 3500xl Genetic Analyzer through capillary electrophoresis; allele calling was conducted using GENEMAPPER version 4.0 (Applied Biosystems, Foster City, CA). The strategy for confirming a QTL was according to the following strategy; a resistance QTL was assumed to be present only when both contiguous markers showed the resistance alleles which accordingly marked as ‘+ +’. Similarly, ‘- -’ represented the absence of the resistance allele, whereas ‘+ -’ indicated that only one of the two flanking markers showed

resistance genotype. For the 3BS QTL as in Sumai 3, the 3AS QTL as in *T. dicoccoides*, and the 3AL QTL as in Frontana, only one closely linked marker was genotyped for declaration of presence or absence of the corresponding QTL. *Rht-B1* and *Rht-D1* were genotyped in the LGC Company (http://www.lgcgroup.com) with the KASP assay.

Table 3.2 FHB markers used for haplotyping the 15th FHBSN

Source of resistance	Chromosome	Plant material or mapping population	Resistance Type	Marker(s)	Reference(s)
Sumai 3	3BS	3BS BAC library of Chinese Spring	II	UMN 10	Liu et al. 2008
	5AS	CM-82036/Remus	I	BARC 186	Anderson 2007, Buerstmayr et al. 2002
				BARC 180	Anderson 2007, Buerstmayr et al. 2002
	6BS	BW278/AC_Foremost	II	GWM 133	Cuthbert et al. 2007
				WMC 179	Cuthbert et al. 2007
Frontana	3A	Frontana/Remus	I	DUPW 227	Steiner et al. 2004
	5A	Frontana/Remus	I	BARC 197	Steiner et al. 2004
				GWM 129	Steiner et al. 2004

Wuhan 1	2DL	Wuhan_1/Maringa	II	WMC 144	Somers et al. 2003
				WMC 245	Somers et al. 2003
	4BS	Wuhan_1/Maringa	II	WMC 238	Somers et al. 2003
				GWN 149	Somers et al. 2003
CJ 9306	2DL	Veery/CJ 9306	II	GWM157	Jiang et al. 2007
				GWM 539	Jiang et al. 2007
T. dicoccoides	3A	Recombinant inbred chromosome line 3A of *T. dicoccoides* in Langdon-16	II	GWM 2	Otto et al. 2002
	7A	Langdon/ LDN-DIC 7A(742)	II	BARC 121	Kumar et al. 2007
				WMC 488	Kumar et al. 2007

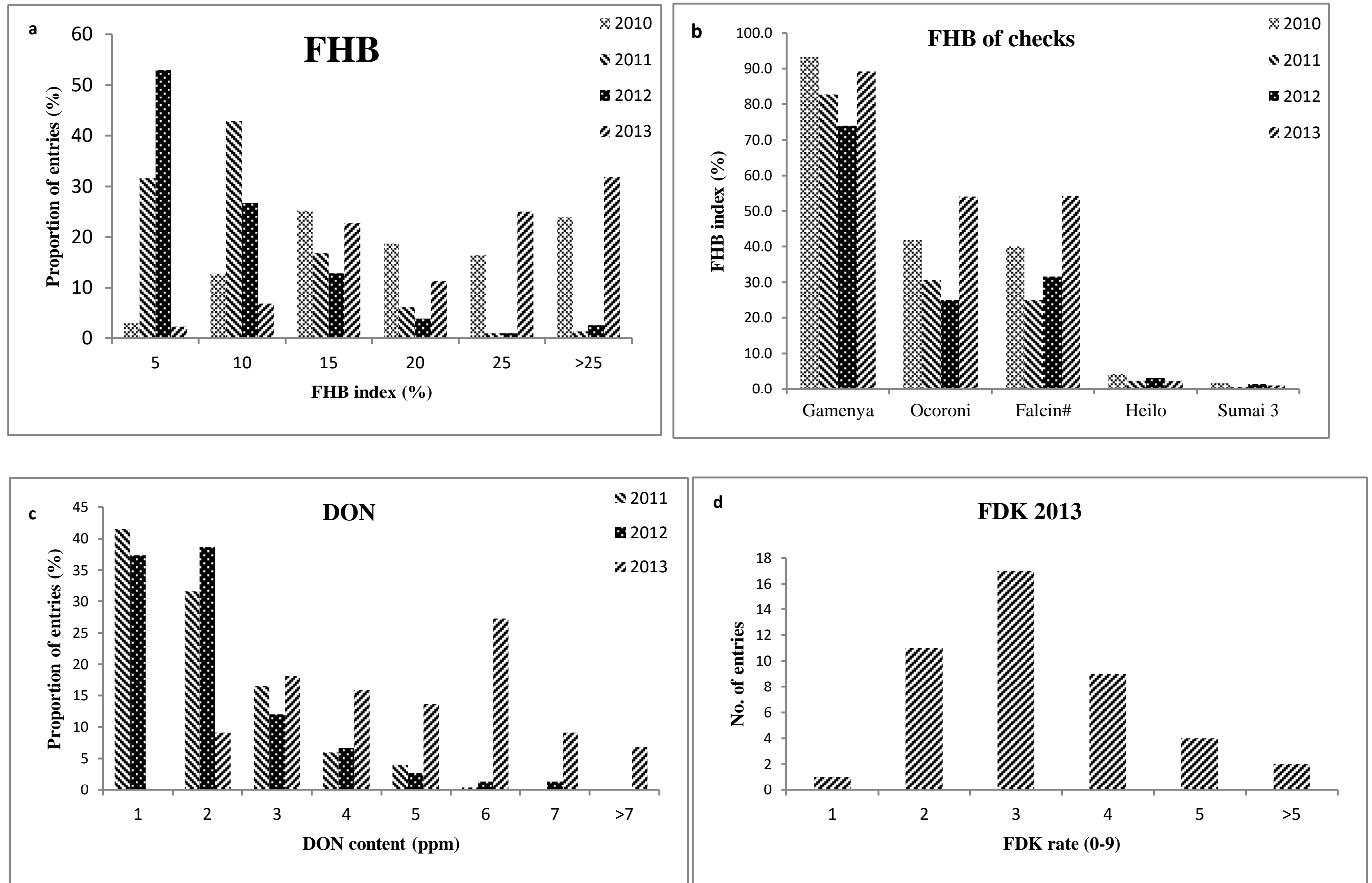
3.3. Results

ANOVA results indicated significant 'year' effect for FHB, having the largest mean square (MS) value that was almost two times higher than that of the 'entry' effect; this was more significant for DON, where the year MS was 60 times higher than the entry MS (Table 3.3), ascribable to both the different disease levels in El Batán across years and the positive selection error. Nevertheless, the 'entry' effect was significant for all the three FHB parameters at $P<0.001$ level, and so did the genotype-by-environment effects for FHB and DON. FHB exhibited the highest heritability estimate of 0.82, followed by FDK of 0.81 and DON of 0.69.

In 2010, FHB index ranged from 0.6% to 83.5%, but over 75% of the entries had a value lower than 25% (Fig. 3.1a). The two resistant checks, Sumai 3 and Heilo, showed very low disease index, whereas the two moderately susceptible checks, Ocoroni and Falcin#, exhibited FHB index around 40%, and the susceptible check Gamenya had the highest disease severity (>90%) (Fig. 3.1b). Based on the field screening data, 1,109 lines with an FHB index lower than 15%, DH less than 80 days and PH lower than 110 cm, were selected for further screening in 2011.

In 2011, the disease level was lower than in the previous year, which was evidenced from the disease distribution patterns of both the candidates (Fig. 3.1a) and checks (Fig. 3.1b). Accordingly, DON distribution skewed markedly to the direction of low value, with 90% of the lines having DON content less than 3 ppm (Fig. 3.1c). Selection was made to retain 311 entries showing FHB index <5 % and DON content <3 ppm. The disease level in 2012 was similar to that in 2011, and finally 44 resistant lines with FHB index <2.5% and DON content <1.5 ppm were selected and compiled as the 15th FHBSN, wherein maximum two sister lines per cross were included (Table 3.4).

Fig. 3.1 Frequency distribution of FHB index, DON content and FDK in different years in El Batán, Mexico



Note: for the FHB chart (**a**), 2794 lines were evaluated in 2010, 1109 in 2011, 311 in 2012, and 44 in 2013; for the DON chart (**c**), 301 lines were measured in 2011, 75 in 2012, and 44 in 2013; for the FDK chart (**d**), only the 44 entries of the 15th FHBSN were evaluated.

In 2013, the average FHB index of all genotypes was 23.1%, much higher than those in previous years (Tables 3.4 and S2, Fig. 3.1a), indicating a high disease pressure. DON content was also much higher than in 2011 and 2012 (Fig. 3.1c), with the highest value of 7.9 ppm, even higher than that of the susceptible check Gamenya (6.1 ppm). However, FDK values ranging from 1.0 to 5.5 (Fig. 3.1d) were not as high as would be predicted from the DON values.

Table 3.3 Analysis of variance of the 15th FHBSN evaluated in El Batán for FHB index, DON content, FDK and their heritability estimates

Trait	Source	DF	MS	*F* value	*Pr*>F	Heritability
FHB	Entry	45	627.46	24.56	<0.001	0.82
	Year	2	11747.55	459.86	<0.001	
	Rep(year)	3	37.12	1.45	0.2302	
	Entry*Year	90	127.19	4.98	<0.001	
	Error	135	25.55			
DON	Entry	45	6.81	6.22	<0.001	0.69
	Year	1	415.34	379.02	<0.001	
	Rep(year)	2	0.80	0.73	0.4850	
	Entry*Year	45	2.69	2.46	<0.001	
	Error	90	1.10			
FDK	Entry	45	3.85	5.21	<0.001	0.81
	Rep	1	0.27	0.37	0.5471	
	Error	45	0.74			

Note: only the 15th FHBSN entries and the two checks (Sumai 3 and Gamenya) were included in this analysis. FHB data were from 2011, 2012 and 2013, DON from 2012 and 2013, and FDK from 2013. FHB 2010 and DON 2011 were not included since the experiments were not replicated. All the data were measured in El Batán, Mexico.

In the Netherlands, the disease severity was very high with a grand mean FHB severity of 43.6%, wherein only 12 lines exhibited values less than 30%. Under the natural infection in Toluca, 2013, the entries showed very low disease with an average FHB index of merely 2% for all genotypes, while 0 for Sumai 3 and 56% for Gamenya (Table S1). Although no artificial inoculation was used in Canada, the severity was notably higher than Toluca under the natural infection, having a grand mean FHB severity of 29.6%. In Norway, a grand mean FHB severity of 34.9% was obtained, with 10 lines showed more severe infection than Gamenya; but there were 18 lines being statistically non-significantly different from Sumai 3. Similar disease distribution happened in Uruguay, with the only marked difference being the lower grand mean value of 17.5%. Whereas in Japan 2014, where spawn inoculation was applied, the grand mean FHB severity was 19.2%, and only 7 lines had FHB severity >30% (Tables 3.4 and S1).

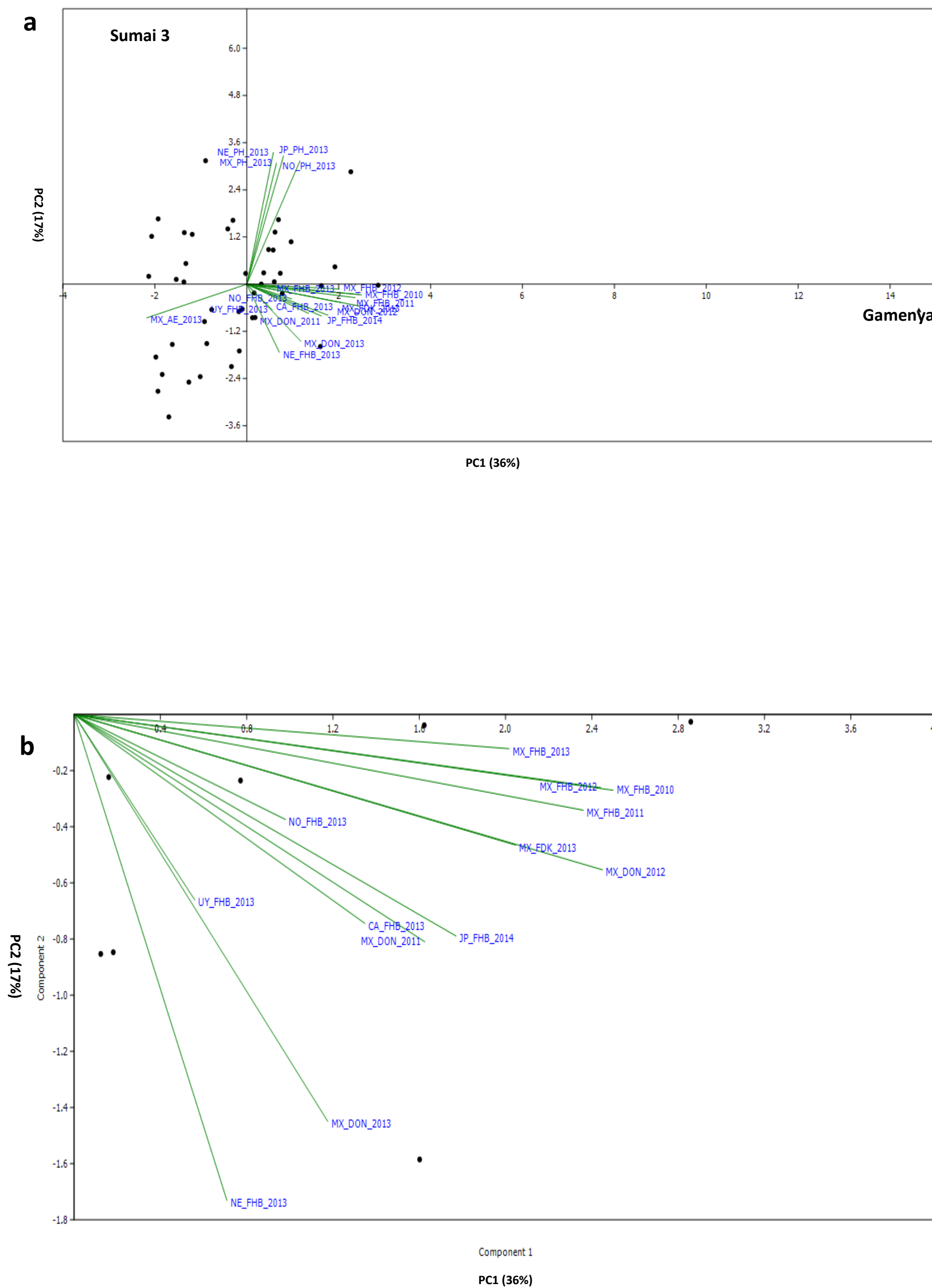
Across year/environment, Sumai 3 and Gamenya performed quite consistently, being the (or among the) most resistant and the most susceptible, respectively. On the other hand, no line other than Sumai 3 was consistently being highly resistant in all the experiments; but there were several lines being resistant in most experiments, which can easily be identified by the composite index, such as FRNCLN/HEILO//FRNCLN (CIMMYT germplasm bank identifier, GID, 6340966), WAXWING*2/TUKURU*2//HEILO (GID 6340862), ATTILA/PASTOR/3/ATTILA/BAV92//PASTOR/4/PBW343*2/TUKURU (GID 6000696), etc. (Table 3.4).

As expected, Sumai 3 and Gamenya were scattered away from the 15th FHBSN entries in the biplot, wherein the most promising lines were found in the second quadrant (Fig. 3.2), including the lines nominated above. Regarding the correlation among FHB traits, those evaluated in El Batán were highly interrelated and their vectors clustered together in the biplot, with only DON 2011 and DON 2013 being outliers. Of the five international locations, Norway, Japan, and Canada were more similar to El Batán than Uruguay and the Netherlands, although positive correlation was always evidenced between these locations and El Batán.

AE, a trait associated with Type I resistance, ranged between 5.5 and 8.0 for the entries in El Batán, whereas a low AE of 3.0 was observed for Gamenya (Table S1). Negative correlation was observed between AE and most FHB traits (Fig. 3.2). PH did not differ greatly among the

entries in different locations and showed marginally negative correlation with FHB traits in the most cases.

Fig. 3.2 Biplot of the 15th FHBSN based on principal component analysis (PCA) on FHB parameters, PH and AE values



Note: Both the original one (**a**) and its magnified part showing the FHB related vectors (**b**) are shown. Cosine of the angle between vectors indicates correlation between variables in the dimension of the first two principal components (PCs).

The haplotyping results proposed that 24 (55%) of the genotypes carried the 4BS QTL as in Wuhan 1, which was the most frequent QTL in this nursery. Another frequent one was the 7A QTL as in *T. dicoccoides*, which was noticed in five (11%) of the genotypes. In contrast, the resistance QTLs on chromosomes 3B, 5A and 6B as in Sumai 3 and 3A as in *T. dicoccoides* were not detected in any of the genotypes (Table 3.5). It is noteworthy that 15 (34%) of the genotypes appeared to carry none of the ten QTLs examined. Results of allelic variation at *Rht-B1* and *Rht-D1* are presented in Table S1. All but one of the entries had the *Rht-B1b* dwarfing allele, whereas none had the *Rht-D1b* dwarfing allele, i.e. most entries were of the *Rht-B1b/Rht-D1a* genotype.

Table 3.4 Phenotypic data of the 15th Fusarium Head Blight Screening Nursery (15th FHBSN)

GID	Entry name	Mexico (El Batán)								International locations					Composite index
		FHB 2010	FHB 2011	DON 2011	FHB 2012	DON 2012	FHB 2013	DON 2013	FDK 2013	NO 2013	UY 2013	NE 2013	CA 2013	JP 2014	
6342075	ATTILA/3*BCN*2//BAV92/3/HEILO	7.6	5.2	2.8	1.4	1.2	10.9	5.0	2.5	31.6	36.0	55.0	30.0	10.0	24
6340362	ATTILA/3*BCN*2//BAV92/3/HEILO/4/CHIBIA//PRLII/CM65531/3/SKAUZ/BAV92	12.7	4.9	0.6	1.3	0.2	13.4	3.0	2.5	16.6	6.0	55.0	10.0	18.0	15
6000734	ATTILA/BAV92//PASTOR/3/ATTILA*2/PBW65/4/ATTILA/PASTOR	14.3	2.9	1.3	3.2	0.5	24.6	6.7	2.5	43.2	15.0	37.0	10.0	15.0	21
6000696	ATTILA/PASTOR/3/ATTILA/BAV92//PASTOR/4/PBW343*2/TUKURU	2.4	0.5	0.3	3.5	0.9	20.8	2.2	3.0	37.9	2.0	10.0	10.0	7.0	10
6343618	BABAX/LR42//BABAX*2/3/PAVON 7S3,+LR47/4/HEILO	3.8	6.4	1.4	0.6	1.0	35.9	4.5	1.0	46.4	64.0	60.0	30.0	9.0	35
6342108	BABAX/LR42//BABAX*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ/5/HEILO	12.5	3.9	1.8	3.0	0.9	20.8	4.4	2.0	22.9	2.0	37.0	30.0	13.0	15
6340565	CHIBIA//PRLII/CM65531/3/SKAUZ/BAV92*2/4/GONDO/CBRD	8.9	2.7	0.4	2.7	1.0	20.3	1.7	3.5	19.3	14.0	17.0	10.0	6.0	13
6340604	CHIBIA//PRLII/CM65531/3/SKAUZ/BAV92/4/HEILO/5/FRET2/KUKUNA//FRET2	8.9	4.1	2.9	1.7	2.4	23.9	5.3	4.0	28.4	0.3	65.0	50.0	24.0	15
6000632	CNO79//PF70354/MUS/3/PASTOR/4/BAV92/5/ATTILA*2/PBW65/6/PBW343*2/TUKURU	2.0	3.1	0.2	1.9	2.5	30.0	3.9	3.0	48.6	8.0	25.0	30.0	30.0	25
6342187	FRET2/KUKUNA//FRET2/3/HEILO	10.3	3.7	1.6	1.4	0.6	28.6	3.0	2.5	17.0	24.0	65.0	50.0	6.0	25
6340649	FRET2/KUKUNA//FRET2/3/HEILO/4/BLOUK #1	2.8	3.6	2.3	1.9	0.7	17.4	1.9	2.0	30.5	42.0	50.0	30.0	14.0	28
6340966	FRNCLN/HEILO//FRNCLN	11.3	4.4	0.7	3.3	0.5	4.3	2.4	4.0	27.7	1.0	17.0	10.0	11.0	8
6001555	GOUBARA-1/2*SOKOLL	0.9	0.5	2.2	2.1	1.5	25.4	6.2	3.0	43.1	40.0	25.0	30.0	9.0	26
6001364	KABY/BAV92/3/CROC_1/AE.SQUARROSA (224)//OPATA/4/PASTOR/FLORKWA-1//BAV92	2.3	1.7	1.0	3.3	0.8	51.9	3.4	4.0	29.0	7.0	55.0	50.0	30.0	31
6000673	KABY/BAV92/3/CROC_1/AE.SQUARROSA (224)//OPATA/4/WHEAR/5/ATTILA/BAV92//PASTOR	14.3	1.4	1.3	1.8	1.0	11.0	6.0	2.0	50.0	8.0	37.0	30.0	25.0	22
6176474	KACHU #1/4/CROC_1/AE.SQUARROSA (205)//KAUZ/3/SASIA/5/KACHU	8.6	1.8	1.2	3.1	1.4	15.2	6.0	1.5	24.8	10.0	25.0	10.0	52.0	19
6342246	KAUZ*2/MNV//KAUZ/3/MILAN/4/BAV92/5/HEILO	4.3	3.1	0.6	3.1	0.7	12.7	3.6	2.5	23.9	7.0	55.0	50.0	6.0	18
6340672	KAUZ*2/MNV//KAUZ/3/MILAN/4/BAV92/5/HEILO/6/CHIBIA//PRLII/CM65531/3/SKAUZ/BAV92	2.1	3.4	1.0	1.8	1.5	13.1	5.3	2.0	22.1	18.0	37.0	50.0	9.0	21
6340708	KAUZ*2/MNV//KAUZ/3/MILAN/4/BAV92/5/HEILO/6/CHIBIA//PRLII/CM65531/3/SKAUZ/BAV92	10.3	3.4	2.0	2.3	1.3	22.1	4.7	2.5	25.5	28.0	50.0	70.0	12.0	30
6340845	MUNAL//SHA3/CBRD/3/PAURAQ	7.3	4.3	1.3	1.8	0.4	10.9	2.7	1.5	35.9	21.0	60.0	10.0	12.0	20
6342263	OASIS/SKAUZ//4*BCN*2/3/PASTOR/4/HEILO	8.1	3.9	0.9	2.7	1.1	13.9	6.0	2.0	38.9	0.3	55.0	10.0	51.0	12
6342266	OASIS/SKAUZ//4*BCN*2/3/PASTOR/4/HEILO	8.2	3.8	0.2	2.5	0.5	8.5	2.3	2.0	35.2	5.0	50.0	50.0	9.0	18
6340765	PBW343/WBLL1//PANDION/3/HEILO/4/PAURAQ	7.4	2.9	1.5	2.6	0.7	21.8	3.8	3.0	29.8	8.0	17.0	30.0	13.0	18

6340803	PFAU/WEAVER*2//BRAMBLING/3/HEILO/4/WAXWING*2/TUKURU	3.6	6.7	1.4	2.4	0.9	39.3	4.5	2.0	26.6	8.0	65.0	30.0	11.0	24
6343651	PFAU/WEAVER*2//TRANSFER#12,P88.272.2/3/HEILO	17.9	2.7	0.9	1.4	0.7	16.0	2.6	2.5	19.1	18.0	50.0	30.0	9.0	20
5999927	PROINTA SUPERIOR/4/RL6043/4*NAC//PASTOR/3/BAV92/5/KLEIN SAGITARIO	0.9	0.8	1.1	3.8	1.6	36.6	5.3	4.5	49.8	12.0	60.0	10.0	15.0	24
6000034	QG 4.37A/4/MILAN/KAUZ//PRINIA/3/BAV92/5/MILAN/KAUZ//PRINIA/3/BAV92	3.7	1.7	1.2	2.1	1.2	24.3	5.4	4.0	35.9	16.0	25.0	50.0	8.0	23
6000970	SOKOLL*2/ROLF07	13.6	0.6	0.4	2.3	1.9	28.0	7.8	3.0	53.1	12.0	37.0	50.0	24.0	30
6000906	SOKOLL*2/TROST	3.9	2.5	0.7	3.3	1.4	45.2	7.7	5.5	41.6	24.0	55.0	50.0	56.0	44
6001180	SOKOLL//FRTL/2*PIFED	8.0	2.9	1.1	3.1	3.1	39.8	5.9	3.0	55.4	30.0	25.0	30.0	40.0	35
6000931	SOKOLL//PBW343*2/KUKUNA/3/ATTILA/PASTOR	7.1	2.1	1.0	1.7	1.1	27.4	5.4	4.0	56.2	48.0	55.0	30.0	24.0	38
6001093	SOKOLL/ROLF07	9.0	1.7	1.9	3.0	2.2	58.7	7.9	5.0	42.3	24.0	25.0	30.0	52.0	36
6000939	SW89-5124*2/FASAN/3/ALTAR 84/AE.SQ//2*OPATA/4/ARREHANE	11.2	0.6	1.5	2.4	1.7	24.4	7.0	3.5	34.6	45.0	37.0	30.0	13.0	29
6342336	TAM200/PASTOR//TOBA97/3/HEILO	6.5	1.6	1.4	2.8	2.2	7.7	5.0	2.5	45.0	1.0	25.0	50.0	21.0	14
6342353	TAM200/PASTOR//TOBA97/3/HEILO	18.7	3.3	0.6	1.1	0.5	11.8	1.3	4.5	48.2	18.0	65.0	10.0	13.0	21
6343684	THELIN/2*WBLL1//HEILO	18.6	4.5	1.0	3.0	0.7	5.8	1.6	2.5	35.9	16.0	60.0	10.0	12.0	17
6342383	TOBA97/PASTOR//HEILO	10.0	3.7	2.0	1.8	1.1	24.7	4.4	4.0	26.0	30.0	55.0	50.0	6.0	26
5999807	VORB/4/D67.2/PARANA 66.270//AE.SQUARROSA (320)/3/CUNNINGHAM	2.7	2.2	0.0	1.5	1.0	35.2	5.5	5.5	35.2	6.0	55.0	30.0	24.0	26
6340858	WAXWING*2/TUKURU*2//HEILO	3.9	2.5	1.3	2.0	2.5	39.4	4.4	3.0	42.1	36.0	50.0	30.0	12.0	32
6340862	WAXWING*2/TUKURU*2//HEILO	11.1	4.2	0.7	1.7	0.5	11.3	2.6	1.5	32.9	1.0	50.0	10.0	3.0	9
6343369	WBLL1*2/BRAMBLING*2//GONDO/TNMU	16.4	3.8	1.3	2.7	3.1	15.9	5.9	1.5	22.6	21.0	60.0	10.0	25.0	22
6342460	WBLL1*2/KIRITATI//HEILO	9.7	4.1	2.0	1.5	1.1	20.5	3.6	3.5	31.1	28.0	65.0	30.0	13.0	28
6343743	WBLL1*2/KUKUNA//HEILO	7.3	4.3	0.6	0.6	0.7	12.9	2.4	2.5	34.0	12.0	17.0	10.0	13.0	15
5999852	YAR/AE.SQUARROSA (518)/3/PRL/SARA//TSI/VEE#5/4/ATTILA/5/BERKUT	1.1	2.4	2.5	2.7	0.9	18.9	3.2	4.5	59.1	9.0	37.0	10.0	25.0	21
10004	**SUMAI #3 (Resistant check)**	**1.5**	**1.4**	**0.1**	**1.0**	**0.0**	**1.0**	**0.3**	**1.0**	7.6	1.0	3.0	10.0	7.0	3
5536	**GAMENYA (Susceptible check)**	**68.0**	**78.7**	**3.9**	**72.0**	**7.5**	**60.4**	**6.1**	**8.5**	44.6	24.0	70.0	70.0	65.0	52

Note: additional information to this table is available in Table S1. Phenotyping data include FHB index (%), DON content (ppm) and FDK (%) from El Batán , Mexico, FHB severity (%) from Norway (NO), the Netherlands (NE), Canada (CA), and Japan (JP), and FHB index (%) from Uruguay (UY). The composite index was the sixth root of the product of FHB2013 (in El Batán), NO2013, UY2013, NE2013, CA2013, and JP2014.

Table 3.5 Haplotyping data of the 15th Fusarium Head Blight Screening Nursery (15th FHBSN)

GID	Entry name	WU 2D	CJ 2D	FR 3A	SU 3B	DI 3A	WU 4B	SU 5A	FR 5A	SU 6B	DI 7A
6342075	ATTILA/3*BCN*2//BAV92/3/HEILO	- -	- -	-	-	-	+ -	- -	+ -	- -	+ -
6340362	ATTILA/3*BCN*2//BAV92/3/HEILO/4/CHIBIA//PRLII/CM65531/3/SKAUZ/BAV92	- -	- -	-	-	-	+ +	- -	- -	- -	+ +
6000734	ATTILA/BAV92//PASTOR/3/ATTILA*2/PBW65/4/ATTILA/PASTOR	- -	- -	-	-	-	+ +	- -	+ -	NA	- -
6000696	ATTILA/PASTOR/3/ATTILA/BAV92//PASTOR/4/PBW343*2/TUKURU	- -	- -	-	-	-	+ -	- -	+ -	- -	+ -
6343618	BABAX/LR42//BABAX*2/3/PAVON 7S3, +LR47/4/HEILO	- -	- -	-	-	-	+ +	- -	+ -	- -	- -
6342108	BABAX/LR42//BABAX*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ/5/HEILO	- -	- -	-	-	-	+ +	- -	+ +	- -	+ +
6340565	CHIBIA//PRLII/CM65531/3/SKAUZ/BAV92*2/4/GONDO/CBRD	- -	- -	-	-	-	+ -	- -	- -	- -	+ -
6340604	CHIBIA//PRLII/CM65531/3/SKAUZ/BAV92/4/HEILO/5/FRET2/KUKUNA//FRET2	- -	- -	-	-	-	- -	- -	- -	- -	+ -
6000632	CNO79//PF70354/MUS/3/PASTOR/4/BAV92/5/ATTILA*2/PBW65/6/PBW343*2/TUKURU	- -	- -	-	-	-	- -	- -	+ -	- -	+ -
6342187	FRET2/KUKUNA//FRET2/3/HEILO	- -	- -	-	-	-	+ +	- -	+ +	- -	+ -
6340649	FRET2/KUKUNA//FRET2/3/HEILO/4/BLOUK #1	- -	- -	-	-	-	+ +	- -	+ -	- -	+ -
6340966	FRNCLN/HEILO//FRNCLN	- -	- -	-	-	-	+ +	- -	+ -	- -	+ -
6001555	GOUBARA-1/2*SOKOLL	- -	- -	-	-	-	+ -	- -	- -	- -	- -
6001364	KABY/BAV92/3/CROC_1/AE.SQUARROSA (224)//OPATA/4/PASTOR/FLORKWA-1//BAV92	- -	+ -	+	-	-	- -	- -	+ -	- -	+ -
6000673	KABY/BAV92/3/CROC_1/AE.SQUARROSA (224)//OPATA/4/WHEAR/5/ATTILA/BAV92//PASTOR	- -	- -	-	-	-	- -	- -	+ -	- -	+ -
6176474	KACHU #1/4/CROC_1/AE.SQUARROSA (205)//KAUZ/3/SASIA/5/KACHU	- -	- -	-	-	-	+ +	- -	+ -	- -	- -
6342246	KAUZ*2/MNV//KAUZ/3/MILAN/4/BAV92/5/HEILO	NA	- -	NA	-	-	+ +	NA	+ -	NA	+ -
6340672	KAUZ*2/MNV//KAUZ/3/MILAN/4/BAV92/5/HEILO/6/CHIBIA//PRLII/CM65531/3/SKAUZ/BAV92	- -	- -	-	-	-	+ +	- -	- -	- -	- -
6340708	KAUZ*2/MNV//KAUZ/3/MILAN/4/BAV92/5/HEILO/6/CHIBIA//PRLII/CM65531/3/SKAUZ/BAV92	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
6340845	MUNAL//SHA3/CBRD/3/PAURAQ	+ +	- -	-	-	-	+ -	- -	+ -	- -	- -
6342263	OASIS/SKAUZ//4*BCN*2/3/PASTOR/4/HEILO	- -	- -	-	-	-	+ +	- -	+ -	- -	+ -
6342266	OASIS/SKAUZ//4*BCN*2/3/PASTOR/4/HEILO	- -	- -	-	-	-	+ +	- -	+ -	- -	+ -
6340765	PBW343/WBLL1//PANDION/3/HEILO/4/PAURAQ	- -	- -	-	-	-	+ -	- -	-	- -	+ +
6340803	PFAU/WEAVER*2//BRAMBLING/3/HEILO/4/WAXWING*2/TUKURU	- -	- -	-	-	-	+ +	- -	-	- -	- -
6343651	PFAU/WEAVER*2//TRANSFER#12,P88.272.2/3/HEILO	- -	- -	-	-	-	+ +	- -	+ -	- -	+ -
5999927	PROINTA SUPERIOR/4/RL6043/4*NAC//PASTOR/3/BAV92/5/KLEIN SAGITARIO	- -	- -	-	-	-	+ -	- -	- -	- -	- -
6000034	QG 4.37A/4/MILAN/KAUZ//PRINIA/3/BAV92/5/MILAN/KAUZ//PRINIA/3/BAV92	+ -	- -	-	-	-	+ +	- -	- -	- -	+ +
6000970	SOKOLL*2/ROLF07	+ -	- -	-	-	-	+ +	- -	- -	- -	+ +
6000906	SOKOLL*2/TROST	- -	- -	-	-	-	+ -	- -	- -	+ -	+ -
6001180	SOKOLL//FRTL/2*PIFED	- -	- -	-	-	-	+ -	- -	+ -	- -	+ -
6000931	SOKOLL//PBW343*2/KUKUNA/3/ATTILA/PASTOR	- -	- -	-	-	-	+ -	- -	- -	- -	+ -
6001093	SOKOLL/ROLF07	- -	- -	-	-	-	+ +	- -	- -	- -	+ -
6000939	SW89-5124*2/FASAN/3/ALTAR 84/AE.SQ//2*OPATA/4/ARREHANE	- -	- -	-	-	-	+ +	- -	- -	- -	+ -
6342336	TAM200/PASTOR//TOBA97/3/HEILO	+ +	- -	-	-	-	- -	- -	+ -	- -	+ -
6342353	TAM200/PASTOR//TOBA97/3/HEILO	- -	- -	-	-	-	+ +	- -	+ -	- -	- -
6343684	THELIN/2*WBLL1//HEILO	+ -	- -	-	-	-	+ +	- -	+ -	- -	- -
6342383	TOBA97/PASTOR//HEILO	- -	- -	-	-	-	+ -	- -	+ -	- -	+ -
5999807	VORB/4/D67.2/PARANA 66.270//AE.SQUARROSA (320)/3/CUNNINGHAM	+ -	- -	-	-	-	+ +	- -	- -	+ -	+ -
6340858	WAXWING*2/TUKURU*2//HEILO	- -	- -	-	-	-	+ -	- -	+ -	- -	+ -
6340862	WAXWING*2/TUKURU*2//HEILO	- -	- -	-	-	-	+ -	- -	- -	- -	+ -
6343369	WBLL1*2/BRAMBLING*2//GONDO/TNMU	- -	- -	-	-	-	+ -	- -	- -	- -	- -
6342460	WBLL1*2/KIRITATI//HEILO	- -	- -	-	-	-	+ +	- -	+ -	- -	+ -
6343743	WBLL1*2/KUKUNA//HEILO	- -	- -	-	-	-	+ +	- -	+ -	NA	- -
5999852	YAR/AE.SQUARROSA (518)/3/PRL/SARA//TSI/VEE#5/4/ATTILA/5/BERKUT	- -	- -	-	-	-	+ +	- -	- -	+ -	+ -
10004	**SUMAI #3 (Resistant check)**	- -	+ +	-	+	-	- -	+ +	- -	+ +	+ +
5536	**GAMENYA (Susceptible check)**	+ -	- -	-	-	-	+ -	- -	- -	- -	- -

Note: information on the sizes of PCR products is available in Table S1. Data from 17 markers linked to 10 validated QTLs are presented, where WU stands for Wuhan 1, CJ for CJ 9306, FR for Frontana, SU for Sumai 3, and DI for T. diccocoides. '+ +' denotes the presence of the QTL supported by both flanking markers; '+ -', supported by only one marker; '- -' putative absence of a QTL; 'NA', not analyzed. For SU_3B, DI_3A, and FR_3A, only one flanking marker was applied to predict the presence/absence of QTL.

3.4. Discussion

To successfully identify novel FHB resistance resources, an effective screening protocol is crucial, in which genetic background and haplotyping results must be taken into consideration in addition to FHB parameters, to maintain good level of diversity. FHB resistance could be best estimated in the field by FHB index since it considers both severity and incidence (Wilcoxson et al. 1992). The selection for the 15th FHBSN was mainly based on FHB index and DON concentration, with an attention on the maintenance of genetic diversity based on pedigree information, in accordance with the selection for our previous FHBSNs (He et al. 2013a; He et al. 2013b). Screening on DH and PH was done in 2010 primarily to discard very late and tall lines.

Although the 15th FHBSN accessions generally exhibited low levels of infection in both El Batán and Toluca (Mexican environments), many turned out to be susceptible in other five locations, due to a significant genotype-by-environment interaction, which could be caused by one or all of the following reasons; different inoculation protocols, field management, weather condition, *Fusarium* isolates etc. Many studies have shown that FHB resistance in wheat is horizontal, not species- nor strain-specific (Van Eeuwijk et al. 1995; Mesterhazy et al. 1999; Mesterhazy et al. 2005). Therefore, the differences in FHB levels resulting from variation in *Fusarium* isolates or species used throughout this study may not explain the resistance variation across locations, although significant differences in aggressiveness have been reported in *Fusarium* isolates/species of different geographic origins (Malihipour et al. 2012).

The environmental effects are obliviously seen in 2013 at El Batan where the high precipitation during the epidemic season (227.1 mm, July-August) led to more FHB epidimic than those in

the 2011 and 2012 seasons (August-September), with 39.5 and 126.7 mm of precipitation scored, respectively (El Batán weather station, CIMMYT). Though it is anticipated that the misting system could provide sufficient micro-environmental moisture and thus rainfall would not significantly contribute to increased disease development; results from El Batán 2013 rejected this hypothesis or at least raised questions about it. The reason could be due to the rain-splash facilitated pathogen spread; even though spray inoculation was adopted in this experiment, huge quantity of *Fusarium* pathogen was expected to be present in the soil after the long-term use of the field as FHB screening nursery, leading to what is called background infection. Similar situation was also found in Ontario, Canada (Tamburic-Ilincic et al. 2013) and in Nebraska, USA (Nopsa et al. 2012). Thus planting in the year was advanced half month compared to previous years, after realizing that early planting in El Batán usually leads to higher FHB disease pressure (He et al. 2014). This highlights the importance of multi-locational and/or multiple years FHB evaluation, whereby the potential resistant germplasm are exposed to diverse epidemic environments, facilitating the identification of genotypes with durable resistance as well as the selection of locally adapted lines useful to national breeding programs. As mentioned above, several lines were consistently resistant across environments and thus could be used as resistance sources; although their resistance is not as high as that of Sumai 3, especially under high disease pressure.

Generally, mycotoxin content is the most important FHB trait regarding food safety; but it is also laborious and costly to evaluate compared to FHB and FDK, especially in mass screening programs where thousands of accessions are tested annually. Accordingly, it is obligatory to do a couple of field evaluations to reduce the total number of accessions to be tested for DON content by excluding lines which have high FHB index and/or FDK. This indirect screening strategy is based on the controversial association among different FHB parameters, most notably between FHB index and DON content. However the conclusions from different studies are debateable and the correlation between FHB index and DON ranges from no significant association to strong positive correlation (Paul et al. 2005, 2006). In the present study, DON content appeared to be the least stable FHB parameter across years in El Batán compared to the more stable FHB index as shown in the biplot, and no high correlation of FHB/DON and FDK/DON were found. The main reason for this could be ascribed to a lack of major QTL conditioning both field FHB and DON/FDK resistance, e.g. *Fhb1*, as proposed by Lu et al.

(2013). Additionally, the temporal separation of evaluations for different FHB traits, invisible infections and wide diversity present in the studied lines are all possible reasons could have caused the lack of expected correlations. This implies that low FHB and FDK do not necessarily lead to low DON, which complied well with our phenotypic data. Therefore, varieties with low FHB and FDK should be further tested for DON to determine their resistance components.

AE has been reported to be negatively correlated with FHB/DON and to be part of the Type I resistance after spawn and spray inoculation (Lu et al. 2013). In the present study, all the 15th FHBSN entries exhibited high AE rates, which could have conferred good Type I resistance that protected the materials very well in the low epidemic years of 2011 and 2012; but the protection was not sufficient in 2013 in El Batán, Norway, the Netherlands and Canada, implying weaker Type II resistance.

Unlike the 13th and 14th FHBSN, where the 2DL QTL as in Wuhan 1 was the predominant one (He et al. 2013a; He et al. 2013b), the 15th FHBSN suggested a high frequency of 55% of the 4BS QTL as in Wuhan 1. Although both were found in Wuhan 1, the 2DL QTL conferred Type II resistance, whereas the 4BS one contributed Type I resistance (Somers et al. 2003). The latter has been fine mapped by Xue et al. (2010) and designated as *Fhb4*. The QTL on 7A chromosome as in *T. dicoccoides* was the second frequent QTL, contributing to Type II resistance (Kumar et al. 2007); but it was found only in five lines. Considering also the very low frequencies or absence of other QTLs, the haplotyping results proposed a clear non-Sumai 3 resistance background of the 15th FHBSN, which lacked major Type II resistance QTLs such as 3BS (*Fhb1*) and 6BS (*Fhb2*) as in Sumai 3 (Cuthbert et al. 2006; Cuthbert et al. 2007). CIMMYT has devoted great efforts on the identification and utilization of non-Sumai 3 resistance since the last decade (He et al. 2013a), which was very successful as shown by the haplotyping results. *Fhb1* and *Sr2* are linked in repulsion (Flemmig, 2012), thus the deployment of *Sr2* in high proportions of CIMMYT germplasm due to Ug99 (stem rust) threat has possibly further resulted in eliminating *Fhb1* gene from CIMMYT germplasm. Based on 2013 results, wherein very high disease was observed, it is imperative to introduce resistance genes/QTLs of both Sumai 3 and non-Sumai 3 origins, particularly the two Type II resistance genes *Fhb1* and *Fhb2*, into the CIMMYT germplasm to increase the resistance level.

It is well known from previous studies that the dwarfing genes *Rht-B1b* and *Rht-D1b* are associated with FHB susceptibility due to either genetic linkage, pleiotropic effect, or disease escape (Hilton et al. 1999; Schmolke et al. 2005; Holzapfel et al. 2008; Yan et al. 2011). However, the negative effect of *Rht-D1b* is more significant than that of *Rht-B1b*, and it has thus been advised to utilize the latter to achieve a desirable plant height at a relatively low cost of increasing FHB susceptibility (Miedaner and Voss, 2008; Srinivasachary et al. 2009). According to our results, it was *Rht-B1b* instead of *Rht-D1b* that prevalent in this nursery, which is the ideal in terms of FHB resistance.

Taken together the phenotypic and genotypic data, this study demonstrated that the 15th FHBSN entries have generally good Type I and Type IV resistance, but lower Type III resistance. Although there was no direct evidence, their Type II resistance level may not be high, considering the high disease levels in El Batán, Norway, the Netherlands, and Canada in 2013, as well as the absence of major Type II resistance QTLs. Strategies are being adopted involving pyramiding of Sumai 3 and non Sumai 3 resistance in CIMMYT germplasm and breaking the repulsive linkage of *Sr2* and *Fhb1*. Resistant genotypes identified in this study could be successfully utilized by breeders as donors of novel FHB resistance in an attempt to diversifying and/or pyramiding FHB resistance.

3.5. Acknowledgements

The helpful assistance of Francisco Lopez and Javier Segura with field trials and Nerida Lozano for her efforts on strain identification and inoculum preparation is highly acknowledged. Financial support by the CGIAR Research Program on Wheat is gratefully acknowledged. Field testing of the 15th FHBSN nursery in Norway was financed through grants from the Research Council of Norway (NFR 208340 and NFR 224833).

3.6. References

Agostinelli AM, Clark AJ, Brown-Guedira G, Van Sanford DA (2012) Optimizing phenotypic and genotypic selection for Fusarium head blight resistance in wheat. Euphytica 186:115-126.

Audenaert K, Van Broeck R, Bekaert B, De Witte F, Heremans B, Messens K, Hofte M, Haesaert G (2009) Fusarium head blight (FHB) in Flanders: population diversity, inter-species associations and DON contamination in commercial winter wheat varieties. Eur J Plant Pathol 125:445-458.

Bai G, Shaner G (1994) Scab of wheat: Prospects for control. Plant Dis 78:760-766.

Bai G, Shaner G (2004) Management and resistance in wheat and barley to Fusarium head blight. Annu Rev Phytopathol 42:135-161.

Bai GH, Desjardins AE, Plattner RD (2002) Deoxynivalenol-nonproducing *Fusarium graminearum* causes initial infection, but does not cause disease spread in wheat spikes. Mycopathologia 153:91-98.

Brunner K, Paris MPK, Paolino G, Burstmayr H, Lemmens M, Berthiller F, Schuhmacher R, Krska R, Mach RL (2009) A reference-gene-based quantitative PCR method as a tool to determine *Fusarium* resistance in wheat. Anal Bioanal Chem 395:1385-1394.

Buerstmayr H, Adam G, Lemmens M (2012) Resistance to head blight caused by *Fusarium* spp. in wheat. In: Sharma I (ed) Disease resistance in wheat. CABI, Wallingford, Oxfordshire, UK; Cambridge, MA, pp 236-276.

Buerstmayr H, Ban T, Anderson JA (2009) QTL mapping and marker-assisted selection for Fusarium head blight resistance in wheat: a review. Plant Breed 128:1-26.

Buerstmayr H, Lemmens M, Hartl L, Doldi L, Steiner B, Stierschneider M, Ruckenbauer P (2002) Molecular mapping of QTLs for Fusarium head blight resistance in spring wheat. I. Resistance to fungal spread (type II resistance). Theoretical and applied genetics 104:84-91.

Champeil A, Dore T, Fourbet JF (2004) Fusarium head blight: epidemiological origin of the effects of cultural practices on head blight attacks and the production of mycotoxins by *Fusarium* in wheat grains. Plant Sci 166:1389-1415.

Culler MD, Miller-Garvin JE, Dill-Macky R (2007) Effect of extended irrigation and host resistance on deoxynivalenol accumulation in *Fusarium*-infected wheat. Plant Dis 91:1464-1472.

Cuthbert PA, Somers DJ, Brulé-Babel A (2007) Mapping of *Fhb2* on chromosome 6BS: a gene controlling Fusarium head blight field resistance in bread wheat (*Triticum aestivum* L.). Theoretical and applied genetics 114:429-437.

Cuthbert PA, Somers DJ, Thomas J, Cloutier S, Brulé-Babel A (2006) Fine mapping *Fhb1*, a major gene controlling Fusarium head blight resistance in bread wheat (*Triticum aestivum* L.). Theoretical and applied genetics 112:1465-1472.

Draeger R, Gosman N, Steed A, Chandler E, Thomsett M, Srinivasachary, Schondelmaier J, Buerstmayr H, Lemmens M, Schmolke M, Mesterhazy A, Nicholson P (2007) Identification of QTLs for resistance to Fusarium head blight, DON accumulation and associated traits in the winter wheat variety Arina. Theoretical and applied genetics 115:617-625.

Dubin HJ, Gilchrist L, Reeves J, McNab A (1997) Fusarium head scab, global status and future prospects. Mexico, D.F.:CIMMYT

Duveiller E, Mezzalama M, Murakami J, Lewis J, Ban T (2008) Global Fusarium networking. Cereal Res Commun 36:11-19.

European Commission (2006) Commission Regulation (EC) No. 1881/2006 setting maximum levels of certain contaminants in foodstuffs. Off J Eur Union L364:5–24.

Fernando WGD, Paulitz TC, Seaman WL, Dutilleul P, Miller JD (1997) Head blight gradients caused by *Gibberella zeae* from area sources of inoculum in wheat field plots. Phytopathology 87:414-421.

Flemmig EL (2012) Molecular markers to deploy and characterize stem rust resistance in wheat. M.S. thesis, North Carolina State University

Graham S, Browne RA (2009) Anther extrusion and Fusarium head blight resistance in European wheat. J Phytopathol 157:580-582.

Hammer Ø, Harper D, Ryan P (2001) PAST: Paleontological Statistics Software Package for education and data analysis. Palaeontolia Electronica 4:1-9.

He X, Singh PK, Duveiller E, Dreisigacker S, Singh RP (2013a) Development and characterization of International Maize and Wheat Improvement Center (CIMMYT) germplasm for Fusarium head blight resistance. In: Alconada TM, Chulze SN (eds) Fusarium Head Blight in Latin America. pp 241-262

He X, Singh PK, Duveiller E, Schlang N, Dreisigacker S, Singh RP (2013b) Identification and characterization of international Fusarium head blight screening nurseries of wheat at CIMMYT, Mexico. Eur J Plant Pathol 136:123-134.

He X, Singh PK, Schlang N, Duveiller E, Dreisigacker S, Payne T, He Z (2014) Characterization of Chinese wheat germplasm for resistance to Fusarium head blight at CIMMYT, Mexico. Euphytica 195:383-395.

He ZH, van Ginkel M, Gilchrist L, Rajaram S (2000) Progress of China/CIMMYT shuttle breeding and germplasm exchange aimed at combining high yield potential with scab resistance. Paper presented at the Proceedings of the National Fusarium Head Blight Forum, Cincinnati, USA.

Hilton, Jenkinson, Hollins, Parry (1999) Relationship between cultivar height and severity of Fusarium ear blight in wheat. Plant Pathology 48:202-208.

Holzapfel J, Voss HH, Miedaner T, Korzun V, Haberle J, Schweizer G, Mohler V, Zimmermann G, Hartl L (2008) Inheritance of resistance to Fusarium head blight in three European winter wheat populations. Theoretical and applied genetics 117:1119-1128.

Jansen C, von Wettstein D, Schafer W, Kogel KH, Felk A, Maier FJ (2005) Infection patterns in barley and wheat spikes inoculated with wild-type and trichodiene synthase gene disrupted *Fusarium graminearum*. Proc Natl Acad Sci U S A 102:16892-16897.

Khonga EB, Sutton JC (1988) Inoculum production and survival of *Gibberella zeae* in maize and wheat residues. Canadian Journal of Plant Pathology-Revue Canadienne De Phytopathologie 10:232-239.

Kubo K, Fujita M, Kawada N, Nakajima T, Nakamura K, Maejima H, Ushiyama T, Hatta K, Matsunaka H (2013) Minor differences in anther extrusion affect resistance to Fusarium head blight in wheat. J Phytopathol 161:308-314.

Kumar S, Stack RW, Friesen TL, Faris JD (2007) Identification of a novel Fusarium head blight resistance quantitative trait locus on chromosome 7A in tetraploid wheat. Phytopathology 97:592-597.

Leonard KJ, Bushnell WR (2003) Fusarium head blight of wheat and barley. APS Press, St. Paul, Minn.

Li HP, Wu AB, Zhao CS, Scholten O, Loffler H, Liao YC (2005) Development of a generic PCR detection of deoxynivalenol- and nivalenol-chemotypes of *Fusarium graminearum*. FEMS Microbiol Lett 243:505-511.

Liu SY, Hall MD, Griffey CA, McKendry AL (2009) Meta-analysis of QTL associated with Fusarium head blight resistance in wheat. Crop Sci 49:1955-1968.

Lu Q, Lillemo M, Skinnes H, He X, Shi J, Ji F, Dong Y, Bjornstad A (2013) Anther extrusion and plant height are associated with Type I resistance to Fusarium head blight in bread wheat line 'Shanghai-3/Catbird'. Theoretical and applied genetics 126:317-334.

Lu, Q, Szabo-Hever, A, Bjørnstad, Å, Lillemo, M, Semagn, K, Mesterhazy, A, Ji, F, Shi, J, Skinnes, H (2011) Two major resistance quantitative trait loci are required to counteract the increased susceptibility to Fusarium head blight of the dwarfing gene in wheat. Crop Sci 51:2430-2438.

Malihipour A, Gilbert J, Piercey-Normore M, Cloutier S (2012) Molecular phylogenetic analysis, trichothecene chemotype patterns, and variation in aggressiveness of Fusarium isolates causing head blight in wheat. Plant Dis 96:1016-1025.

McMullen M, Bergstrom G, De Wolf E, Dill-Macky R, Hershman D, Shaner G, Van Sanford D (2012) A unified effort to fight an enemy of wheat and barley: Fusarium head blight. Plant Dis 96:1712-1728.

Mesterhazy A, Bartok T, Mirocha CG, Komoroczy R (1999) Nature of wheat resistance to Fusarium head blight and the role of deoxynivalenol for breeding. Plant Breed 118:97-110.

Mesterhazy A, Bartok T, Kaszonyi G, Varga M, Toth B, Varga J (2005) Common resistance to different *Fusarium* spp. causing *Fusarium* head blight in wheat. Eur J Plant Pathol 112:267-281.

Miedaner T (1997) Breeding wheat and rye for resistance to Fusarium diseases. Plant Breed 116:201-220.

Miedaner T, Reinbrecht C, Lauber U, Schollenberger M, Geiger HH (2001) Effects of genotype and genotype-enviromment interaction on deoxynivalenol accumulation and resistance to Fusarium head blight in rye, triticale, and wheat. Plant Breed 120:97-105.

Miedaner T, Voss HH (2008) Effect of dwarfing genes on Fusarium head blight resistance in two sets of near-isogenic lines of wheat and check cultivars. Crop Sci 48:2115-2122.

Nicholson P, Simpson DR, Weston G, Rezanoor HN, Lees AK, Parry DW, Joyce D (1998) Detection and quantification of *Fusarium culmorum* and *Fusarium graminearum* in cereals using PCR assays. Physiol Mol Plant Pathol 53:17-37.

Nopsa JFH, Baenziger PS, Eskridge KM, Peiris KHS, Dowell FE, Harris SD, Wegulo SN (2012) Differential accumulation of deoxynivalenol in two winter wheat cultivars varying in FHB phenotype response under field conditions. Canadian Journal of Plant Pathology-Revue Canadienne De Phytopathologie 34:380-389.

Paul PA, Lipps PE, Madden LV (2005) Relationship between visual estimates of Fusarium head blight intensity and deoxynivalenol accumulation in harvested wheat grain: a meta-analysis. Phytopathology 95:1225-1236.

Paul PA, Lipps PE, Madden LV (2006) Meta-analysis of regression coefficients for the relationship between Fusarium head blight and deoxynivalenol content of wheat. Phytopathology 96:951-961.

Salas B, Steffenson B, Casper H, Tacke B, Prom L, Fetch Jr T, Schwarz P (1999) *Fusarium* species pathogenic to barley and their associated mycotoxins. Plant Dis 83:667-674.

Schuelke M (2000) An economic method for the fluorescent labeling of PCR fragments. Nat Biotechnol 18:233-234.

Schmolke M, Zimmermann G, Buerstmayr H, Schweizer G, Miedaner T, Korzun V, Ebmeyer E, Hartl L (2005) Molecular mapping of Fusarium head blight resistance in the winter wheat population Dream/Lynx. Theoretical and applied genetics 111:747-756.

Skinnes H, Semagn K, Tarkegne Y, Marøy AG, Bjørnstad Å (2010) The inheritance of anther extrusion in hexaploid wheat and its relationship to Fusarium head blight resistance and deoxynivalenol content. Plant Breed 129:149-155.

Somers DJ, Fedak G, Savard M (2003) Molecular mapping of novel genes controlling Fusarium head blight resistance and deoxynivalenol accumulation in spring wheat. Genome 46:555-564.

Srinivasachary, Gosman N, Steed A, Simmonds J, Leverington-Waite M, Wang Y, Snape J, Nicholson P (2008) Susceptibility to Fusarium head blight is associated with the *Rht-D1b* semi-dwarfing allele in wheat. Theoretical and applied genetics 116:1145-1153.

Srinivasachary, Gosman N, Steed A, Hollins TW, Bayles R, Jennings P, Nicholson P (2009) Semi-dwarfing *Rht-B1* and *Rht-D1* loci of wheat differ significantly in their influence on resistance to Fusarium head blight. Theoretical and applied genetics 118:695-702.

Stack RW, McMullen MP (1994) A visual scale to estimate severity of Fusarium head blight in wheat. In: North Dakota State University Extension Service. p 1095.

Steffenson B, Leonard K, Bushnell W (2003) Fusarium head blight of barley: impact, epidemics, management, and strategies for identifying and utilizing genetic resistance. In: Leonard K, Bushnell W (eds) Fusarium head blight of wheat and barley. pp 241-295.

Tamburic-Ilincic L, Muckle A, Schaafsma A (2013) Interaction between Quantitative Trait Loci (QTL) for Fusarium head blight (FHB) resistance and *Fusarium graminearum* 15-ADON and 3-ADON chemotypes in spring wheat. Proceedings of the 12th European Fusarium Seminar, Bordeaux, France, May 12-16

Van Eeuwijk FA, Mesterhazy A, Kling CI, Ruckenbauer P, Saur L, Burstmayr H, Lemmens M, Keizer LCP, Maurin N, Snijders CHA (1995) Assessing non-specificity of resistance in wheat to head blight caused by inoculation with European strains of *Fusarium culmorum, F. graminearum* and *F. nivale* using a multiplicative model for interaction. Theoretical and applied genetics 90:221-228.

Wilcoxson RD, Busch RH, Ozmon EA (1992) Fusarium head blight resistance in spring wheat cultivars. Plant Dis 76:658-661.

Xue S, Li GQ, Jia HY, Xu F, Lin F, Tang MZ, Wang Y, An X, Xu HB, Zhang LX, Kong ZX, Ma ZQ (2010) Fine mapping *Fhb4*, a major QTL conditioning resistance to Fusarium infection in bread wheat (*Triticum aestivum* L.). Theoretical and applied genetics 121:147-156.

Yan W, Li HB, Cai SB, Ma HX, Rebetzke GJ, Liu CJ (2011) Effects of plant height on type I and type II resistance to fusarium head blight in wheat. Plant Pathology 60:506-512.

Identification of New Sources of Resistance to Fungal Leaf and Head Blight Diseases of Wheat

Mohamed Osman

Chapter 4

Identification of New Sources of Resistance to Fungal Leaf and Head Blight Diseases of Wheat

Abstract

Tan spot (TS), Stagonospora nodorum blotch (SNB), spot blotch (SB), and Septoria tritici blotch (STB) caused by *Pyrenophora tritici-repentis*, *Parastagonospora nodorum*, *Cochliobolus sativus* and *Zymoseptoria tritici*, respectively, are the four important fungal leaf spotting diseases of wheat with global importance. These diseases reduce the photosynthetic area of leaves resulting in poor grain filling and lower yields; particularly when the penultimate and flag leaves are severely infected. Fusarium head blight (FHB) is another important fungal disease that infects wheat heads causing significant yield and quality deterioration including mycotoxins contamination. Host plant resistance is one of the main strategies in the management of these diseases. To identify new resistant sources to TS, SNB, STB, SB and FHB, 110 wheat cultivars and advanced breeding lines from different geographic origins were evaluated in growth chambers and field nurseries at the International Maize and Wheat Improvement Center (CIMMYT), Mexico. These materials have been previously evaluated for their field FHB resistance (combination of type I and II resistance) and performed well, but in the current study they were tested in greenhouse with point inoculation to confirm exclusively their type II resistance. The frequency of resistant lines was similar for TS, SNB and STB with 45, 40 and 33 genotypes, respectively. However, only 11 lines showed resistance to SB while 51 genotypes showed FHB type II resistance. Two genotypes of Chinese origin, NANJING 8611 and NANJING 4840, exhibited broad-spectrum resistance to all of the studied diseases and were not significantly different ($P \leq 0.01$) from the resistant check of the respective diseases. The multiple disease resistant accessions identified in this study could be utilized in breeding programs aimed at improving wheat resistance to either individual or multiple leaf and head blight diseases.

Key Words: Tan spot, Septoria tritici blotch, spot blotch, Stagonospora nodorum blotch, Fusarium head blight, host resistance

4.1. Introduction

Wheat (*Triticum aestivum* L.) is one of the most important crops that has been the major staple food of the societies for 1,000 years (Curtis et al. 2002). Leaf spotting diseases of wheat is a complex of diseases that produce similar symptoms on leaves, involving tan spot (TS) caused by *Pyrenophora tritici-repentis* (Died.) Drechs. [ana. *Drechslera tritici-repentis* (Died.) Shoem.], Stagonospora nodorum blotch (SNB) caused by *Parastagonospora* [syn. ana. *Stagonospora*; teleo. *Phaeosphaeria*] *nodorum* (Berk.) Quaedvlieg, Verkley & Crous (Quaedvlieg et al. 2013), spot blotch (SB) caused by *Cochliobolus sativus* (Ito & Kuribayashi) Drechsler ex Dastur [ana. *Bipolaris sorokiniana* (Sacc.) Shoemaker] and Septoria tritici blotch (STB) caused by *Zymoseptoria tritici* (Desm.) Quaedvlieg & Crous [syn. ana. *Septoria tritici* Rob. ex Desm.; teleo. *Mycosphaerella graminicola* (Fuckel) J. Schrot.] (Quaedvlieg et al. 2011), in addition to other diseases of less importance (Fernandez et al. 1999; Gilbert and Woods 2001; King et al. 1983; May et al. 2014). Fusarium head blight (FHB), another devastating disease of wheat and other small grains, is mainly caused by *Fusarium graminearum* species complex (FGSC) and has the potential to severely damage yield and quality including contamination with mycotoxins.

Leaf spotting is an important and devastating disease complex in major wheat growing regions and yield losses under favourable conditions can be higher than 50% (Duveiller and Sharma 2012; Goodwin 2012; Gurung et al. 2012; Singh et al. 2010), for which the major reasons could be the reduction in the number of tillers (Duveiller 2004) and in the photosynthetic area of the leaves (King et al. 1983; Zuckerman et al. 1997). Susceptible germplasm usually show poor grain filling, lower test weight (Hosford and Busch 1973), kernel shrivelling (McKendry et al. 1995), and lower number of kernels per head (Shabeer 1988), leading to serious quantity losses besides quality deterioration represented by grain shrivelling, red smudge, salmon-pink or red discoloration and black point (Fernandez et al. 2010; Hosford and Busch 1973; May et al. 2014; Singh et al. 2010, 2012a).

FHB disease often results in 30 to 40% yield loss, however under favorable conditions losses up to 70% have been observed for susceptible cultivars. When infected kernels are planted further losses can be caused resulting from poor germination, poor seedling vigor, and seedling blight (Parry et al. 1995). FHB infected seeds are commonly

referred to as tombstones due to their small, shriveled, and white or chalky appearance. FHB infected grains are primarily contaminated with two important mycotoxins: deoxynivalenol (DON) and zearalenone that have been shown to be harmful to humans and animals (Bai and Shaner 2004). FHB resistance is quantitative and at least five types of resistance have been recognized so far, i.e. resistance against initial infection (type I), resistance to fungal spreading in the infected head (type II), resistance to DON accumulation (type III), resistance to Fusarium damaged kernels (FDK) formation (type IV) and resistance to yield reduction (type V) (Buerstmayr et al. 2012).

All of these pathogens belong to ascomycetes and propagate both asexually and sexually. Conidia/pycniospores (asexual spores) are wind-borne and disseminate by rain splash allowing disease to move up the canopy. The ascospores (sexual spores) are discharged from pseudothecia which develop on infected crop stubble wherein the fungus overwinters (Acharya et al. 2011; Buerstmayr et al. 2012; Gilbert and Woods 2001; Goodwin 2012). The high pathogenic diversity within pathogen populations, due to factors such as mutation, recombination, gene flow or migration and selection (Eyal 1999; Friesen et al. 2006; McDonald and Linde 2002; Singh et al. 2010), give them the advantage of a rapid response to changing environmental factors including host resistance and fungicide treatments (Amand et al. 2003; Cowger and Mundt 2002; Eyal 1999).

Changes in agricultural practices including increased adoption of conservation agriculture, intensified wheat production, monoculture or short crop rotations, and commercial cultivation of susceptible cultivars have led to increased leaf and head blight diseases (Acharya et al. 2011; Duveiller and Sharma 2012; McDonald and Linde 2002; Singh et al. 2010). For FHB, increased maize-wheat rotations have further aggravated the situation. The rapid changes in pathogen virulence, resistance to fungicides and climate change have further promoted the prevalence and severity of these diseases (Strelkov and Lamari 2003). Accordingly, several disease management strategies have been proposed including cultural practices, fungicides and resistant cultivars (Bhathal et al. 2003; Duveiller et al. 2005; May et al. 2014). Cultivation of resistant cultivars is the most cost-effective, sustainable, and environmentally friendly approach for wheat production under reduced tillage and appropriate crop-rotation (Duveiller 2004; Eyal 1999; Singh et al. 2010). Availability of diverse germplasm with broad spectrum-resistance to multiple diseases is important to the success of wheat improvement programs (Polák et al. 2002;

Sharma et al. 2013; Singh et al. 2012b), for which the identification of new sources of resistance to multiple diseases is a prerequisite.

The availability of broad-spectrum resistant sources is of great importance due to the fact that CIMMYT wheat breeding lines target different destinations across the globe with different biotic stresses. Despite its obvious importance, only a few studies have been conducted to identify sources of resistance to multiple pathogens of wheat and the resistance genes currently available for resistance breeding are still limited (Ali et al. 2007; Lamari et al. 2005; Singh et al. 2006). The objective of the present study was to identify new sources of broad-spectrum resistance to TS, SNB, SB and STB in genotypes from different geographic origins. Most of these materials have been evaluated for their field resistance to FHB in previous studies (He et al. 2014; Osman et al. 2015); in the present study their type II FHB resistance was exclusively measured in greenhouse experiments to reinforce the previous studies. Together with the information for leaf spotting diseases, the resistant lines identified herein will contribute potentially in enhancing the genetic diversity and aid in developing wheat cultivars with durable resistance to these diseases.

4.2. Materials and Methods

4.2.1. Plant material

Wheat genotypes were selected from 4 geographic regions and compiled in 3 groups: Group1 included 44 advanced breeding lines selected based on multi-environmental FHB tests representing the 15th International FHB Screening Nursery of CIMMYT, Mexico (Osman et al. 2015). Group2 included 50 Chinese lines from CIMMYT gene bank that showed high field resistance to FHB as reported in He et al. (2014). Group3 included 9 Syrian and 8 Italian cultivars, of which 5 and 2 entries, respectively, were durum. All of the 110 genotypes were subjected to field testing for TS, STB and SB, except for Group 3 which was only tested for SB in field. Additionally, all of the lines were evaluated for FHB type II resistance, and for seedling resistance to SNB and TS in the growth chambers at CIMMYT, Mexico (see later for details).

4.2.2. Inoculum preparation

Table 4.1 summarizes information on the isolates used in this study.

4.2.2.1. Tan spot

Inoculum was produced on V8-PDA medium (Lamari and Bernier 1989). Mycelial plugs of 0.5 cm diameter from the stock cultures of *P. tritici-repentis* race 1 were transferred to 10 cm petri plates containing V8-PDA agar (150 ml V8-juice, 10 g PDA, 10 g agar, 3 g $CaCO_3$ and 850 ml distilled water). The cultures were incubated in the dark at 20-22°C for six days, then the mycelium was flattened with the base of a sterile test tube. Furthermore, the plates were incubated under continuous light at room temperature for 24h followed by 24h in the dark in an incubator at 16°C to induce conidiophore and conidia production, respectively. The conidia were suspended in the distilled water by adding about 40 ml of distilled sterile water per plate and gently brushing the plate surface with a camel-hair brush. In order to reduce surface tension, 5 drops of Tween 20 (polyxyethlene sorbitan monolaurate) were added per litre of spore suspension. Spore concentration was estimated with a haemocytometer and adjusted to 4,000 conidia ml^{-1} by adding distilled water.

4.2.2.2. Stagonospora nodorum blotch

The monosporic isolate SN4 of *P. nodorum*, previously recovered from wheat in Mexico, was used for inoculum production on V8-PDA. A mycelium plug stored in the freezer was placed upside-down and wiped in a zigzag way on the surface of each Petri dish containing V8-PDA agar. The plates were incubated at 21°C in 12/12 light-dark system for 8 days. Mature fungal cultures were flooded with sterile distilled water and scraped with a sterile glass slide to loosen and release oozing pycnidiospores. The resulting suspension was filtered through four layers of cheesecloth and the spore concentration was adjusted to 1 x 10^7 spores ml^{-1} using a haemocytometer. Before inoculation, two drops of Tween 20 per 100 ml was added into the inoculum suspension to reduce surface tension and facilitate uniform spore deposition onto leaves after inoculation.

Table 4.1 Fungal isolates used in this study, their origin, medium and inoculum concentration.

Pathogen	Isolate	Origin	Medium	Concentration (Conidia ml^{-1})
P. tritici-repentis	Ptr 1	Mexico	V8-PDA	4x10^3
P. nodorum	SN4	Mexico	V8-PDA	1x10^7
Z. tritici	A mixture of six virulent isolates	Mexico	Yeast malt agar medium	1x10^7
B. sorokiniana	A mixture of four virulent local isolates	Mexico	Sorghum seeds	Not applicable
F. graminearum	A mixture of four highest ranked isolates	Mexico	mung bean agar	70x10^3

4 .2.2.3. Septoria tritici blotch

A mixture of six virulent isolates of *Z. tritici*, collected from naturally infected wheat fields in Mexico, was used to prepare the spore inoculum, which was produced on a medium composed of 4 g of yeast extract, 4 g of malt extract, 18 g agar and 1000 ml of double distilled sterile water. The plates were streaked with 500 µl of fresh pycnidiospore suspension of individual *Z. tritici* isolates, and were then placed on laboratory benches at room temperature for 3 days to produce pycnidiospores. Approx. 25 ml of sterile distilled water was added to each plate and pycnidiospores were collected using a looped wire needle. Spore suspension from the six isolates was mixed and adjusted to 1 x 10^7 spores ml^{-1} using a haemocytometer.

4 .2.2.4. Spot blotch

A mixture of four virulent local isolates of C. sativus that were previously identified and stored at -20ºC was used to produce fresh inoculum. The mycelium plugs (one plug/plate) were plated on V8-PDA for 7 days, with 12/12h photoperiod cycle at 22-25 ºC for fresh culture. Subsequently, the fungal culture was used to inoculate soaked and autoclaved sorghum seeds (a petri dish of fungus per jar of sorghum seed). The jars containing C. sativus inoculated sorghum grains were incubated for approximately six weeks at room temperature and were regularly manually shaken to promote good coverage of the fungus.

4.2.2.5. Fusarium head blight

To evaluate type II FHB resistance of the wheat genotypes, four highest ranked isolates of F. graminearum collected in 2013, selected based on in vitro DON productivity and in planta aggressiveness, were used. These isolates were mixed with a control strain with known aggressiveness to generate the inoculum as described in Osman et al. (2015). Inoculum was produced on mung bean agar medium and adjusted to 70 x 103 macroconidia ml-1 for greenhouse inoculation.

4.3. Seedling evaluation for TS and SNB in the greenhouse

All seedling tests for TS and SNB were conducted under greenhouse conditions at 22°C day and 18°C night temperatures with a 16-hour photoperiod. Each experiment was conducted as a randomized complete block design with four replicates for TS and two replicates for SNB. Each replicate consisted of 110 genotypes with 4 seedlings planted in plastic trays, i.e. each experiment consisted of a total of 16 seedlings per genotype for TS and 8 for SNB. Each experiment was repeated twice. The wheat genotypes Erik and Glenlea were included in each experiment as resistant and susceptible checks respectively to verify the inoculation process. Inoculations were done when the second leaf was fully expanded (14 days after seeding) by spraying the spore suspensions onto the leaves until runoff (about 0.5 ml inoculum per plant) using a hand sprayer. When the plants were almost dry, the trays were moved into a humid chamber for 24 h at 20ºC to facilitate infection, where moist conditions (RH 100%) were maintained by continuous operation of automatic misters. The plants were then transferred back to the greenhouse bench. Disease evaluation for SNB and TS was performed at seven days post-inoculation (DPI) using a 1-5 scale (Feng et al. 2004; Lamari and Bernier 1989).

Disease reactions of 1 and 2 were considered to be resistant because necrosis and chlorosis were minimal on host leaves and lesion expansion is not observed. Disease reaction of 3 was considered an intermediate reaction as lesion expansion is observed. Whereas genotypes having scores 4 and 5 were considered to be susceptible as lesions had expanded and coalesced, and necrosis and chlorosis were abundant.

4.4. Evaluation of FHB type II resistance

Two experiments were performed respectively in spring and winter of 2014. All genotypes were planted in pots in a completely random design without replicates, and individual pots were considered as the experimental units. Seven seeds were initially sown per pot and were thinned to 5 plants at the three leaves stage, under greenhouse conditions of 22°C day and 18°C night temperatures with a 16-hour photoperiod. Inoculations were carried out by delivering 10 µl of spore suspension (about 700 macroconidia) into a central floret at anthesis that was marked in advance to be properly recognized. After injecting the inoculum, each head was labelled with a tag with the date of inoculation and covered by a polyethylene bag for 48 h to maintain humidity. On average 10 spikes were inoculated for each pot. Notes on the numbers of total and infected spikelets were taken at 21 dpi and disease severity was calculated as the percentage of symptomatic spikelets per inoculated head. Mean values of FHB severity for the inoculated plants in each pot were used for statistical analysis (Stack and McMullen 1994), where the two experiments were considered as two replicates. Genotypes with disease severity less than 10% were considered resistant, 10-30% moderately resistant, 30-60% moderately susceptible and more than 60% susceptible.

4.5. Adult plant testing for foliar diseases in the field

4.5.1. Tan spot

Field experiments were conducted in the CIMMYT TS nursery at El Batán (altitude of 2,240 masl, latitude 19°N, with an average annual precipitation of 625 mm), State of Mexico, Mexico. The wheat genotypes were sown and evaluated for TS reaction in the summer season (May to September) of 2014. The experiment was done in 1 m double rows with two replications. Fresh inoculum of conidial suspension at a concentration of 4,000 conidia ml^{-1} was spray inoculated at the tillering stage GS 30 (Zadoks et al. 1974) and repeated after 7 days. Erik and Glenlea were included as resistant and susceptible checks, respectively.

4.5.2. Septoria tritici blotch

The screening nursery was located at Toluca, which is a cool and humid location with an average annual rainfall of 800 mm at an altitude of 2,640 masl, latitude 19°N, in the State of Mexico, Mexico. Sowing was done in May, 2013 in 0.75 m double row plots with two replications. The spray inoculation started from Zadoks GS 30 (stem elongation) and was repeated two more times at 7-day intervals, with a spore suspension of 1 x 10^7 pycnidiospores ml^{-1}. The two checks, Murga and Huirivis, were included in the experiment for STB resistance and susceptibility, respectively.

4.5.3. Spot blotch

The screening nursery was located at the Agua Fria experimental station, State of Puebla, Mexico, at an altitude of 100 masl, latitude 20.5°N, with an average annual precipitation of 1,200mm. The 110 entries were sown in the winter cycle (November to March) of 2013, in 1 m double row plots with two replications. Two susceptible lines (CIANO T 79 and Sonalika) and two resistant lines (Chirya 3 and FRANCOLIN #1) were included as checks in the experiment. For field inoculation, about 25 g of the *B. sorokiniana* colonized sorghum grains were distributed at the base of plants, in the middle of each of the double row at the tillering stage GS 29 (Zadoks et al. 1974).

4.6. Field phenotyping for foliar diseases

A double-digit scoring system (00–99) adopted from Saari and Prescott's severity scale for assessing wheat foliar diseases (Saari and Prescott 1975) was used to visually evaluate disease severity on whole plots. Disease scoring began at the flag leaf sheath opening stage GS 47 (Zadoks et al. 1974). The evaluation was repeated 4 times at 7-day intervals in SB nursery but 5 times for TS and STB at the same interval. The first digit (D1) refers to height of disease movement in canopy from field surface, while the second digit (D2) refers to disease severity estimated by the extent of leaf blotches. Both D1 and D2 were scored on a scale of 1 to 9. We used the following formula to estimate disease severity for each reading:

severity% = (D1/9) x (D2/9) x 100. The area under disease progress curve (AUDPC) was calculated from the four or five disease evaluations, using the following formula:

$$\mathrm{AUDPC} = \sum_{i=1}^{n} [\{(Y_i + Y_{(i+1)})/2\} \times (t_{(i+1)} - t_i)$$

where Yi = disease severity at time ti, t(i+1) - ti = time interval (days) between two disease scores, n = number of observations.

4.7. Data analysis

The R program ver. 3.2.0 (R Core Team, 2015) was used to analyse the phenotypic data. Analysis of variance (ANOVA) was performed separately for each disease. Kendall's rank correlation coefficients, among diseases and between each disease and phenological traits like plant height (PH) and days to heading (DH), were calculated to investigate any possible association.

For field experiments, mean disease score of each genotype and the LSD value ($P \leq 0.01$) with respective to each disease were calculated to identify the significance of differences among entries and checks. Entries with disease values not significantly different from the resistance check were classified as resistant, whereas those not significantly different from the susceptible check were regarded as susceptible, the rest as intermediate or moderately resistant/susceptible.

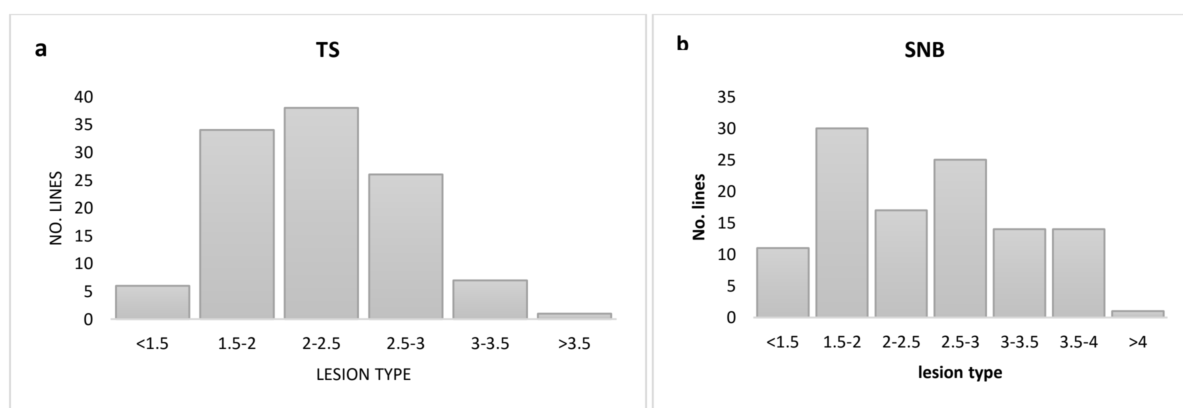
4.8. Results

4.8.1. Greenhouse screening for TS and SNB resistance

Wheat genotypes differed significantly ($P<0.001$) in their reactions to TS and SNB. For TS, 39 genotypes (35%) had a reaction value less or equal to 2 and were classified as resistant, whereas 64 and 7 genotypes showed moderately susceptible and susceptible reactions with reaction values more than 2 and more than 3, respectively (Table 4.2, Fig. 4.1a). Likewise, for SNB, a relatively high proportion of the tested entries (40 genotypes, of which 6 tetraploids) had moderate to high levels of resistance to SNB, while 43 and 27

genotypes were classified as moderately susceptible and susceptible, respectively (Table 4.2, Fig. 4.1b). Eighteen of the tested wheat genotypes exhibited resistance to both SNB and TS indicating their multiple disease resistance.

Fig. 4.1 Frequency distribution of lesion types for: a) tan spot (TS) and b) Stagonospora nodorum blotch (SNB) in greenhouse experiments. Wheat genotypes were classified for their reaction to TS at the two-leaf stage using a linear scale of 1 (resistant, small dark brown spots) to 5 (susceptible, either tan necrosis or chlorosis).

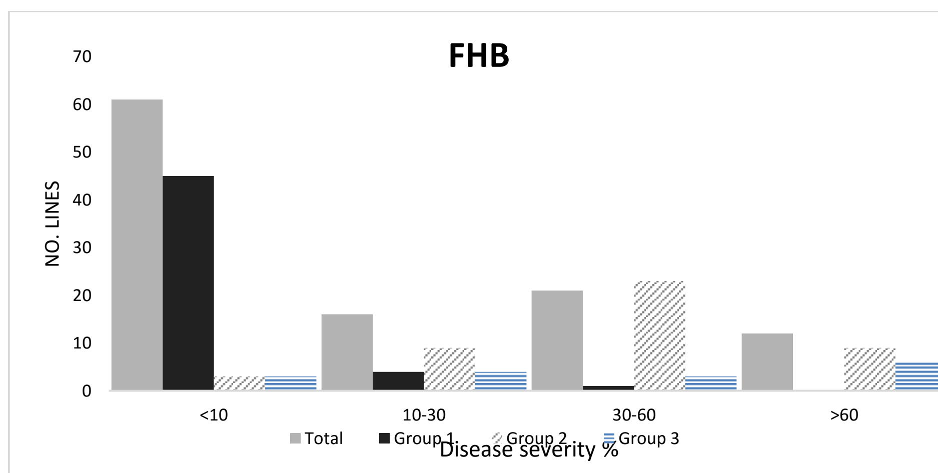


4.8.2. FHB type II resistance

Analysis of variance revealed significant differences ($P<0.001$) in disease severity among genotypes. Phenotypic data are presented in Table 2. FHB severity ranged from 2% in the Chinese line ER63403 to 90% in the Syrian cultivar Sham 7. Most of the Chinese lines showed high level of type II resistance having a grand mean 7.30 % and all but 5 lines were considered as resistant. Disease severity of the 15th FHBSN set was highly variable and ranged from 5.83% in the entry OASIS/SKAUZ//4*BCN*2/3/PASTOR/4/HEILO (CIMMYT germplasm bank identifier, GID, 6342263) to 86.51% in TAM200/PASTOR//TOBA97/3/HEILO (GID 6342353). The grand mean of disease severity in this set was 42.30% indicating rather low type II resistance compared to that of the Chinese set (Fig. 2), and only 3 lines were classified as resistant. Additionally, 4 of the old Italian cultivars, Verna, Frassinetto, Inalettabile and Gentil Rosso, showed type II resistance having disease severities <11%, and 4 of the Syrian cultivars were moderately

resistant/susceptible of which a durum wheat cultivar (Buhous11) had a disease severity of 24%.

Fig. 4.2 Frequency distribution of mean disease severity for FHB type II resistance at 21 days after point inoculation in the greenhouse. Group 1 included 44 CIMMYT advanced breeding lines, Group 2 included 50 Chinese lines, and Group 3 included 9 Syrian and 8 Italian cultivars.



4.8.3. Field screening for TS, STB and SB

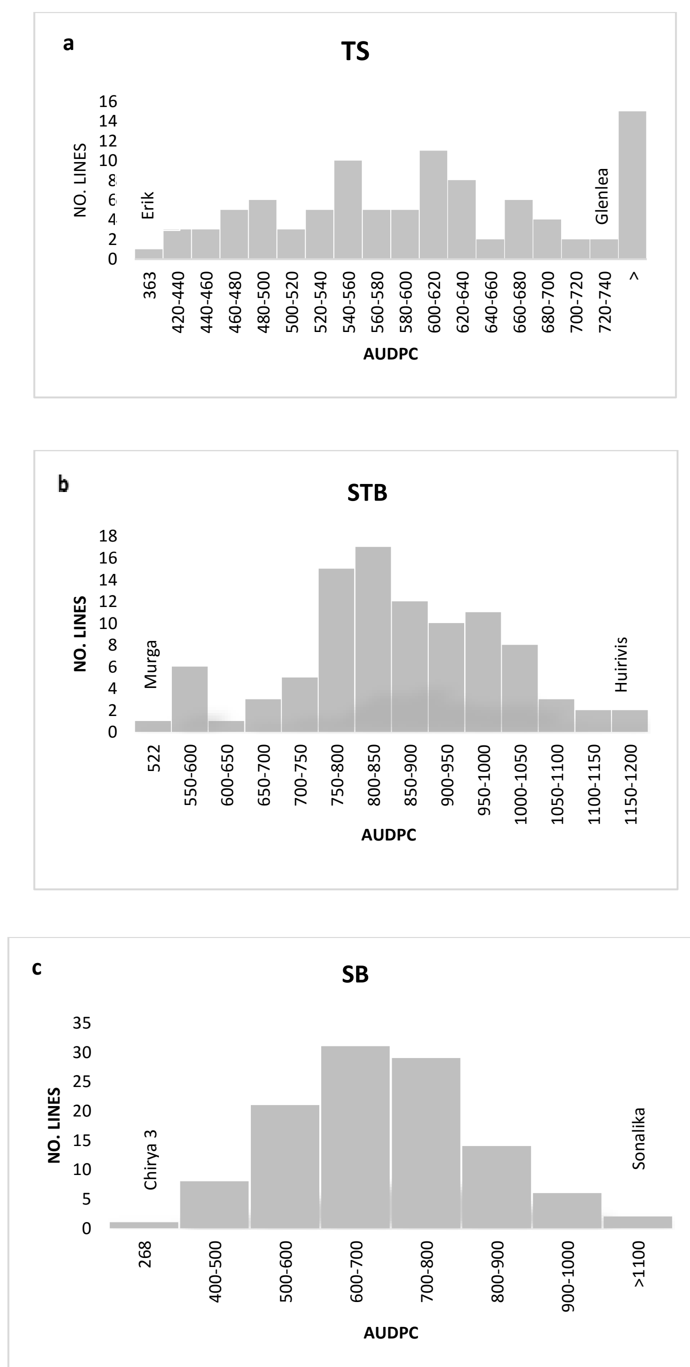
In all experiments significant differences (*P*<0.001) were observed for the three diseases as revealed by ANOVA (Table 4.3). The AUDPC values for TS ranged from 363 for the resistant check Erik to 1,187 in the Chinese line QIN MEI 6 which was significantly more susceptible than the susceptible check Glenlea (Table 4.2), indicating relatively high disease pressure in the nursery. Forty-five genotypes (40%) showed a resistant reaction to TS in the field, but 16 genotypes (14%) had AUDPC values even higher than the susceptible check Glenlea (Fig. 3a).

In the STB nursery, 33 genotypes exhibited a resistant reaction and 25 genotypes showed an intermediate reaction, whereas the susceptible check Huirivis had the highest AUDPC value, from which 36 of the evaluated lines were not significantly different and were classified as susceptible (Table 4.2, Fig. 4.3b).

There was high disease pressure in the SB nursery where only 11 genotypes were identified as resistant, although their AUDPC values were all higher than the resistant check Chirya3 which exhibited the highest level of resistance under field conditions of Agua Fria.

However, only 6 genotypes were similar to Sonalika which had an AUDPC value of 1175 (Table 4.2, Fig. 4.3c). All the remaining lines were of the intermediate class, having AUDPC values significantly lower than the susceptible check Sonalika.

Fig. 4.3 Frequency distribution of field disease severity data (area under disease progress curve, AUDPC) for: a) tan spot (TS), b) Septoria tritici blotch (STB), and c) spot blotch (SB). Disease scores of resistant and susceptible checks for each disease are indicated.



4.8.4. Identification of genotypes with broad-spectrum resistance

Entries with resistance to two or more diseases have been identified in all of the three groups of genotypes. Two Chinese genotypes, NANJING 8611 and NANJING 4840, exhibited broad-spectrum resistance to all of the 5 evaluated diseases. Additional 8 genotypes showed resistance to 4 of the pathogens and 22 genotypes had resistance to 3 of the studied diseases (Table 4.2). However, 13 (12%) of the studied lines have not shown resistance to any of the 5 diseases.

Table 4.2 CIMMYT germplasm bank identifier (GID), entry, origin and disease reaction of wheat genotypes to Spot blotch (SB), Septoria tritici blotch (STB), Tan spot (TS), Stagonospora nodorum blotch (SNB) and Fusarium head blight (FHB).

GID	Entry	Origin	AUDPC-mean			Greenhouse			Resistant to # of diseases
			SB	STB	TS	TS	SNB	FHB	
8297	NANJING 8611	China	477.5	779.9	424.3	1.3	2.0	3.7	5
345449	NANJING 4840	China	490.4	792.9	492.2	1.8	2.0	3.7	5
8285	NANJING 8343	China	522.8	970.1	426.5	1.4	1.6	4.4	4
11006	WUHAN #2	China	447.2	639.5	512.9	1.8	2.7	5.7	4
4751861	SHANGHAI	China	779.9	723.8	433.9	1.4	1.9	5.7	4
9774	SHANGHAI #8	China	505.6	570.4	461.6	1.8	2.3	5.9	4
897779	HXL8144	China	570.4	799.4	534.9	2.0	1.6	9.9	4
6342263	OASIS/SKAUZ//4*BCN*2/3/PASTOR/4/ HEILO	15th FHBSN	840.4	723.8	544.6	2.4	1.8	5.8	4
101140	NING 8331	China	535.8	961.4	464.2	1.8	1.7	2.8	3
78693	SW89.4974	China	615.7	868.5	686.5	1.7	1.9	2.9	3
100328	CHUANYU 10	China	758.3	922.5	489.7	1.8	1.3	3.7	3
78683	SW89.2814	China	553.1	1043.5	469.6	1.9	1.4	4.3	3
357802	LU 95	China	827.5	725.9	599.1	2.6	2.4	5.0	3
266774	NANJING 8176	China	758.3	1017.6	670.4	1.6	1.9	5.6	3
67238	ZHENGJIANG8709	China	546.6	656.8	487.9	2.0	3.3	5.9	3
58675	HAAS3621-2	China	743.2	760.5	568.8	2.5	2.9	6.3	3
67005	HXL7493	China	637.3	598.5	557.6	2.0	3.1	6.4	3
150196	SHAOXING CANHUAMIMAI	China	857.7	1021.9	603.4	1.9	1.4	7.0	3
2409260	NING MAI 50	China	475.3	836.1	470.6	1.8	2.7	7.0	3
80152	SW89-3052	China	585.5	885.8	522.5	1.2	1.6	7.4	3
897822	HAAS8193	China	613.6	987.3	577.0	2.3	1.8	9.1	3
67506	CHUANZHI4331	China	743.2	717.3	482.5	1.8	2.4	9.3	3

6176474	KACHU #1/4/CROC_1/AE.SQUARROSA (205)//KAUZ/3/SASIA/5/KACHU	15th FHBSN	509.9	777.8	448.6	2.0	2.6	21.9	3
5999807	VORB/4/D67.2/PARANA 66.270//AE.SQUARROSA (320)/3/CUNNINGHAM	15th FHBSN	594.1	598.5	617.2	1.8	1.6	35.4	3
5999852	YAR/AE.SQUARROSA (518)/3/PRL/SARA//TSI/VEE#5/4/ATTILA/5/BERKUT	15th FHBSN	650.3	760.5	587.2	2.7	2.0	38.9	3
6000673	KABY/BAV92/3/CROC_1/AE.SQUARROSA (224)//OPATA/4/WHEAR/5/ATTILA/BAV92//PASTOR	15th FHBSN	801.5	589.8	548.4	2.3	1.9	51.7	3
	Inalettabile	Italy	570.4	NA	NA	1.9	1.5	8.6	3
67259	ER63403	China	823.1	1060.8	585.7	2.4	2.4	2.0	2
67278	NING8745	China	764.8	862.0	556.8	1.6	2.3	2.5	2
200830	CP881	China	572.5	849.1	521.1	1.6	2.4	2.5	2
200825	8429.1.1.3	China	656.8	842.6	524.4	1.8	2.9	3.7	2
289485	FUJING 538	China	922.5	812.3	618.5	2.6	2.4	4.1	2
222672	DGB BV84.1406/JIANGSU	China	684.9	913.9	661.7	1.7	2.3	4.3	2
1752956	JIAN85.11//SUZHOU7906/NING8249	China	838.3	900.9	479.3	2.0	2.2	4.7	2
67107	HXL41547	China	795.1	872.8	608.1	2.3	2.0	4.9	2
897701	GANG85-454	China	693.5	1026.2	558.9	2.5	3.6	5.3	2
1370605	NING9131 (X)	China	773.5	821.0	502.8	1.7	2.2	5.5	2
150232	QIN MEI 6	China	613.6	929.0	1187.4	2.2	1.7	5.6	2
1753002	ZUO1330	China	678.4	872.8	538.1	2.0	2.2	5.7	2
66920	HAAS8676	China	844.8	868.5	694.1	2.1	2.0	6.0	2
4701012	SUM3//CS*2/LE.RA/3/YANGMAI 158	China	643.8	855.6	457.5	1.8	2.2	6.7	2
67289	NING89.6812	China	609.3	844.8	576.2	2.7	2.4	7.2	2
102210	LONG MAI16	China	624.4	1006.8	612.0	2.3	1.6	7.8	2
407368	YANG MAI 6	China	691.4	840.4	567.8	1.8	2.7	7.8	2
91861	W226.16	China	697.8	1149.4	459.8	1.3	2.7	9.3	2
80132	SW87-2323	China	429.9	840.4	501.9	2.0	2.8	14.6	2
1370597	TAIGU DERIVATIVE	China	648.1	974.4	553.7	1.9	2.0	18.7	2
78720	80.25	China	715.1	823.1	481.1	1.8	1.8	40.4	2
6340765	PBW343/WBLL1//PANDION/3/HEILO/4/PAURAQ	15th FHBSN	965.7	663.3	685.5	2.7	3.6	7.3	2
6343743	WBLL1*2/KUKUNA//HEILO	15th FHBSN	589.8	803.7	667.1	2.1	2.7	9.1	2
6340565	CHIBIA//PRLII/CM65531/3/SKAUZ/BAV92*2/4/GONDO/CBRD	15th FHBSN	689.2	758.3	564.3	2.3	2.8	13.0	2
6340966	FRNCLN/HEILO//FRNCLN	15th FHBSN	769.1	786.4	601.2	2.4	1.4	19.4	2
6340862	WAXWING*2/TUKURU*2//HEILO	15th FHBSN	775.6	993.8	549.4	2.5	1.6	36.2	2
6000632	CNO79//PF70354/MUS/3/PASTOR/4/BAV92/5/ATTILA*2/PBW65/6/PBW343*2/TUKURU	15th FHBSN	929.0	801.5	630.4	2.3	2.0	41.6	2
6342266	OASIS/SKAUZ//4*BCN*2/3/PASTOR/4/HEILO	15th FHBSN	574.7	771.3	580.7	2.5	3.6	46.5	2
6342246	KAUZ*2/MNV//KAUZ/3/MILAN/4/BAV92/5/HEILO	15th FHBSN	555.2	730.2	489.9	2.0	3.3	47.5	2

6001180	SOKOLL//FRTL/2*PIFED	15th FHBSN	758.3	678.4	996.5	2.1	1.6	50.8	2
6340362	ATTILA/3*BCN*2//BAV92/3/HEILO/4/CHIBIA//PRLII/CM65531/3/SKAUZ/BAV92	15th FHBSN	1065.1	961.4	553.8	1.8	1.4	53.4	2
	Verna	Italy	538.0	NA	NA	1.7	3.0	5.7	2
	Frassineto	Italy	475.3	NA	NA	2.0	2.3	6.6	2
	Svevo [a]	Italy	496.9	NA	NA	2.3	1.4	41.6	2
79990	NINGXIA 88R3438	China	857.7	926.9	609.6	2.1	3.4	3.2	1
64808	FUJING 5114	China	609.3	857.7	662.8	2.4	3.9	4.6	1
103143	SHAN 32109	China	646.0	1041.4	622.8	2.7	3.6	5.0	1
8304	NANJING 8647	China	643.8	1080.2	708.1	2.2	2.8	5.7	1
10079	SUZHOE #9	China	885.8	1056.5	912.2	2.5	3.1	6.6	1
67084	HXL30646	China	745.4	974.4	654.1	2.1	3.3	8.2	1
10074	SUZHOE #3	China	717.3	561.7	838.8	2.3	4.0	15.2	1
6001364	KABY/BAV92/3/CROC_1/AE.SQUARROSA (224)//OPATA/4/PASTOR/FLORKWA-1//BAV92	15th FHBSN	829.6	866.4	554.4	1.7	2.8	13.5	1
6000734	ATTILA/BAV92//PASTOR/3/ATTILA*2/PBW65/4/ATTILA/PASTOR	15th FHBSN	756.2	846.9	614.0	2.4	1.4	18.1	1
6000939	SW89-5124*2/FASAN/3/ALTAR 84/AE.SQ//2*OPATA/4/ARREHANE	15th FHBSN	775.6	767.0	918.0	2.8	3.7	19.4	1
6340672	KAUZ*2/MNV//KAUZ/3/MILAN/4/BAV92/5/HEILO/6/CHIBIA//PRLII/CM65531/3/SKAUZ/BAV92	15th FHBSN	812.3	957.1	675.7	2.6	3.4	21.5	1
6340649	FRET2/KUKUNA//FRET2/3/HEILO/4/BLOUK #1	15th FHBSN	637.3	1028.4	631.5	2.5	3.8	23.7	1
6001555	GOUBARA-1/2*SOKOLL	15th FHBSN	637.3	829.6	834.5	2.1	1.8	26.2	1
6340845	MUNAL//SHA3/CBRD/3/PAURAQ	15th FHBSN	855.6	831.8	772.9	2.9	2.9	32.7	1
6000931	SOKOLL//PBW343*2/KUKUNA/3/ATTILA/PASTOR	15th FHBSN	563.9	922.5	1008.3	2.1	1.6	33.0	1
6342336	TAM200/PASTOR//TOBA97/3/HEILO	15th FHBSN	905.2	836.1	550.3	2.9	2.9	43.9	1
6340858	WAXWING*2/TUKURU*2//HEILO	15th FHBSN	684.9	812.3	633.6	2.3	2.7	44.1	1
6000970	SOKOLL*2/ROLF07	15th FHBSN	710.8	754.0	790.2	2.9	3.6	46.3	1
6000696	ATTILA/PASTOR/3/ATTILA/BAV92//PASTOR/4/PBW343*2/TUKURU	15th FHBSN	667.6	808.0	740.5	2.5	2.6	48.2	1
6001093	SOKOLL/ROLF07	15th FHBSN	792.9	764.8	612.9	2.9	2.7	48.5	1
6342075	ATTILA/3*BCN*2//BAV92/3/HEILO	15th FHBSN	592.0	777.8	725.2	2.8	2.9	52.8	1
6343369	WBLL1*2/BRAMBLING*2//GONDO/TNMU	15th FHBSN	933.3	775.6	698.2	2.9	2.8	62.2	1
6342187	FRET2/KUKUNA//FRET2/3/HEILO	15th FHBSN	738.9	581.2	624.8	2.3	3.6	63.0	1
6000906	SOKOLL*2/TROST	15th FHBSN	641.7	892.3	839.8	3.0	1.9	64.0	1
6343618	BABAX/LR42//BABAX*2/3/PAVON 7S3,+LR47/4/HEILO	15th FHBSN	792.9	762.7	636.7	3.0	3.4	65.2	1
6342460	WBLL1*2/KIRITATI//HEILO	15th FHBSN	624.4	939.8	596.5	2.4	2.7	77.7	1
	G.rosso	Italy	604.9	NA	NA	2.1	3.7	10.3	1
	Buhous11 [a]	Syria	544.4	NA	NA	3.1	1.4	23.7	1
	Sieve	Italy	553.1	NA	NA	2.3	1.6	26.2	1
	Douma3 [a]	Syria	622.2	NA	NA	2.9	1.4	60.5	1

	Andriolo	Italy	490.4	NA	NA	2.9	3.3	62.3	1
	Sham5 [a]	Syria	715.1	NA	NA	3.5	1.7	67.4	1
	Sham9 [a]	Syria	717.3	NA	NA	2.6	1.8	72.9	1
	Sham7 [a]	Syria	529.3	NA	NA	3.3	2.0	89.7	1
401277	KUNG CHIAO	China	728.1	993.8	822.6	2.9	3.1	25.7	0
6343684	THELIN/2*WBLL1//HEILO	15th FHBSN	654.6	993.8	748.1	3.2	2.8	34.8	0
6342108	BABAX/LR42//BABAX*2/4/SNI/TRAP#1 /3/KAUZ*2/TRAP//KAUZ/5/HEILO	15th FHBSN	777.8	907.4	619.6	2.2	3.5	45.5	0
6340708	KAUZ*2/MNV//KAUZ/3/MILAN/4/BAV9 2/5/HEILO/6/CHIBIA//PRLII/CM65531/ 3/SKAUZ/BAV92	15th FHBSN	738.9	1006.8	646.6	2.8	3.1	47.5	0
6000034	QG 4.37A/4/MILAN/KAUZ//PRINIA/3/BAV9 2/5/MILAN/KAUZ//PRINIA/3/BAV92	15th FHBSN	790.7	920.4	628.3	2.6	3.8	49.8	0
6340604	CHIBIA//PRLII/CM65531/3/SKAUZ/BAV 92/4/HEILO/5/FRET2/KUKUNA//FRET2	15th FHBSN	946.3	1162.3	818.3	3.3	3.9	52.2	0
5999927	PROINTA SUPERIOR/4/RL6043/4*NAC//PASTOR/ 3/BAV92/5/KLEIN SAGITARIO	15th FHBSN	682.7	957.1	703.8	3.2	2.3	54.3	0
6343651	PFAU/WEAVER*2//TRANSFER#12,P88.2 72.2/3/HEILO	15th FHBSN	669.8	894.4	680.0	2.3	3.2	66.9	0
6342383	TOBA97/PASTOR//HEILO	15th FHBSN	760.5	896.6	635.4	2.9	2.3	68.7	0
6340803	PFAU/WEAVER*2//BRAMBLING/3/HEIL O/4/WAXWING*2/TUKURU	15th FHBSN	877.2	935.5	616.3	2.8	3.7	72.4	0
6342353	TAM200/PASTOR//TOBA97/3/HEILO	15th FHBSN	630.9	1127.8	909.0	2.3	2.2	86.5	0
	Douma2	Syria	734.6	NA	NA	2.6	3.2	25.7	0
	Sham6	Syria	760.5	NA	NA	2.5	2.6	32.7	0
	Douma4	Syria	879.3	NA	NA	3.0	2.9	54.0	0
	S.capelli [a]	Italy	561.7	NA	NA	3.1	2.6	80.9	0
	Resistant check[b]		268.0	522.0	363.0	1.3	1.1	5.63	
	Susceptible check[c]		1175.0	1200.0	737.0	3.9	4.2	72.4	
	LSD[d]		257.24**[e]	297.57**	236.57**				

a Durum entries

b Resistant checks: Chirya 3, Murga, Erik and Sumai 3 for Spot blotch (SB), Septoria tritici blotch (STB), Tan spot (TS) and Fusarium head blight (FHB) respectively.

c Susceptible checks: Sonalika, Huirivis, Glenlea and PFAU/WEAVER*2//BRAMBLING/3/HEILO/4/WAXWING*2/TUKURU for Spot blotch (SB), Septoria tritici blotch (STB), Tan spot (TS) and Fusarium head blight (FHB) respectively.

d Least significant difference, $p \leq 0.01$.

e Significance codes: ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05

4.8.5. Correlations among measured traits

A significant correlation (r = 0.60, P<0.001) was found between field and greenhouse TS experiments. Likewise, a moderate correlation coefficient of 0.44 (P<0.001) was observed between TS and SNB greenhouse results. Moreover, field TS results exhibited significant association with FHB (r= 0.41, P<0.001) and was the only to have significant correlation with SB (r= 0.27, P<0.01). As for DH and PH, only the former showed significant negative correlations with TS (r=-0.60, P<0.001) and SB (r=-0.36, P<0.001) but not with STB. For PH, however, no significant correlation was found between this trait and any of the diseases in this study.

Table 4.3 Analysis of variance for greenhouse Fusarium head blight (FHB) and field AUDPC values for tan spot (TS), Septoria tritici blotch (STB) and spot blotch (SB), and their heritability estimates.

Trait	Source	DF	MS	*F* value	*Pr*(>F)	Heritability	
FHB	Genotype	111	1212.5	5.476	<0.001		Greenhouse
	Rep	1	522.2	2.359	0.127	0.82	
	Error	111	221.4				
TS	Genotype	95	39539	4.758	<0.001		Field
	Rep	1	24	0.003	0.958	0.79	
	Error	95	8311				
STB	Genotype	95	39276	3.123	<0.001		
	Rep	1	57350	4.560	<0.05	0.68	
	Error	95	12576				
SB	Genotype	111	40173	4.246	<0.001		
	Rep	1	385	0.041	0.84	0.76	
	Error	111	9462				

Note: FHB greenhouse and SB nursery included the complete set of the 3 groups of genotypes and the two respective checks, whereas only Group1 and Group2 were tested in the TS and STB nurseries with the respective checks for each disease.

4.9. Discussion

Wheat is often infected simultaneously by multiple foliar diseases; but the components of the foliar disease complex differ from region to region despite the similar symptoms. For example, TS and SB co-present in South Asia and it is often very difficult to diagnose the pathogen based only on visual symptoms (Duveiller et al. 2005), while STB, TS and SNB co-exist in the United States and all of the four diseases, along with FHB, are common in Canadian prairies (Fernandez et al. 2014; Fernandez et al. 1999; Gilbert and Woods 2001; May et al. 2014). Accordingly, strategies based on multiple disease resistance will reduce risks related to the co-existence of different pathogens in certain environments. Moreover, it may facilitate further breeding efforts on combining resistance to blight diseases with resistance to other diseases (Gurung et al. 2012).

Despite the importance of breeding for host resistance, only a few reports aimed at identifying novel sources of resistance to multiple wheat blighting pathogens (Ali et al. 2007; Friesen et al. 2008; Gurung et al. 2009, 2012; Singh et al. 2006). Moreover, the genetic basis of foliar spotting diseases resistance is narrow, highlighting the need to identify novel resistance genes (Lamari et al. 2005; Singh et al. 2011). There is a great need to search for additional resistance genes and to incorporate them into commercial wheat cultivars, and this can be achieved only by continuous testing of lines from different origins and genetic make-up. Additionally, majority of the resistant materials reported in previous studies (Ali et al. 2007; Gurung et al. 2009, 2012) was identified in greenhouse studies with inoculations at seedling stage. Although greenhouse experiments have many advantages and are cost-eeffective, they cannot replace field evaluations entirely (Singh et al. 2012a). Accordingly, resistant genotypes from greenhouse studies will need to be re-evaluated in field experiments for confirmation. In this study, we evaluated 110 wheat accessions of diverse origins for their resistance against multiple leaf spotting diseases under both greenhouse and field conditions. Results from independent testing of diseases revealed that possibly novel resistance sources with good levels of resistance compared to resistant checks were identified for TS, SNB, SB and STB. The frequency of resistant lines was very similar in TS, STB and SNB with 50, 49 and 39 genotypes respectively, whereas the frequency in SB was much lower with only a few resistant genotypes. The possible reason for the difference could be the low or no selection pressure for SB in the regions where this set of genotypes was developed. The findings of previous studies indicated that wheat lines

with multiple disease resistance are rare. Compared to those studies, the percentage of wheat lines with multiple disease resistant was relatively high in the current study where 26 out of 110 genotypes were resistant to three or more diseases, which could be attributable to the indirect selection during multiple-year evaluation for FHB. Miedaner et al. (2012) noticed a similar phenomenon, where selection for FHB and SNB resistance also led to reduced STB infection.

Breeding for resistance to wheat FHB is the most effective approach to mitigate damage caused by this disease (Buerstmayr et al. 2012). However, this is not an easy task due to the quantitative nature of both pathogen aggressiveness and host resistance and the challenge of available diversity of the proposed types of resistance. In contrast to type II which is rather stable under greenhouse conditions, type I FHB resistance is more elusive, highly affected by environmental conditions during anthesis through early dough stage and may include disease escape (Bai and Shaner 2004; Parry et al. 1995). In field based FHB evaluation, FHB index is usually used as a parameter for disease resistance that combines both disease incidence and severity. However, FHB index is not reliable enough to infer the level of type II resistance of a particular genotype due the fact that symptoms during evaluation process could be resulted from multi- or simultaneous infection events, especially when weather conditions are favourable for infection throughout anthesis and early grain filling stages, thus symptoms do not necessarily reflect the fungal invasion or spread within head. Single floret inoculation technique is usually used to overcome the aforementioned obstacle to estimate type II resistance. Apparently the more the different types of resistance that are present in a particular wheat genotype, the more stable its performance will be under variable disease pressure. Nevertheless type II resistance is only occasionally evaluated at CIMMYT due the huge number of lines screened each year and when done is often used to a limited extent (Schlang and Duveiller 2011), this is also the situation in other mass screening programs where spray inoculation is mainly used (Mesterházy et al. 2008). Evaluation for type II resistance was reconsidered after the distribution of the 15th FHBSN to international institutes (Osman et al. 2015), when it was found that only some lines showed stable FHB resistance across locations hence strategies were designed to minimize the variation in the future nurseries.

According to our results, most of the Chinese genotypes had high type II resistance and this was not unexpected since all of the Chinese genotypes originated from an area in

China where FHB is endemic. Additionally they have gone through multiple cycles of FHB selection in Mexico before being included in the current study (He et al. 2014). However genotypes of the 15th FHBSN tended to have less type II resistance as observed by the relatively high grand mean of disease severity after point inoculation when compared to Chinese lines, despite their good resistance under artificial spray inoculation for several years in Mexico (Osman et al. 2015), leading to the conclusion that type I is generally more pronounced in this nursery compared to type II. This variation may be due to the lack of major FHB QTLs that confer both type I and type II resistance (Osman et al. 2015), emphasizing the idea of independent screening for different FHB resistance types especially in non-Chinese resources, which could be successfully used for developing new varieties with improved FHB tolerance. Nonetheless still 3 lines showed high type II resistance and possible absence of known major QTLs according to our previous haplotyping results (Chapter 3), and thus could be utilized as adapted type II resistance donors. It is noteworthy also that the Italian cultivars possibly represent novel source of resistance and more research is needed to discover their molecular background. Sumai 3 and Frontana are two of the most commonly used FHB resistant sources that have heritable and stable resistance belonging to the Chinese and Brazilian gene pools, respectively (Buerstmayr et al. 2012; Rudd et al. 2001). Interestingly both of these cultivars have an old Italian parent in their pedigree, i.e. Funo in Sumai 3 and Mentana in Frontana (Buerstmayr et al. 2012; Kohli and Diaz de Ackermann 2013), which were developed during 1920's by Nazareno Strampelli, the father of Italian green revolution. Furthermore, the famous Russian cultivar Avrora which is a parent of the FHB resistant Chinese cultivar Ning 7840 can also be traced back to the old Italian cultivar Ardito (Worland et al. 1998). Therefore, some old Italian cultivars were included in this study with the aim to identify promising parents for future crosses. Durum wheat is known to be even more susceptible than bread wheat (Buerstmayr et al. 2012). Although only one durum genotype was identified to be moderately susceptible to FHB, the scarcity of FHB resistant sources makes it important.

Significant correlations among different disease measurements may imply that the tested genotypes, which belong to different origins and pedigrees, have common genetic factors that confer them broad-spectrum resistance to multiple pathogens (Miedaner et al. 2012; Singh et al. 2012b). The late lines are likely to have better resistance to SB, TS and FHB than the early ones based on the significant association between these diseases and DH which is usually ascribed to disease escape (Brown et al. 2015; Buerstmayr et al. 2009;

Francki 2013). But in our study the situation may be different due to the fact that multiple artificial spray inoculation of the plants at the same growth stage have avoided or at least minimised disease escape related to late maturing. Hence other explanation for this phenomenon is critical.

Both quantitative and qualitative resistance to SB (Duveiller and Sharma 2012), STB (Brown et al. 2015), TS (Singh et al. 2010) and SNB (Francki 2013) in wheat have been reported while only quantitative resistance to FHB has been identified (Buerstmayr et al. 2012). Most of the wheat cultivars or advanced breeding lines used in this study had not been previously evaluated for these diseases, and they may have diverse resistance-gene constitutions based on their diverse geographic origin and different pedigrees. Broad-spectrum resistance (BSR) (Miedaner et al. 2012) and multi-disease resistance (MDR) QTLs (Singh et al. 2012b) are concepts used to refer to chromosomal regions harbouring resistance to multiple diseases. In wheat, although resistance gene clusters in specific genomic regions such as chromosomes 3BS and 3DL are well described, recently QTL regions for BSR have been confirmed (Miedaner et al. 2012; Singh et al. 2012b; Zwart et al. 2010). In this study, two Chinese genotypes, NANJING 8611 and NANJING 4840, exhibited resistance to all of the studied diseases and showed acceptable agronomic traits. Both of them showed types I, III and IV FHB resistance and high leaf rust resistance in a previous study (He et al. 2014). These lines could be very useful in the development of mapping populations that focus on resistance to either individual or multiple pathogens. Identifying such lines is of special importance in international breeding centres like CIMMYT wherein thousands of crosses are made annually; since the availability of parents resistant to multiple diseases enables breeders to combat those diseases in a single cross and eventually minimizes their workload. Further genetic studies should be carried out in order to verify their novelty and to determine whether they have single gene with broad-spectrum effect or there are individual genes/QTLs responsible independently for each of the pathogens. The lines susceptible to all of the studied diseases may also be useful in exploring the molecular biology of broad-spectrum vulnerability, promoting our understanding on plant-pathogen interactions.

From breeding perspective, the resistant materials identified in this study are ready to be utilized since most of them had already been proved to have acceptable agronomic performance and good FHB resistance (He et al. 2014; Osman et al. 2015).

4.10. Acknowledgements

The author gratefully acknowledges the financial support for this project from The CGIAR Research Program WHEAT and The Bill and Melinda Gates Foundation, USA through the Durable Rust Resistance Wheat project.

4.11. Literature cited

Acharya, K., Dutta, A. K., & Pradhan, P. (2011). *Bipolaris sorokiniana* (Sacc.) Shoem.: The most destructive wheat fungal pathogen in the warmer areas. *Aust J Crop Sci*, *5*, 1064–1071

Ali, S., Singh, P. K., McMullen, M. P., Mergoum, M., & Adhikari, T. B. (2007). Resistance to multiple leaf spot diseases in wheat. *Euphytica*, *159*, 167–179

Amand, O., Calay, F., Coquillart, L., Legat, T., Bodson, B., Moreau, J. M., & Maraite, H. (2003). First detection of resistance to QoI fungicides in *Mycosphaerella graminicola* on winter wheat in Belgium. *Comm Agric Applied Biological Sci*, *68*, 519–531

Bai, G., & Shaner, G. (2004). Management and resistance in wheat and barley to Fusarium head blight. *Annu Rev Phytopathol*, *42*, 135–161

Bhathal, J. S., Loughman, R., & Speijers, J. (2003). Yield reduction in wheat in relation to leaf disease from Yellow (Tan) spot and Septoria nodorum blotch. *Eur J Plant Pathol*, *109*, 435–443

Brown, J. K. M., Chartrain, L., Lasserre-Zuber, P., & Saintenac, C. (2015). Genetics of resistance to *Zymoseptoria tritici* and applications to wheat breeding. *Fungal Gen Biol*, *79*, 33–41

Buerstmayr, H., Adam, G., & Lemmens, M. (2012). Resistance to head blight caused by *Fusarium* spp. in wheat. In I. Sharma (Ed.), *Disease Resistance in Wheat* (pp. 236–276). CABI, Wallingford, UK

Cowger, C., & Mundt, C. C. (2002). Aggressiveness of *Mycosphaerella graminicola* isolates from susceptible and partially resistant wheat cultivars. *Phytopathol*, *92*, 624–630

Curtis, B. C., Rajaram, S., & Macpherson, H. G. (Eds.). (2002). *Bread wheat: Improvement and production.* Rome: FAO

Duveiller, E. (2004). Controlling foliar blights of wheat in the rice-wheat systems of Asia. *Plant Dis*, *88*, 552–556

Duveiller, E., Kandel, Y. R., Sharma, R. C., & Shrestha, S. M. (2005). Epidemiology of foliar blights (spot blotch and tan spot) of wheat in the plains bordering the Himalayas. *Phytopathol*, *95*, 248–256

Duveiller, E., & Sharma, R. C. (2012). Wheat resistance to spot blotch or foliar blight. In I. Sharma (Ed.), *Disease Resistance in Wheat* (pp. 120–135). CABI, Wallingford, UK

Eyal, Z. (1999). The Septoria tritici and Stagonospora nodorum blotch diseases of wheat. *EurJ Plant Pathol*, *105*, 629–641

Eyal, Z., Scharen, A. L., Prescott, J. M., & Ginke, M. van. (1987). *The Septoria Diseases of Wheat: Concepts and methods of disease management*. CIMMYT, Mexico

Feng, J., Ma, H., & Hughes, G. R. (2004). Genetics of resistance to Stagonospora nodorum blotch of hexaploid wheat. *Crop Sci*, *44*, 2043-2048

Fernandez, M. R., Clarke, F. R., Knox, R. E., Clarke, J. M., & Singh, A. K. (2010). Quantification of effects of leaf spotting diseases on grain yield and market quality of durum wheat using near-isogenic lines. *Can J Plant Pathol*, *32*, 177–187

Fernandez, M. R., Fox, S. L., Hucl, P., & Singh, A. K. (2014). Leaf spotting reaction of spring common, durum and spelt wheat, and Kamut under organic management in western Canada. *Can J Plant Sci*, *94*, 929–935

Fernandez, M. R., McConkey, B. G., & Zentner, R. P. (1999). Effects of tillage method and fallow frequency on leaf spotting diseases of spring wheat in the semiarid Canadian prairies. *Soil Til Res 50*, 259–269

Francki, M. G. (2013). Improving *Stagonospora nodorum* resistance in wheat: A review. *Crop Sci*, *53*, 355–365

Friesen, T. L., Stukenbrock, E. H., Liu, Z., Meinhardt, S., Ling, H., Faris, J. D., et al. (2006). Emergence of a new disease as a result of interspecific virulence gene transfer. *Nature Gen*, *38*, 953–956

Friesen, T. L., Xu, S. S., & Harris, M. O. (2008). Stem Rust, Tan Spot, Stagonospora nodorum blotch, and Hessian fly resistance in langdon durum–*Aegilops tauschii* synthetic hexaploid wheat lines. *Crop Sci 48*, 1062-1070

Gilbert, J., & Woods, S. M. (2001). Leaf spot diseases of spring wheat in Southern Manitoba farm fields under conventional and conservation tillage. *Can J Plant Sci*, *81*, 551–559

Goodwin, S. B. (2012). Resistance in wheat to septoria diseases caused by *Mycosphaerella graminicola* (*Septoria tritici*) and *Phaeosphaeria* (*Stagonospora*) *nodorum*. In I. Sharma (Ed.), *Disease Resistance in Wheat* (pp. 151–159). CABI, Wallingford, UK

Gurung, S., Bonman, J. M., Ali, S., Patel, J., Myrfield, M., Mergoum, M., et al. (2009). New and diverse sources of multiple disease resistance in wheat. *Crop Sci*, *49*, 1655–1666

Gurung, S., Hansen, J. M., Bonman, J. M., Gironella, A. I. N., & Adhikari, T. B. (2012). Multiple disease resistance to four leaf spot diseases in winter wheat accessions from the USDA national small grains collection. *Crop Sci*, *52*, 1640–1650

He, X., Singh, P. K., Schlang, N., Duveiller, E., Dreisigacker, S., Payne, T., & He, Z. (2014). Characterization of Chinese wheat germplasm for resistance to Fusarium head blight at CIMMYT, Mexico. *Euphytica*, *195*, 383–395

Hosford, R. M., & Busch, R. H. (1973). Losses in wheat caused by *Pyrenophora trichostoma* and *Leptosphaeria avenaria* f. sp. *triticea*. *Phytopathol*, *64*, 184–187

King, J. E., Jenkins, J. E. E., & Morgan, W. A. (1983). The estimation of yield losses in wheat from severity of infection by *Septoria* species. *Plant Pathol*, *32*, 239–249

Lamari, L., & Bernier, C. C. (1989). Evaluation of wheat lines and cultivars to tan spot [*Pyrenophora tritici-repentis*] based on lesion type. *Can J Plant Pathol*, *11*, 49–56

Lamari, L., McCallum, B. D., & DePauw, R. M. (2005). Forensic pathology of Canadian bread wheat: The case of tan spot. *Phytopathol*, *95*, 144–152

May, W. E., Fernandez, M. R., Selles, F., & Lafond, G. P. (2014). Agronomic practices to reduce leaf spotting and Fusarium kernel infections in durum wheat on the Canadian prairies. *Can J Plant Sci*, *94*, 141–152

McDonald, B. A, & Linde, C. (2002). Pathogen population genetics, evolutionary potential, and durable resistance. *Annu Rev Phytopathol*, *40*, 349–379

McKendry, A. L., Henke, G. E., & Finney, P. L. (1995). Effects of Septoria leaf blotch on soft red winter wheat milling and baking quality. *Cereal Chem*, *72*, 142–146

Mesterházy, A., Tóth, B., Bartók, T., & Varga, M. (2008). Breeding strategies against FHB in winter wheat and their relation to type I resistance. *Cereal Res Comm*, *36*, 37–43

Miedaner, T., Risser, P., Paillard, S., Schnurbusch, T., Keller, B., Hartl, L., et al. (2012). Broad-spectrum resistance loci for three quantitatively inherited diseases in two winter wheat populations. *Mol Breed*, *29*, 731–742

Osman, M., He, X., Singh, R. P., Duveiller, E., Lillemo, M., Pereyra, S. A., et al. (2015). Phenotypic and genotypic characterization of CIMMYT's 15th international Fusarium head blight screening nursery of wheat. *Euphytica*, *205*, 521-537

Parry, D. W., Jenkinson, P., & McLeod, L. (1995). Fusarium ear blight (scab) in small grain cereals—a review. *Plant Pathol*, *44*, 207–238

Polák, J., Bartoš, P., & Republic, C. (2002). Natural sources of plant disease resistance and their importance in the breeding. *Czech J Gen Plant Breed*, *38*, 146–149

Quaedvlieg, W., Kema, G. H. J. H. J., Groenewald, J. Z. Z., Verkley, G. J. M. J. M., Seifbarghi, S., Razavi, M., et al. (2011). *Zymoseptoria* gen. nov.: A new genus to accommodate *Septoria*-like species occurring on graminicolous hosts. *Persoonia: Mol Phyl Evolu Fungi*, *26*, 57–69

Quaedvlieg, W., Verkley, G. J. M., Shin, H. D., Barreto, R. W., Alfenas, A. C., Swart, W. J., et al. (2013). Sizing up *Septoria*. *Stud Mycol*, *75*, 307–390

Rudd, J. C., Horsley, R. D., McKendry, A. L., & Elias, E. M. (2001). Host plant resistance genes for Fusarium head blight: Sources, mechanisms, and utility in conventional breeding systems. *Crop Science*, *41*, 620–627

Saari, E. E., & Prescott, J. M. (1975). A scale for appraising the foliar intensity of wheat disease. *Plant Dis Report*, *59*, 377–380

Schlang, N., & Duveiller, E. (2011). Current approaches and utilization of new screening techniques for evaluation of FHB resistance at CIMMYT. *Plant Breed Seed Sci*, *64*, 89–103

Shabeer, A. (1988). Tan spot effects on yield and yield components relative to growth stage in winter wheat. *Plant Dis*, *72*, 599–602

Sharma, S., Upadhyaya, H. D., Varshney, R. K., & Gowda, C. L. L. (2013). Pre-breeding for diversification of primary gene pool and genetic enhancement of grain legumes. *Fron Plant Sci*, *4*, 1–14

Singh, P. K., Crossa, J., Duveiller, E., Singh, R. P., & Djurle, A. (2015). Association mapping for resistance to tan spot induced by *Pyrenophora tritici-repentis* race 1 in CIMMYTs historical bread wheat set. *Euphytica*, DOI 10.1007/s10681-015-1528-7

Singh, P. K., Duveiller, E., & Singh, R. P. (2011). Evaluation of CIMMYT germplasm for resistance to leaf spotting diseases of wheat. *Czech J Gen Plant Breed*, *47*, S102–S108

Singh, P. K., Duveiller, E., & Singh, R. P. (2012a). Resistance breeding for tan spot (*Pyrenophora tritici-repentis*) of wheat. In I. Sharma (Ed.), *Disease Resistance in Wheat* (pp. 136–150). CABI, Wallingford, UK

Singh, P. K., Mergoum, M., Adhikari, T. B., Kianian, S. F. & Elias, E. M. (2007). Chromosomal location of genes for seedling resistance to tan spot and Stagonospora nodorum blotch in tetraploid wheat. *Euphytica*, *155*, 27-34.

Singh, P. K., Mergoum, M., Ali, S., Adhikari, T. B., Elias, E. M., & Hughes, G. R. (2006). Identification of new sources of resistance to tan spot, Stagonospora nodorum blotch, and Septoria tritici blotch of wheat. *Crop Sci*, *46*, 2047–2053

Singh, P. K., Singh, R. P., Duveiller, E., Mergoum, M., Adhikari, T. B., & Elias, E. M. (2010). Genetics of wheat–*Pyrenophora tritici-repentis* interactions. *Euphytica*, *171*, 1–13

Singh, S., Hernandez, M. V., Crossa, J., Singh, P. K., Bains, N. S., Singh, K., & Sharma, I. (2012b). Multi-trait and multi-environment QTL analyses for resistance to wheat diseases. *PLoS ONE*, *7*, e38008

Strelkov, S. E., & Lamari, L. (2003). Host–parasite interactions in tan spot [*Pyrenophora tritici-repentis*] of wheat. *Can J Plant Pathol*, *25*, 339–349

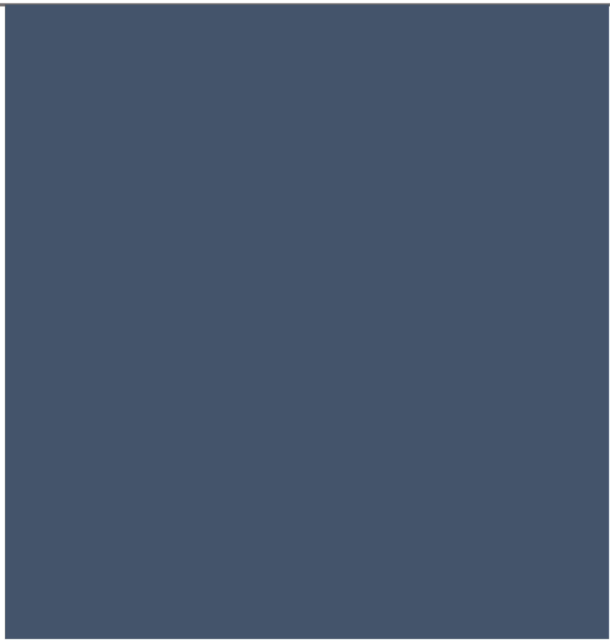
Worland, A. J., Korzun, V., Roder, M. S., Ganal, M. W., & Law, C. N. (1998). Genetic analysis of the dwarfing gene *Rht8* in wheat. Part II. The distribution and adaptive significance of allelic variants at the *Rht8* locus of wheat as revealed by microsatellite screening. *Theor Appl Genet*, *96*, 1110–1120

Zadoks, J. C., Chang, T. T., & Konzak, C. F. (1974). A decimal code for the growth stages of cereals. *Weed Res*, *14*, 415–421

Zhu, Z., Bonnett, D., Ellis, M., Singh, P., Heslot, N., Dreisigacker, S., et al. (2014). Mapping resistance to spot blotch in a CIMMYT synthetic-derived bread wheat. *Mol Breed*, *34*, 1215–1228

Zuckerman, E., Eshel, A., & Eyal, Z. (1997). Physiological aspects related to tolerance of spring wheat cultivars to Septoria tritici blotch. *Phytopathol*, *87*, 60–65

Zwart, R. S., Thompson, J. P., Milgate, A. W., Bansal, U. K., Williamson, P. M., Raman, H., & Bariana, H. S. (2010). QTL mapping of multiple foliar disease and root-lesion nematode resistances in wheat. *Mol Breed*, *26*, 107–124



Chapter 5

General discussion

Mohamed Osman

Chapter 6

General Discussion

One aim of this study was to identify the predominant cereal FHB causing pathogen and investigate its trichothecene chemotype composition in Mexico. Both morphological and molecular techniques confirmed that *F. graminearum* is the major species causing FHB in Mexico which is consistent with a previous study in Mexico (He et al., 2013) and with other studies in North America (Goswami and Kistler, 2004; Ward et al., 2008). Although a few previous reports studied the genetic variation of Mexican FHB related *Fusarium* isolates using chemical analysis (Miller et al., 1991) and molecular markers (He et al., 2013; Malihipour et al., 2012); this study provided the first detailed report on the incidence of type-B trichothecene genotypes of toxigenic FHB related *Fusarium* populations in Mexico. According to our results, both NIV and DON chemotypes were detected in isolates obtained from different locations and/or years in the states of Mexico, Puebla, Jalisco and Michoacan, whereas only DON chemotype was found in the other locations. Miedaner et al. (2000) reported that these species possess a high level of genetic plasticity, evidenced by the high level of genetic variation in aggressiveness and other characteristics, that may threaten resistant wheat genotypes. Shifts in chemo-genotypes within *Fusarium* species have been observed in different reports. For example, Ward et al., (2008) detected a shift from 15- to 3-ADON producing isolates in North America, which have the potential to produce a higher quantity of this mycotoxin and are reported to be more aggressive. In the Netherlands, a slight increase in NIV frequency, which is more toxic to humans and animals, was detected by Waalwijk et al., (2003). Continues inspection of populations is required to detect such events, which might pose a threat to the imported FHB-resistant lines that are generated in different countries using a limited number of resistance genes. Hence, continues inspection of populations is required to detect such events, which might pose a threat to the imported FHB-resistant lines that are generated in different countries using a limited number of resistance genes.

Breeding for resistance to wheat FHB is the most effective approach to mitigate damage caused by this disease (Buerstmayr et al. 2012). However, this is not an easy task due to the quantitative nature of both pathogen aggressiveness and host resistance and the challenge of available diversity of the proposed types of resistance. In contrast to type II which is rather stable under greenhouse conditions, type I FHB resistance is more elusive, highly affected by environmental conditions during anthesis through early dough stage and may include disease escape (Bai and Shaner 2004; Parry et al. 1995). According to our results, most of the Chinese

genotypes had high type II resistance and this was not unexpected since all of the Chinese genotypes originated from an area in China where FHB is endemic. Additionally, they have gone through multiple cycles of FHB selection in Mexico before being included in the current study (He et al. 2014). However, genotypes of the 15th FHBSN tended to have less type II resistance as observed by the relatively high grand mean of disease severity after point inoculation when compared to Chinese lines, despite their good resistance under artificial spray inoculation for several years in Mexico (Osman et al. 2015), leading to the conclusion that type I is generally more pronounced in this nursery compared to type II. This variation may be due to the lack of major FHB QTLs that confer both type I and type II resistance (Osman et al. 2015), emphasizing the idea of independent screening for different FHB resistance types especially in non-Chinese resources, which could be successfully used for developing new varieties with improved FHB tolerance. Although the 15th FHBSN accessions generally exhibited low levels of infection in both El Batán and Toluca (Mexican environments), many turned out to be susceptible in other five locations, due to a significant genotype-by-environment interaction, which could be caused by one or all of the following reasons; different inoculation protocols, field management, weather condition, *Fusarium* isolates, etc. Many studies have shown that FHB resistance in wheat is horizontal, not species- nor strain-specific (Van Eeuwijk et al. 1995; Mesterhazy et al. 1999; Mesterhazy et al. 2005). Therefore, the differences in FHB levels resulting from variation in *Fusarium* isolates or species used throughout this study may not explain the resistance variation across locations, although significant differences in aggressiveness have been reported in *Fusarium* isolates/species of different geographic origins (Malihipour et al. 2012). Previous reports have demonstrated that resistance efficiency depends upon the level of resistance i.e. highly FHB resistant genotypes remain unaffected under almost all epidemic conditions though the performance of moderately resistant and susceptible germplasm is greatly impacted by environmental conditions and disease pressure (Mesterhazy 1995; Miedaner et al. 2001). However, the moderately resistant genotypes might have several QTL with small to medium effects. This group is potentially significant for breeders because they are mostly adapted, and can be used to improve FHB resistance. Unlike the 13th and 14th FHBSN, where the 2DL QTL as in Wuhan 1 was the predominant one (He et al. 2013a; He et al. 2013b), the 15th FHBSN suggested a high frequency of 55% of the 4BS QTL as in Wuhan 1. Although both were found in Wuhan 1, the

2DL QTL conferred Type II resistance, whereas the 4BS one contributed Type I resistance (Somers et al. 2003). The latter has been fine mapped by Xue et al. (2010) and designated as Fhb4. The QTL on 7A chromosome as in T. dicoccoides was the second frequent QTL, contributing to Type II resistance (Kumar et al. 2007); but it was found only in five lines. Considering also the very low frequencies or absence of other QTLs, the haplotyping results proposed a clear non-Sumai 3 resistance background of the 15th FHBSN, which lacked major Type II resistance QTLs such as 3BS (*Fhb1*) and 6BS (*Fhb2*) as in Sumai 3 (Cuthbert et al. 2006; Cuthbert et al. 2007). CIMMYT has devoted great efforts on the identification and utilization of non-Sumai 3 resistance since the last decade (He et al. 2013a), which was very successful as shown by the haplotyping results.

Wheat is often infected simultaneously by multiple foliar diseases, but the components of the foliar disease complex differ from region to region despite the similar symptoms. For example, TS and SB co-present in South Asia, and it is often very hard to diagnose the pathogen based only on visual symptoms (Duveiller et al. 2005). Whereas STB, TS, and SNB co-exist in the United States and all of the four diseases, along with FHB, are common in Canadian prairies (Fernandez et al. 2014; Fernandez et al. 1999; Gilbert and Woods 2001; May et al. 2014). Accordingly, strategies based on multiple disease resistance will reduce risks related to the co-existence of different pathogens in certain environments. Moreover, it may facilitate further breeding efforts on combining resistance to blight diseases with resistance to other diseases (Gurung et al. 2012). One of the absolute requirements in international wheat breeding institutes is to incorporate multiple disease resistance while maintaining favorable gene complexes responsible for regional adaptation and acceptable agronomic traits. Gene pyramiding is a valuable tool to accumulate different resistance genes of various diseases into a single wheat genotype. However, breeding costs could be too high, and thus, the MAS advantage may be dropped by the high cost since it is speculated that molecular markers for multiple disease resistance may be more costly than phenotypic selection. This study showed that multiple disease resistance can be identified in cimmyt wheat germplasm by using carefully characterized pathogens. Such screens also demonstrate the efficacy of identifying such genotypes in various breeding programs and sustainable disease management. Therefore, the most beneficial strategy is to investigate existing germplasm to identify genotypes with acceptable levels of resistance to the majority of the most significant diseases before using

other costly techniques. Most of the wheat cultivars or advanced breeding lines used in this study had not been previously evaluated for these diseases, and they may have diverse resistance-gene constitutions based on their diverse geographic origin and different pedigrees. Broadspectrum resistance (BSR) (Miedaner et al. 2012) and multi-disease resistance (MDR) QTLs (Singh et al. 2012b) are concepts used to refer to chromosomal regions harbouring resistance to multiple diseases. In wheat, although resistance gene clusters in specific genomic regions such as chromosomes 3BS and 3DL are well described, recently QTL regions for BSR have been confirmed (Miedaner et al. 2012; Singh et al. 2012b; Zwart et al. 2010). In this study, two Chinese genotypes, NANJING 8611 and NANJING 4840, exhibited resistance to all of the studied diseases and showed acceptable agronomic traits. Both of them showed types I, III and IV FHB resistance and high leaf rust resistance in a previous study (He et al. 2014). These lines could be very useful in the development of mapping populations that focus on resistance to either individual or multiple pathogens.

Table S1.2 *Fusarium* isolates studied in Chapter 2, their ID, origin, year of collection and chemotype.

Isolate ID	Species	City	State	Crop	Year of collection	Chemotype
Fg-1	*F.graminearum*	Tepatitlan	Jalisco	Wheat	2013	NIV
Fg-2	*F.graminearum*	Boximo	State of Mexico	Wheat	2010	NIV
Fg-3	*F.graminearum*	Agua Fria	Puebla	Wheat	2009	NIV
Fg-4	*F.graminearum*	Agua Fria	Puebla	Wheat	2009	NIV
Fg-5	*F.graminearum*	Tlaxcala	Tlaxcala	Barley	1995	15-AcDON
Fg-6	*F.graminearum*	Tlaxcala	Tlaxcala	Barley	1995	15-AcDON
Fg-7	*F.graminearum*	Patzcuaro	Michoacan	Wheat	1997	15-AcDON
Fg-8	*F.graminearum*	Jesús María	Jalisco	Wheat	1997	15-AcDON
Fg-9	*F.graminearum*	Tepatitlan	Jalisco	Wheat	1997	15-AcDON
Fg-10	*F.graminearum*	Tepatitlan	Jalisco	Wheat	1997	15-AcDON
Fg-11	*F.graminearum*	Tepatitlan	Jalisco	Wheat	1997	15-AcDON
Fg-12	*F.graminearum*	Tepatitlan	Jalisco	Wheat	1997	15-AcDON
Fg-13	*F.graminearum*	Tepatitlan	Jalisco	Wheat	1997	15-AcDON
Fg-14	*F.graminearum*	Batan	State of Mexico	Wheat	1998	15-AcDON
Fg-15	*F.graminearum*	Batan	State of Mexico	Wheat	1998	15-AcDON
Fg-16	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-17	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-18	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-19	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-20	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-21	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-22	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-23	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-24	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-25	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-26	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-27	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-28	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-29	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-30	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-31	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-32	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-33	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-34	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-35	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-36	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-37	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-38	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-39	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-40	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-41	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-42	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-43	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-44	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-45	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-46	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-47	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-48	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-49	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-50	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-51	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-52	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-53	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-54	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-55	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-56	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-57	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-58	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON

Fg-59	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-60	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-61	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-62	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-63	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-64	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-65	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-66	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-67	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-68	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-69	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-70	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-71	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-72	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-73	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-74	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-75	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-76	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-77	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-78	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-79	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-80	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-81	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-82	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-83	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-84	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-85	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-86	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-87	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-88	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-89	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-90	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-91	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-92	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-93	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-94	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-95	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-96	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-97	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-98	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-99	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-100	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-101	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-102	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-103	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-104	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-105	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-106	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-107	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-108	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-109	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-110	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-111	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-112	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-113	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-114	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-115	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-116	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-117	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-118	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-119	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-120	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON

Fg-121	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-122	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-123	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-124	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-125	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-126	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-127	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-128	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-129	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-130	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-131	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-132	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-133	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-134	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-135	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
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Fg-137	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
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Fg-139	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
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Fg-141	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
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Fg-144	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
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Fg-147	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
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Fg-150	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
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Fg-154	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-155	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-156	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-157	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-158	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-159	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-160	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-161	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
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Fg-173	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
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Fg-179	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-180	*F.graminearum*	Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-181	*F.graminearum*	Toluca	State of Mexico	Corn	2005	15-AcDON
Fg-182	*F.graminearum*	Toluca	State of Mexico	Corn	2005	15-AcDON

Fg-183	*F.graminearum*	Toluca	State of Mexico	Corn	2005	15-AcDON
Fg-184	*F.graminearum*	Toluca	State of Mexico	Corn	2005	15-AcDON
Fg-185	*F.graminearum*	Toluca	State of Mexico	Corn	2005	15-AcDON
Fg-186	*F.graminearum*	Toluca	State of Mexico	Corn	2005	15-AcDON
Fg-187	*F.graminearum*	Toluca	State of Mexico	Corn	2005	15-AcDON
Fg-188	*F.graminearum*	Toluca	State of Mexico	Corn	2005	15-AcDON
Fg-189	*F.graminearum*	Toluca	State of Mexico	Corn	2005	15-AcDON
Fg-190	*F.graminearum*	Toluca	State of Mexico	Corn	2005	15-AcDON
Fg-191	*F.graminearum*	Toluca	State of Mexico	Corn	2005	15-AcDON
Fg-192	*F.graminearum*	Toluca	State of Mexico	Corn	2005	15-AcDON
Fg-193	*F.graminearum*	Toluca	State of Mexico	Corn	2005	15-AcDON
Fg-194	*F.graminearum*	Toluca	State of Mexico	Corn	2005	15-AcDON
Fg-195	*F.graminearum*	Toluca	State of Mexico	Corn	2005	15-AcDON
Fg-196	*F.graminearum*	Toluca	State of Mexico	Corn	2005	15-AcDON
Fg-197	*F.graminearum*	Toluca	State of Mexico	Corn	2005	15-AcDON
Fg-198	*F.graminearum*	Toluca	State of Mexico	Corn	2005	15-AcDON
Fg-199	*F.graminearum*	Patzcuaro	Michoacan	Wheat	2006	15-AcDON
Fg-200	*F.graminearum*	Patzcuaro	Michoacan	Wheat	2006	15-AcDON
Fg-201	*F.graminearum*	Patzcuaro	Michoacan	Wheat	2006	15-AcDON
Fg-202	*F.graminearum*	Patzcuaro	Michoacan	Wheat	2006	15-AcDON
Fg-203	*F.graminearum*	Patzcuaro	Michoacan	Wheat	2006	15-AcDON
Fg-204	*F.graminearum*	Patzcuaro	Michoacan	Wheat	2006	15-AcDON
Fg-205	*F.graminearum*	Patzcuaro	Michoacan	Wheat	2006	15-AcDON
Fg-206	*F.graminearum*	Patzcuaro	Michoacan	Wheat	2006	15-AcDON
Fg-207	*F.graminearum*	Patzcuaro	Michoacan	Wheat	2006	15-AcDON
Fg-208	*F.graminearum*	Patzcuaro	Michoacan	Wheat	2006	15-AcDON
Fg-209	*F.graminearum*	Patzcuaro	Michoacan	Wheat	2006	15-AcDON
Fg-210	*F.graminearum*	Patzcuaro	Michoacan	Wheat	2006	15-AcDON
Fg-211	*F.graminearum*	Batan	State of Mexico	Wheat	2007	15-AcDON
Fg-212	*F.graminearum*	Toluca	State of Mexico	Wheat	2007	15-AcDON
Fg-213	*F.graminearum*	Toluca	State of Mexico	Wheat	2007	15-AcDON
Fg-214	*F.graminearum*	Toluca	State of Mexico	Wheat	2007	15-AcDON
Fg-215	*F.graminearum*	Toluca	State of Mexico	Wheat	2007	15-AcDON
Fg-216	*F.graminearum*	Toluca	State of Mexico	Wheat	2007	15-AcDON
Fg-217	*F.graminearum*	Toluca	State of Mexico	Wheat	2007	15-AcDON
Fg-218	*F.graminearum*	Batan	State of Mexico	Wheat	2008	15-AcDON
Fg-219	*F.graminearum*	Batan	State of Mexico	Wheat	2008	15-AcDON
Fg-220	*F.graminearum*	Batan	State of Mexico	Wheat	2008	15-AcDON
Fg-221	*F.graminearum*	Batan	State of Mexico	Wheat	2008	15-AcDON
Fg-222	*F.graminearum*	Batan	State of Mexico	Wheat	2008	15-AcDON
Fg-223	*F.graminearum*	Batan	State of Mexico	Wheat	2008	15-AcDON
Fg-224	*F.graminearum*	Batan	State of Mexico	Wheat	2008	15-AcDON
Fg-225	*F.graminearum*	Batan	State of Mexico	Wheat	2008	15-AcDON
Fg-226	*F.graminearum*	Batan	State of Mexico	Wheat	2008	15-AcDON
Fg-227	*F.graminearum*	Batan	State of Mexico	Wheat	2008	15-AcDON
Fg-228	*F.graminearum*	Batan	State of Mexico	Wheat	2008	15-AcDON
Fg-229	*F.graminearum*	Guanajuato	State of Mexico	Wheat	2008	15-AcDON
Fg-230	*F.graminearum*	Boximo	State of Mexico	Wheat harinero	2010	15-AcDON
Fg-231	*F.graminearum*	Boximo	State of Mexico	Wheat harinero	2010	15-AcDON
Fg-232	*F.graminearum*	Boximo	State of Mexico	Wheat harinero	2010	15-AcDON
Fg-233	*F.graminearum*	Boximo	State of Mexico	Wheat harinero	2010	15-AcDON
Fg-234	*F.graminearum*	Toluca	State of Mexico	Wheat	2010	15-AcDON
Fg-235	*F.graminearum*	Toluca	State of Mexico	Wheat Duro	2010	15-AcDON
Fg-236	*F.graminearum*	Toluca	State of Mexico	Wheat Duro	2010	15-AcDON
Fg-237	*F.graminearum*	Toluca	State of Mexico	Wheat Duro	2010	15-AcDON
Fg-238	*F.graminearum*	Toluca	State of Mexico	Wheat Duro	2010	15-AcDON
Fg-239	*F.graminearum*	Toluca	State of Mexico	Triticale	2010	15-AcDON
Fg-240	*F.graminearum*	Batan	State of Mexico	Wheat	2011	15-AcDON
Fg-241	*F.graminearum*	Batan	State of Mexico	Wheat	2011	15-AcDON
Fg-242	*F.graminearum*	Batan	State of Mexico	Wheat harinero	2011	15-AcDON
Fg-243	*F.graminearum*	Batan	State of Mexico	Wheat harinero	2011	15-AcDON
Fg-244	*F.graminearum*	Juchitepec	State of Mexico	Wheat harinero	2012	15-AcDON

Fg-245	*F.graminearum*	Oaxaca	Oxaca	Wheat	2012	15-AcDON
Fg-246	*F.graminearum*	Batan	State of Mexico	Wheat	2013	15-AcDON
Fg-247	*F.graminearum*	Batan	State of Mexico	Wheat	2013	15-AcDON
Fg-248	*F.graminearum*	Batan	State of Mexico	Wheat	2013	15-AcDON
Fg-249	*F.graminearum*	Batan	State of Mexico	Wheat	2013	15-AcDON
Fg-250	*F.graminearum*	Batan	State of Mexico	Wheat	2013	15-AcDON
Fg-251	*F.graminearum*	Batan	State of Mexico	Wheat	2013	15-AcDON
Fg-252	*F.graminearum*	Batan	State of Mexico	Wheat	2013	15-AcDON
Fg-253	*F.graminearum*	Batan	State of Mexico	Wheat	2013	15-AcDON
Fg-254	*F.graminearum*	Batan	State of Mexico	Wheat	2013	15-AcDON
Fg-255	*F.graminearum*	Batan	State of Mexico	Wheat	2013	15-AcDON
Fg-256	*F.graminearum*	Batan	State of Mexico	Wheat	2013	15-AcDON
Fg-257	*F.graminearum*	Batan	State of Mexico	Wheat	2013	15-AcDON
Fg-258	*F.graminearum*	Batan	State of Mexico	Wheat	2013	15-AcDON
Fg-259	*F.graminearum*	Batan	State of Mexico	Wheat	2013	15-AcDON
Fg-260	*F.graminearum*	Batan	State of Mexico	Wheat	2013	15-AcDON
Fg-261	*F.graminearum*	Batan	State of Mexico	Wheat	2013	15-AcDON
Fg-262	*F.graminearum*	Batan	State of Mexico	Wheat	2013	15-AcDON
Fg-263	*F.graminearum*	Batan	State of Mexico	Wheat	2013	15-AcDON
Fg-264	*F.graminearum*	Batan	State of Mexico	Wheat	2013	15-AcDON
Fg-265	*F.graminearum*	Batan	State of Mexico	Wheat	2013	15-AcDON
Fg-266	*F.graminearum*	Batan	State of Mexico	Wheat	2013	15-AcDON
Fg-267	*F.graminearum*	Batan	State of Mexico	Wheat	2013	15-AcDON
Fg-268	*F.graminearum*	Batan	State of Mexico	Wheat	2013	15-AcDON
Fg-269	*F.graminearum*	Batan	State of Mexico	Wheat	2013	15-AcDON
Fg-270	*F.graminearum*	Batan	State of Mexico	Wheat	2013	15-AcDON
Fg-271	*F.graminearum*	Batan	State of Mexico	Wheat	2013	15-AcDON
Fg-272	*F.graminearum*	Batan	State of Mexico	Wheat	2013	15-AcDON
Fg-273	*F.graminearum*	Batan	State of Mexico	Wheat	2013	15-AcDON
Fg-274	*F.graminearum*	Batan	State of Mexico	Wheat	2013	15-AcDON
Fg-275	*F.graminearum*	Oaxaca	Oxaca	Wheat	2013	15-AcDON
Fg-276	*F.graminearum*	Oaxaca	Oxaca	Wheat	2013	15-AcDON
Fg-277	*F.graminearum*	Oaxaca	Oxaca	Wheat	2013	15-AcDON
Fg-278	*F.graminearum*	Oaxaca	Oxaca	Wheat	2013	15-AcDON
Fg-279	*F.graminearum*	Oaxaca	Oxaca	Wheat	2013	15-AcDON
Fg-280	*F.graminearum*	Oaxaca	Oxaca	Wheat	2013	15-AcDON
Fg-281	*F.graminearum*	Oaxaca	Oxaca	Wheat	2013	15-AcDON
Fg-282	*F.graminearum*	Oaxaca	Oxaca	Wheat	2013	15-AcDON
Fg-283	*F.graminearum*	Oaxaca	Oxaca	Wheat	2013	15-AcDON
Fg-284	*F.graminearum*	Oaxaca	Oxaca	Wheat	2013	15-AcDON
Fg-285	*F.graminearum*	Oaxaca	Oxaca	Wheat	2013	15-AcDON
Fg-286	*F.graminearum*	Oaxaca	Oxaca	Wheat	2013	15-AcDON
Fg-287	*F.graminearum*	Oaxaca	Oxaca	Wheat	2013	15-AcDON
Fg-288	*F.graminearum*	Oaxaca	Oxaca	Wheat	2013	15-AcDON
Fg-289	*F.graminearum*	Oaxaca	Oxaca	Wheat	2013	15-AcDON
Fg-290	*F.graminearum*	Oaxaca	Oxaca	Wheat	2013	15-AcDON
Fg-291	*F.graminearum*	Oaxaca	Oxaca	Wheat	2013	15-AcDON
Fg-292	*F.graminearum*	Oaxaca	Oxaca	Wheat	2013	15-AcDON
Fg-293	*F.graminearum*	Oaxaca	Oxaca	Wheat	2013	15-AcDON
Fg-294	*F.graminearum*	Oaxaca	Oxaca	Wheat	2013	15-AcDON
Fg-295	*F.graminearum*	Oaxaca	Oxaca	Wheat	2013	15-AcDON
Fg-296	*F.graminearum*	Oaxaca	Oxaca	Wheat	2013	15-AcDON
Fg-297	*F.graminearum*	Oaxaca	Oxaca	Wheat	2013	15-AcDON
Fg-298	*F.graminearum*	Oaxaca	Oxaca	Wheat	2013	15-AcDON
Fg-299	*F.graminearum*	Oaxaca	Oxaca	Wheat	2013	15-AcDON
Fg-300	*F.graminearum*	Oaxaca	Oxaca	Wheat	2013	15-AcDON
Fg-301	*F.graminearum*	Oaxaca	Oxaca	Wheat	2013	15-AcDON
Fg-302	*F.graminearum*	Oaxaca	Oxaca	Wheat	2013	15-AcDON
Fg-303	*F.graminearum*	Oaxaca	Oxaca	Wheat	2013	15-AcDON
Fg-304	*F.graminearum*	Oaxaca	Oxaca	Wheat	2013	15-AcDON
Fg-305	*F.graminearum*	Oaxaca	Oxaca	Wheat	2013	15-AcDON
Fg-306	*F.graminearum*	Oaxaca	Oxaca	Wheat	2013	15-AcDON

Fg-307	*F.graminearum*	Oaxaca	Oxaca	Wheat	2013	15-AcDON
Fg-308	*F.graminearum*	Oaxaca	Oxaca	Wheat	2013	15-AcDON
Fg-309	*F.graminearum*	Oaxaca	Oxaca	Wheat	2013	15-AcDON
Fg-310	*F.graminearum*	Oaxaca	Oxaca	Wheat	2013	15-AcDON
Fg-311	*F.graminearum*	Oaxaca	Oxaca	Wheat	2013	15-AcDON
Fg-312	*F.graminearum*	Oaxaca	Oxaca	Wheat	2013	15-AcDON
Fg-313	*F.graminearum*	Oaxaca	Oxaca	Wheat	2013	15-AcDON
Fg-314	*F.graminearum*	Tepatitlan	Jalisco	Wheat	2013	15-AcDON
Fg-315	*F.graminearum*	Tepatitlan	Jalisco	Wheat	2013	15-AcDON
Fg-316	*F.graminearum*	Tepatitlan	Jalisco	Wheat	2013	15-AcDON
Fg-317	*F.graminearum*	Tepatitlan	Jalisco	Wheat	2013	15-AcDON
Fg-318	*F.graminearum*	Tepatitlan	Jalisco	Wheat	2013	15-AcDON
Fg-319	*F.graminearum*	Tepatitlan	Jalisco	Wheat	2013	15-AcDON
Fg-320	*F.graminearum*	Tepatitlan	Jalisco	Wheat	2013	15-AcDON
Fg-321	*F.graminearum*	Tepatitlan	Jalisco	Wheat	2013	15-AcDON
Fg-322	*F.graminearum*	Tepatitlan	Jalisco	Wheat	2013	15-AcDON
Fg-323	*F.graminearum*	Tepatitlan	Jalisco	Wheat	2013	15-AcDON
Fg-324	*F.graminearum*	Tepatitlan	Jalisco	Wheat	2013	15-AcDON
Fg-325	*F.graminearum*	Tepatitlan	Jalisco	Wheat	2013	15-AcDON
Fg-326	*F.graminearum*	Tepatitlan	Jalisco	Wheat	2013	15-AcDON
Fg-327	*F.graminearum*	Tepatitlan	Jalisco	Wheat	2013	15-AcDON
Fg-328	*F.graminearum*	Tepatitlan	Jalisco	Wheat	2013	15-AcDON
Fg-329	*F.graminearum*	Tepatitlan	Jalisco	Wheat	2013	15-AcDON
Fg-330	*F.graminearum*	Tepatitlan	Jalisco	Wheat	2013	15-AcDON
Fg-331	*F.graminearum*	Tepatitlan	Jalisco	Wheat	2013	15-AcDON
Fg-332	*F.graminearum*	Toluca	State of Mexico	Wheat	2013	15-AcDON
Fg-333	*F.graminearum*	Toluca	State of Mexico	Wheat	2013	15-AcDON
Fg-334	*F.graminearum*	Toluca	State of Mexico	Wheat	2013	15-AcDON
Fg-335	*F.graminearum*	Toluca	State of Mexico	Wheat	2013	15-AcDON
Fg-336	*F.graminearum*	Toluca	State of Mexico	Wheat	2013	15-AcDON
Fg-337	*F.graminearum*	Toluca	State of Mexico	Wheat	2013	15-AcDON
Fg-338	*F.graminearum*	Toluca	State of Mexico	Wheat	2013	15-AcDON
Fg-339	*F.graminearum*	Toluca	State of Mexico	Wheat	2013	15-AcDON
Fg-340	*F.graminearum*	Toluca	State of Mexico	Wheat	2013	15-AcDON
Fg-341	*F.graminearum*	Toluca	State of Mexico	Wheat	2013	15-AcDON
Fg-342	*F.graminearum*	Toluca	State of Mexico	Wheat	2013	15-AcDON
Fg-343	*F.graminearum*	Toluca	State of Mexico	Wheat	2013	15-AcDON
Fg-344	*F.graminearum*	Toluca	State of Mexico	Wheat	2013	15-AcDON
Fg-345	*F.graminearum*	Toluca	State of Mexico	Wheat	2013	15-AcDON
Fg-346	*F.graminearum*	Toluca	State of Mexico	Wheat	2013	15-AcDON
Fg-347	*F.graminearum*	El Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-348	*F.graminearum*	El Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-349	*F.graminearum*	El Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-350	*F.graminearum*	El Batan	State of Mexico	Wheat	2005	15-AcDON
Fg-351	*F.graminearum*	El Batan	State of Mexico	Wheat	2005	15-AcDON
Fc-1	*F.crokwellense*	Patzcuaro	Michoacan	Wheat	2006	NIV
Fc-2	*F.crokwellense*	Patzcuaro	Michoacan	Wheat	2006	NIV
Fc-3	*F.crokwellense*	Patzcuaro	Michoacan	Wheat	2006	NIV
Fc-4	*F.crokwellense*	Patzcuaro	Michoacan	Wheat	2006	NIV
Fc-5	*F.crokwellense*	Patzcuaro	Michoacan	Wheat	2006	NIV
Fc-6	*F.crokwellense*	Patzcuaro	Michoacan	Wheat	2006	NIV
Fc-7	*F.crokwellense*	Patzcuaro	Michoacan	Wheat	2006	NIV
Fc-8	*F.crokwellense*	Patzcuaro	Michoacan	Wheat	2006	NIV
Fc-9	*F.crokwellense*	Patzcuaro	Michoacan	Wheat	2006	NIV
Fc-10	*F.crokwellense*	Patzcuaro	Michoacan	Wheat	2006	NIV
Fc-11	*F.crokwellense*	Patzcuaro	Michoacan	Wheat	2006	NIV
Fc-12	*F.crokwellense*	Patzcuaro	Michoacan	Wheat	2006	NIV
Fc-13	*F.crokwellense*	Patzcuaro	Michoacan	Wheat	2006	NIV
Fc-14	*F.crokwellense*	Patzcuaro	Michoacan	Wheat	2006	NIV
Fc-15	*F.crokwellense*	Patzcuaro	Michoacan	Wheat	2006	NIV
Fc-16	*F.crokwellense*	Patzcuaro	Michoacan	Wheat	2006	NIV
Fc-17	*F.crokwellense*	Patzcuaro	Michoacan	Wheat	2006	NIV

Fc-18	*F.crokwellense*	Patzcuaro	Michoacan	Wheat	2006	NIV
Fc-19	*F.crokwellense*	Patzcuaro	Michoacan	Wheat	2006	NIV
Fc-20	*F.crokwellense*	Patzcuaro	Michoacan	Wheat	2006	NIV
Fc-21	*F.crokwellense*	Patzcuaro	Michoacan	Wheat	2006	NIV
Fc-22	*F.crokwellense*	Patzcuaro	Michoacan	Wheat	2006	NIV
Fc-23	*F.crokwellense*	Patzcuaro	Michoacan	Wheat	2006	NIV
Fc-24	*F.crokwellense*	Patzcuaro	Michoacan	Wheat	2006	NIV
Fc-25	*F.crokwellense*	Patzcuaro	Michoacan	Wheat	2006	NIV
Fc-26	*F.crokwellense*	Patzcuaro	Michoacan	Wheat	2006	NIV
Fc-27	*F.crokwellense*	Patzcuaro	Michoacan	Wheat	2006	NIV
Fc-28	*F.crokwellense*	Patzcuaro	Michoacan	Wheat	2006	NIV
Fc-29	*F.crokwellense*	Patzcuaro	Michoacan	Wheat	2006	NIV
Fc-30	*F.crokwellense*	Patzcuaro	Michoacan	Wheat	2006	NIV
Fc-31	*F.crokwellense*	Patzcuaro	Michoacan	Wheat	2006	NIV
Fc-32	*F.crokwellense*	Patzcuaro	Michoacan	Wheat	2006	NIV
Fc-33	*F.crokwellense*	Patzcuaro	Michoacan	Wheat	2006	NIV
Fc-34	*F.crokwellense*	Patzcuaro	Michoacan	Wheat	2006	NIV
Fc-35	*F.crokwellense*	Patzcuaro	Michoacan	Wheat	2006	NIV
Fc-36	*F.crokwellense*	Batan	State of Mexico	Wheat	2013	NIV
Fc-37	*F.crokwellense*	Batan	State of Mexico	Wheat	2013	NIV