Phenotypic and SSR-based characterization of new sources of *Fusarium* head blight resistance in wheat

by

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Dissertation summary

Wheat (*Triticum* spp.) is one of the most important cereal crops produced worldwide. South Africa is the largest wheat producer in sub-Saharan Africa (SSA). However, wheat production in South Africa is affected by many biotic, abiotic and socio-economic constraints. Among the biotic stresses, *Fusarium* head blight (FHB) is one of the most important fungal diseases of wheat caused predominantly by *Fusarium graminearum.* Various strategies have been proposed to control FHB epidemics. Genetic control, which includes host plant resistance, is currently the most economically and environmentally friendly approach for controlling FHB. Breeding for FHB resistant wheat cultivars provides the potential for long-term, sustainable control of FHB. Consequently, a pre-breeding of wheat has been undertaken at the Agricultural Research Council-Small Grain Institute (ARC-SGI) in collaboration with global and regional wheat researchers to develop FHB resistant genetic pool. A larger number of genetic resources was acquired from collaborators and about 778 new recombinant inbred lines (RILs) were developed through designed crosses and continuous selfing and selection. As part of this initiative, this study was undertaken with the following objectives: 1) to determine the field response of the 778 newly developed RILs and standard check varieties of wheat for FHB resistance and to identify sources of resistance for breeding or direct production, and 2) to determine FHB resistance among 76 wheat lines using field based phenotyping and to determine the genetic background of 11 selected most resistant lines and four susceptible checks using simple sequence repeat (SSR) markers in order to identify novel FHB resistance sources.

A total of 778 RILs were field evaluated for their FHB reaction across four environments, along with resistant and susceptible checks, Sumai 3 and SST 806, respectively. The analysis of variance showed significant differences among the genotypes, the testing environments and their interactions. FHB resistance was found to be heritable with heritability estimate of 64%. Among the 778 RILs evaluated, 6% had an infection rate <20%, suggesting high FHB resistance among the lines. Overall, five RILs were selected as new sources of high FHB resistance. These lines were 681 (Buff/1036/71), 134 (Duzi/910/8), 22 (Bav/910/22), 717 (Bav/937/8) and 133

(Duzi/910/7) with FHB scores comparable to the resistant check. These lines can be recommended for further breeding or direct production.

Seventy three wheat genotypes were obtained from CIMMYT. These lines were phenotypically characterized for FHB reaction and 14% of the most resistant lines with economic agronomic traits were selected for further characterization using 24 SSR markers linked to known FHB resistance genes. Haplotype profiles of the selected FHB resistant wheat lines were compared to known sources of FHB resistance in order to identify and characterize possible genes conferring resistance in the lines. Three CIMMYT entries were found to have allelic similarities with the FHB resistant lines. These entries included #937, #936 and #930 with FHB resistance genes such as *Fhb1, Fhb5* and *Fhb2,* respectively. The rest of the selected CIMMYT lines showed no similarities to the known resistance sources, implying that they hold novel FHB resistance genes. This will allow for the enrichment of the FHB resistant genepool and diversification of FHB resistance sources. The selected wheat lines are valuable genetic resources for FHB resistance breeding programs.

Declaration

I, Cwengile Chumisa Dweba, declare that

1. The research reported in this dissertation, except where otherwise indicated, is my original research.
2. This dissertation has not been submitted for any degree or examination at any other university.
3. This dissertation does not contain other scientists' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other scientists.
4. This dissertation does not contain other scientists' writing, unless specifically acknowledged as being sourced from other scientists. Where other written sources have been quoted, then their words have been re-written but the general information attributed to them has been referenced.
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Signed……Date…….

Cwengile Chumisa Dweba (Candidate)

As the candidate's supervisor(s), I/We have approved this dissertation for submission**.**

Signed……Date…….

Prof Hussein A. Shimelis

Signed……Date…….

Prof Toi J. Tsilo

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Dedication

This dissertation is dedicated to the love of my life, my baby girl **Yande Oluthando Dweba**. You bring so much joy and colour into my life, may you grow up to be an amazing woman of God.

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Dissertation introduction

Background

Wheat (*Triticum aestivum* L.) is one of the most important cereal crops globally. The mean global consumption of wheat stands at 2.4 billion tonnes per annum representing more than one-third of the minimum food requirements of adults (FAO, 2016). In South Africa, wheat is the second most important grain crop next to maize (Meyer and Kirsten, 2005) which is widely cultivated across the Western Cape, Northern Cape and Free State Provinces (GrainSA, 2016). Wheat cultivation in South Africa started in the Western Cape Province in the 1600s (DAFF, 2010). Presently, the wheat industry contributes to 3% of the gross value of agricultural production creating significant employment opportunities. In South Africa there were about 3800 to 4000 commercial wheat producers with a total capital investment of approximately R 3 billion (DAFF, 2005). Presently the country produces roughly 2 million tonnes of wheat per annum of which 1.2 million tons is being produced under irrigation (ARC, 2014).

Sub-Saharan African (SSA) countries have seen patterns of rapid increase in wheat consumption (Jayne et al., 2010). However, the current level of wheat production is too low to meet wheat consumption in the region (Negassa et al., 2013). South Africa is the leading wheat producer in SSA though wheat production has declined over 10 the past years in the country. Thus, the local demand for wheat is met through imports from Europe, South America and China (DAFF, 2012). The low level of wheat production and productivity in South Africa is attributed to various constraints including biotic, abiotic and socio-economic factors (DAFF, 2013; Negassa et al., 2013).

Constraints to wheat production

Wheat production and productivity is highly limited due to biotic factors including diseases, pests and weeds (Oerke, 2006). Among the abiotic factors, drought and soil infertility are the principal factors limiting wheat production (Hailu et al., 2015; Daryanto et al., 2016). A wide array of biotic stresses affect wheat production leading to significant yield and quality losses. Grain yield losses reaching 100% have been reported due to wheat pathogens during high disease pressure and favourable environmental conditions

(Afzal et al., 2007). The most common wheat diseases include: *Fusarium* head blight caused by *Fusarium graminearum* (Schmale and Bergstrom, 2003), common bunt caused by *Tilletia tritica* and *T. laevis* (Mathre, 2000); loose smut caused by *Ustilago tritici* (Wiese et al., 2000); karnal bunt caused by *T. indica* (Department of Agriculture, 2001); powdery mildew caused by *Blumeria graminis* (syn. *Erysiphe graminis* f. sp. *tritici*) (Briceno-Felix et al., 2008) and the wheat rusts. Of all the wheat pathogens rusts are the most studied owing to their detrimental effects on both grain quality and yield (Pretorius et al., 2007; Figlan et al., 2014). The three major wheat rusts include stripe rust caused by *Puccinia striiformis f. fp. tritici*; stem rust caused by *P. graminis* Pers. *f. sp. tritici*; and leaf rust caused *P. triticina.* Of the wheat pathogens, *F. graminearum,* is the most destructive wheat disease inflicting considerable quality and yield losses with adverse effects to human and animal health upon consumption of infected grain. *Fusarium* head blight also known as scab, caused by the members of the *F. graminearum* species complex or *Fg* complex, is the most devastating fungal disease affecting wheat, barley and maize and ranked fourth among the top ten plant fungal pathogens (Dean et al., 2012). The causal organisms of FHB include *F. graminearum, F. culmorum* and *F. crookwellense* (Schamele and Bergstrom, 2003; McMullen et al., 2008). *Fusarium* spp. have both sexual and asexual life cycles and follow a generalized life cycle where haploid mycelial structures are formed in both developmental stages (Ma et al., 2013). During the asexual life cycle, the mycelial structures produce three types of mitotic spores, *viz.* microconidia produced from conidiophores, macroconidia produced from sporodochium and clamydospres produced on and within hyphae and macroconidia. This occurs under favourable conditions, typically with temperatures of 25 to 28^0 C and high relative humidity of >90% (Gilbert and Tekauz, 2000; Goswami and Kistler, 2004).

Fusarium head blight infection and disease development begins at the florets during anther extrusion stage and progresses to advanced symptoms which include premature bleaching of the heads due to loss of chlorophyll. This is followed by development of water soaked lesions on the spikelet (Bushnell et al., 2003; Trail, 2009) (Figure 0.1). Infected wheat heads or spikes lack water and nutrients leading to the production of shrived grains. Infected wheat grains are tan, tan-orange, brown or dark brown in colour showing shrinkled and thin appearance (Figure 0.1) Wheat grains infected by FHB contain

mycotoxins produced by the *Fusarium* spp. The predominant forms of mycotoxins include Deoxynivalenol (DON) and Zearalenone (ZEA) and pose major health risks to humans and animals upon consumption of infected wheat products (Bottalico and Perrone, 2002).

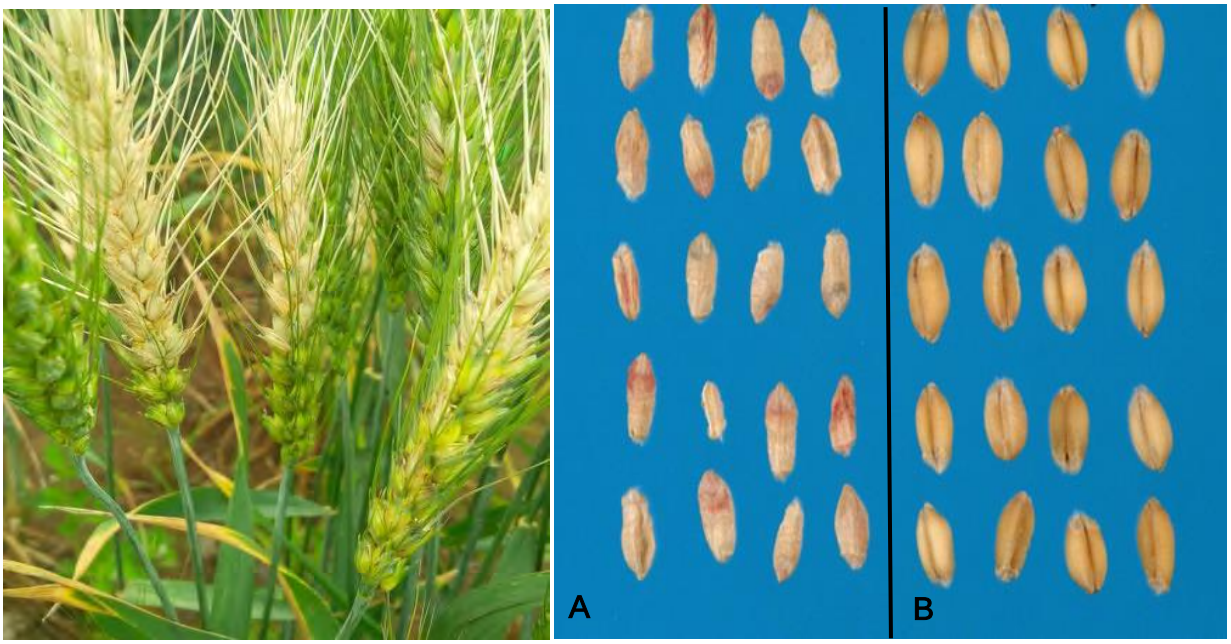

Figure 0.1: Wheat heads showing FHB infection in the field (left photo) and differences between FHB infected wheat kernels (A) and healthy kernels (B) (Schamale and Bergstrom, 2003)

Fusarium head blight has been reported in epidemic proportions globally. Initially the disease was described as a threat to wheat and barley production in England in 1884 (Goswami and Kistler, 2004). In South Africa FHB caused high level of disease outbreaks resulting in significant economic losses (Makandar, 2006; Kriel and Pretorius, 2008; Salgado et al., 2015). With the rapidly increasing global temperatures, further FHB outbreaks are predicted to occur in epidemic proportions (Shah et al., 2014).

Various strategies are suggested for the control FHB including the use of fungicides, cultural practices and genetic control through host plant resistance (HPR) (Pirgozliev et al., 2003). Several cultural methods can be used to reduce the intensity of an epidemic or provide long term partial control. The use of early maturing cultivars, early planting and destruction of volunteer wheat and other susceptible grasses, can be effective in reducing

the amount of initial inoculums and early infections (Dill-Macky, 2008). Genetic control of FHB remains the most sustainable and environmentally friendly strategy. Research efforts are directed towards breeding for FHB resistant cultivars to achieve long-lasting control (Mesterhazy et al., 2003). However, FHB resistance breeding is currently hindered by the quantitative nature of the trait, limited understanding of FHB pathogenesis and large size of the host genome (Mesterhazy et al., 2003). Host plant resistance is the corner stone for integrated disease management (IDM) that holds the potential for reduced use of fungicides. Currently the deployment of HPR genes is the focal point of FHB resistance research. The use of molecular markers compliments classical breeding programs, therefore, identification of new molecular markers linked to resistance genes is required in order to enhance the efficiency of breeding programs and subsequently to develop elite wheat lines with long-lasting FHB resistance.

Rationale

Fusarium head blight or scab is a devastating disease of wheat and barley. The disease causes major quality and yield losses in wheat and poses health risks to humans and animals. In South Africa, FHB causes estimated yield losses of up to 70% in wheat. Resistance breeding is currently the most effective and environmentally friendly approach to control FHB. However, there are currently limited FHB resistant genetic resources available in South Africa. Thus, deploying cultivars with resistance genes and searching for new resistance sources are important pre-requisites in FHB resistance breeding. The Agricultural Research Council-Small Grain Institute (ARC-SGI), in collaboration with the International Maize and Wheat Improvement Centre (CIMMYT) and regional collaborators, has consequently actively undertaken a wheat pre-breeding program. Thus far, the program has developed 778 recombinant inbred lines (RILs) of wheat for FHB resistance breeding through designed crosses of FHB resistant donors and local FHB susceptible wheat parents followed by continuous selfing and selection.

Aims and objectives of the study

The overall aim of this study was to determine FHB resistance of diverse, newly developed RILs of wheat using field phenotyping and diagnostic SSR markers.

Furthermore, the study aimed to select the most promising FHB resistant lines for resistance breeding.

The specific research objectives of the study were:

1. To determine the field response of the 778 newly developed RILs and standard check varieties of wheat for FHB resistance and to identify sources of resistance for breeding or direct production, and

2. To determine FHB resistance among 76 wheat lines using field based phenotyping and to determine the genetic background of 11 selected most resistant lines and four susceptible checks using simple sequence repeat (SSR) markers in order to identify novel FHB resistance sources.

Dissertation outline

The dissertation includes three chapters written in the form of discrete research papers, each following the format of a stand-alone research paper. This is the dominant format adopted by the University of KwaZulu-Natal. As such there is some unavoidable repetition of references and some introductory information between chapters. The structure of the dissertation is outlined below:

Chapter	Title
-	Dissertation Introduction
1	Literature review
2	Field response of newly developed recombinant inbred lines of wheat for *Fusarium* head blight resistance
3	Haplotype comparison of new sources of *Fusarium* head blight resistance in wheat
-	An overview of the research findings

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CHAPTER 1

1. Literature review

Abstract

Fusarium head blight (FHB) is one of the main fungal diseases of grain crops such as wheat, barley and maize. The member species of the *Fusarium graminearum* complex produce mycotoxins that cause quality and yield reductions, as well as human and animal health risks. Resistance breeding, integrated with chemical and or cultural control practices has the potential for sustainable control of FHB. However, breeding for FHB resistance has been slow due to limited genetic gains from conventional breeding, requiring complementary genomic tools to explore and manipulate genetic resources. Breeding for FHB resistance in wheat is also hampered by the quantitative nature of the trait, limited understanding of FHB pathogenesis and the large size of the host genome. This paper highlights the state of knowledge on FHB severity, pathogenesis and genetic control strategies. Available genomic technologies used to uncoil the underlying mechanisms of virulence in the dominant FHB species, *F. graminearum,* are further outlined. Interdisciplinary collaboration is required for successful development and deployment of FHB resistant genotypes to wheat growers.

Keywords: Genetic control, Genomics, *Fusarium* head blight, *Fusarium graminearum,* Wheat

1.1. Introduction

Fusarium head blight (FHB) also known as scab, caused by members of the *Fusarium graminearum* species complex or *Fg* complex, is one of the most devastating fungal diseases of grain crops including wheat, barley and maize. The *Fg* complex comprises of more than 16 species (O'Donnell et al., 2004), that infect a range of hosts (van der Lee et al., 2015). These fungal species produce various mycotoxins, notably deoxynivalenol (DON) and zearalenone (ZEA) that are toxic to humans and animals, respectively (Darwish et al., 2014). The predominant species, *F. graminearum* (teleomorph *Gibberella zeae*), is currently ranked fourth among plant fungal pathogens based on its scientific and economic importance (Dean et al., 2012). The infection biology of *F. graminearum* is yet to be fully understood, but some important aspects of the infectious process have been resolved. In addition, signal transduction pathways which promote invasive growth, sexual reproduction and adaptive stress responses contributing to FHB symptoms have been examined (Gu et al., 2015).

The past decade has witnessed major FHB outbreaks causing significant economic losses in cereal crops globally (Kriel and Pretorius, 2008; Lilleboe, 2011; Makandar et al., 2006; McMullen et al., 2012; Salgado et al., 2015). Given the current global warming associated with increased temperatures, major epidemics of FHB are likely to occur in the near future particularly under high humidity conditions, optimal for disease development (Shah et al., 2014). Global climate indices and models could be used to monitor FHB development. This could assist in determining the distribution of the *Fg* complex across the major cereal production agro-ecologies to allow effective monitoring of the occurrence of the disease. Currently, several groups have catalogued the distribution of *Fg* complex members and their chemotype composition from various parts of the world (Przemieniecki et al., 2014; van der Lee et al., 2015). Previous studies attempted to reconcile phylogenetic with chemotypic properties to provide distribution trajectories that are linked to possible mycotoxicoses. Combined, these studies have provided a better picture of the epidemiology of FHB across the globe which can be a useful guide when devising disease management strategies.

Effective management of FHB cannot be achieved through the use of a single control strategy because each has its own limitations. Employing different control strategies including cultural, biological, chemical and host plant resistance are all powerful tools for FHB management. Genetic control, involving breeding for resistance, when integrated with other control methods mentioned, has the potential to be a sustainable FHB control solution. To date, breeding efforts that include integration of conventional approaches with genomic tools such as quantitative trait loci (QTL) mapping have revealed about 52 QTL conferring FHB resistance that are distributed on all wheat chromosomes, except 7D (Buerstmayr et al., 2009). Consequently, targeted breeding and deployment of resistance genes in breeding programs has gained momentum. Nonetheless, underlying factors influencing FHB resistance breeding including the pathogen and its virulence mechanisms, environmental factors promoting pathogenesis, as well as the host and its resistance mechanisms should be understood for effective control of the disease. Further, various agronomic traits including plant height and flowering biology during anthesis (anther retention/exclusion) should be evaluated together with FHB sensitivity scores to deduce their association with the development of the disease (Malihipour et al., 2016). For instance, various studies have shown that plant height and anther exclusion are negatively correlated to FHB severity, suggesting that tall genotypes that do not retain their anthers could have some levels of resistance to FHB (Yan et al., 2011; Lu et al., 2013; Moidu et al., 2015). This review highlights FHB pathogenesis, infection mechanisms, chemotypic distribution and control options available for integrated management of FHB. Emphasis is given to resistance breeding as a key pillar to a sustainable control strategy.

1.2. The impact of *F. graminearum* and its mycotoxins

Fusarium head blight was first described as a major threat to wheat and barley in England in 1884 (Goswami and Kistler, 2004). Since then, numerous epidemics have been reported worldwide, costing millions to billions of US dollars in some parts of the world including the US (McMullen et al., 2012). Natural toxins in grain lots, feed and general food chain are commonly plant secondary metabolites, bacterial toxins, pycotoxins and mycotoxins that require constant monitoring (Berthiller et al., 2013). Likewise, existence

of *F. graminearum* toxins in cereal grains and animal feeds have long been of global concern (Pleadin et al., 2013; da Rocha et al., 2014).

Due to mycotoxin production by the *Fg* complex species, chemotyping is key in monitoring the impact of FHB mycotoxins on human and livestock health. Type B trichotecenes, deoxynivalenol (DON), nivalenol (NIV) and their derivatives particularly 3-acetyl and 15-acetyl deoxynivalenol (3ADON and 15ADON) and 4-acetyl nivalenol (4ANIV) are some of the most important fungal toxins. As such, research have set out to understand some of the mechanisms underlying the clinical outcomes of FHB mycotoxins. Table 1.1 summarizes the geographic regions where some key FHB species dominate as reviewed by van der Lee et al., (2015). The trend shows that 15-DON is the dominant FHB chemotype globally.

The FHB incidence was highly associated with type B trichothecenes including DON (Boutigny et al., 2012). Concentrations of DON were also found to be up to 2356 µg/kg, which is beyond the regulatory limit, of 1000 µg/kg, in commercial compound feed samples supplied by the Animal Feed Manufacturers Association (AFMA) of South Africa between 2010 and 2011 (Njobeh et al., 2012). Evidently, trichothecenes such as DON are the most common mycotoxins of *F. graminearum* found as contaminants in foods and feeds in sub-Saharan Africa (Stoev et al., 2010; Rodrigues et al., 2011;Njobeh et al., 2012; Darwish et al., 2014). It is, therefore, important to study the physical factors promoting FHB pathotypes to allow monitoring of the disease, particularly in developing countries to limit mycotoxin levels in food and feeds.

Table 1.1: Global distribution of members Fg complex and their salient chemotypic properties

Source of FHB	Country	Chemotype
Fusarium graminearum	Argentina	15-ADON
F. graminearum	Australia	DON and 3ADON
F. graminearum	Brazil	15-ADON
F. graminearum	Canada	3-ADON
F. asiaticum	China	15-ADON
F. graminearum	Denmark	15-ADON
F. graminearum	England	15-ADON
F. aethiopicum	Ethiopia	15-ADON
F. graminearum	Finland	3-ADON
F. graminearum	France	15-ADON
F. graminearum	Iran	15-ADON
F. graminearum	Italy	15-ADON
F. graminearum and *F. asiaticum*	Japan	15-ADON, 3-ADON and NIV
F. chlamydosporum, F. boothii, F. poae, F. scirpi, F. arthrosporioides and F. graminearum	Kenya	DON
F. graminearum	Netherlands	15-ADON
F. graminearum and *F. cortaderiae*	New Zealand	15-ADON and NIV
F. graminearum	Russia	3-ADON and 15-ADON
F. graminearum	South Africa	15-ADON and NIV
F. asiaticum and *F. graminearum*	South Korea	NIV and DON
F. asiaticum	Urugauy	NIV
F. graminearum	USA	15-ADON, 3-ADON and NIV
F. graminearum	Wales	15-ADON

Only dominant species are indicated. Adapted from van der Lee et al., (2015).

Recently, a new field studying the plant metabolites of mycotoxins from plants infected by *F. graminearum,* also known as masked mycotoxins, has gained significant interest. Plants are able to convert the chemical structure of mycotoxins as a defence mechanism

to prevent xenobiotic effects (Galaverna et al., 2009). For instance, plants can convert the *Fusarium* toxins DON and ZEA by Glucosyltransferase enzymes into deoxynivalenol-3-glucoside and zearalenone-14-glucoside, respectively, which are not virulent factors (Berthiller et al., 2015). These substances are often not the primary target of researchers when analyzing toxic compounds in plants. However, there is a chance that chemical reactions during food or feed processing or digestion can revert the masked mycotoxins back to their original toxicological states, which can cause significant human and livestock health problems (Berthiller et al., 2013). Alternatively, some of these hidden mycotoxins could pose health hazards in their present forms. More research on the detection and monitoring of these *F. graminearum* masked mycotoxins is becoming just as essential in wheat grain and bi-products as these mycotoxins can accumulate in significant quantities during FHB disease development without notable negative effects on yield or quality.

Global contamination of food and feeds with mycotoxins is an important problem, with trichothecenes and zearalenone being among the mycotoxins of great agro-economic importance (Zain et al., 2012). Limited research has been conducted to assess the economic losses due to FHB. However, there are widespread reports on the health impacts of FHB infected food and feed to both humans and livestock. Health impacts due to consumption of *Fusarium*-infected cereals include food poisoning symptoms such as diarrhea, abdominal pain and headache in humans, while symptoms such as emaciation are observed in animals fed with FHB-infected feeds (Wegulo, 2012). Development of resistant cultivars and availability of proper infrastructures such as processing, storage and transportation facilities, as well as skilled human resources should be the overriding considerations to minimize the risks associated with FHB mycotoxins. Notably, the risks of major FHB epidemics significantly increase when the relative humidity increases to above 70%. Further, precise prediction of weather conditions is vital to assist farmers and researchers in putting strategic measures in place to minimize disease development and hence, yield and quality losses by FHB. Prediction models such as the Boosted Regression Trees (BRTs) have been developed to enhance FHB forecasting (Shah et al., 2014). For instance, the University of Delaware has a Fusarium head blight prediction center, where signing up to their website allows access to the tools that predict severe FHB epidemics (http://www.wheatscab.psu.edu/).

1.3. *Fusarium* head blight (FHB) pathogenesis

1.3.1. Infectious life cycle of F. graminearum

Fusarium spp. have both sexual and asexual life cycles and follow a generalized life cycle as summarized in Figure 1.1. Haploid mycelial structures are formed in both developmental stages (Ma et al., 2013). During the asexual life cycle, the mycelial structures produce three types of mitotic spores, viz. microconidia produced from conidiophores, macroconidia produced from sporodochium and clamydospres produced on and within hyphae and macroconidia. Mycelia can either form through apomixes, which is restricted to homothallic species, or self-sterile heterothallic species (Figure 1.1). Both sexual orientations result in airborne spores which infect the floral tissues and contaminate the grain with mycotoxins (Ma et al., 2013). *Fusarium* pathogens proliferate and spread rapidly intracellularly followed by development of FHB symptoms involving necrosis and bleaching of heads resulting in shriveled kernels. The pathways of spikelet to spikelet colonization in wheat has been visualized in depth using ultrastructural cellular morphology of wheat cells (Brown et al., 2010). Upon inoculation, invasive mycelia of the FHB spread throughout the spikelet, down into the rachial node and ultimately up and down the rachis until FHB symptoms are clear. It still remains unclear whether *F. graminearum* is a true hemibiotroph or not. Such information will help researchers to give management recommendations that minimize overwintering of the pathogen.

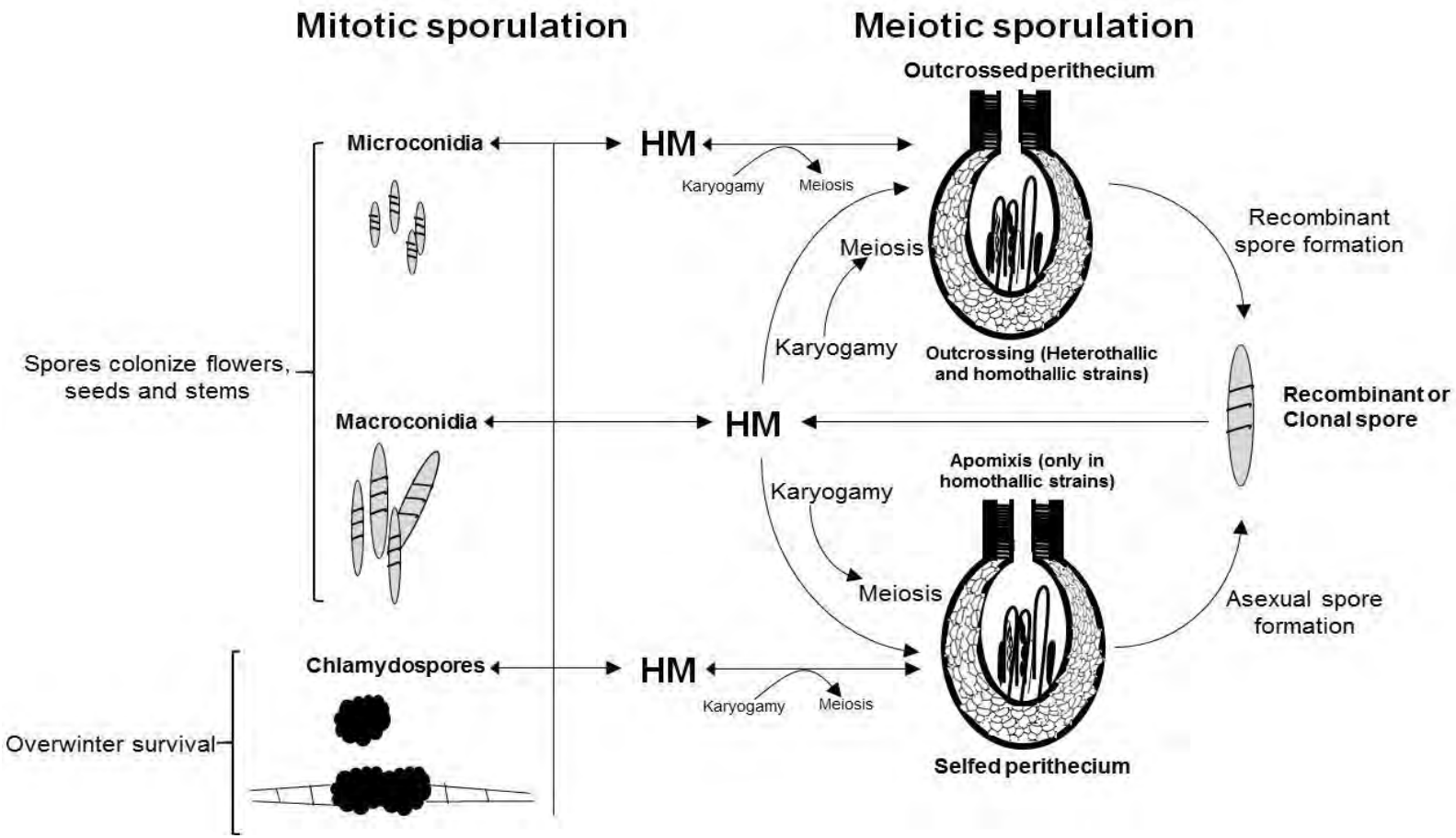

Figure 1.1: Generalized life cycle of *Fusarium* spp. Following plasmogamy and karyogamy, outcorossed and selfed perithecium respectively produce recombinant and clonal meiotic spores. These form haploid mycelium (HM) which in turn form three types of mitotic spores. While conida (micro- or macroconidia) can colonize the host, chlamydospores, in addition to direct colonization of the crop, can overwhinter and develop into perithecium to restart the cycle when conditions are favorable

1.3.2. Virulence in F. graminearum

Host-pathogen interaction is typified by an evolutionary arms race where one species develops mechanisms against another for survival. Fungal pathogens evolve faster than the host, and can bypass plant resistance barriers due to virulence shifts owing to mutation and genetic recombination. Following genome sequencing of *F. graminearum* isolate PH-1 (Cuomo et al., 2007), a larger number of virulent genes were detected. Recently, King et al., (2015) re-sequenced and annotated the PH-1 genome and identified important regions such as AT rich sequences and a retroviral transposon, possibly contributing to the success of this species as a pathogen. Other *Fusarium* species with sequenced genomes are listed by Ma et al., (2013). The sequenced genome of *F. graminearum*, has helped to define some important aspects of *Fusarium* pathogenomics. Table 1.2 summarizes some of the important genes identified through functional genomics with major roles in *F. graminearum* virulence. Genetic determinants, orthologues (where available) and functional conservations in other pathogenic species are useful genomic references. Functional analysis through mutant characterization forms part of the large molecular tool box used to directly and indirectly control disease consequences imposed by FHB.

Table 1.2: Selected genes with major roles in *F. graminearum* virulence

Gene	Role in *F. graminearum*	Orthologues in fungal phytopathogens[a]	Orthologues in human fungal pathogens[b]	Function conserved[c, d]	Reference
FgCID1	Required for the phosphorylation of the carboxy-terminal domain of RNA polymerase II	*F. verticillioides*	*C. albicans*	Yes, but roles may vary in some species	Zhou et al., (2010)
FgFGL1	Encodes lipase gene for the hydrolytic decomposition of triacylglycerols into glycerol and free fatty acids	*F. oxysporum*	n.r.	Yes	Voigt et al., (2005)
FgGPMK1	Regulates conidiation, conidium germination, filamentous growth, sexual reproduction and virulence	*F. oxysporum* and *F. verticillioides*	*C. albicans* and other *Candida* species (CGD)	Yes, but roles may vary in some species	Jenczmionka et al., (2003)
FgHOG1	Regulates sexual reproduction, stress and infection	*M. oryzae* and *Alternaria alternate*	*C. albicans*, *C. neoformans* and *Aspergillus fumigatus*	Yes	Nguyen et al., (2012)
FgILV-2/6	Catalytic (*ILV-2*) and regulatory (*ILV-6*) subunit of AHAS	*M. oryzae* and other fungi	*C. albicans* (CGD)	Yes	Liu et al., (2015)
FgSTE12	Key transcription factor activated by FgGmpk1p	*M. grisea* and *Neuorspora cressa*	*C. albicans* (CGD) and *C. neoformans*	Yes, but roles may vary in some species	Gu et al., (2015)
FgTRI5	Encodes trichodiene synthase required for trichothecene biosynthesis	*Trichoderma arundinaceum* and *T. brevicompactum*	n.r.	Yes	Boenisch and Schäfer (2011)

[a]Only selected fungal phytopathogens are listed; [b]Only selected human fungal pathogens are listed; [c]Basic kinase functions (activation or deactivation by phosphorylation) are conserved. Downstream effectors may differ in other pathogens; [d]Some genes may display additional functions in other fungi. For instance, *STE12* also plays a role in capsule and melanin synthesis in *C. neoformans* (Jung et al., 2015); n.r. – not reported; CGD – Candida genome database.

Deoxynivalenol is a key virulence factor giving the pathogen a “stealth” infection ability associated with FHB infection. Therefore, suppressing its biosynthesis upon host infection will permit virulence reduction. A number of genes encode key proteins involved in the biosynthesis of trichothecenes such as DON. These include the Tri-cluster, which have been studied both in the anamorphic and teleomorphic forms of the FHB pathogens. The cluster houses the *FgTRI5* gene that encodes the enzyme trichodiene synthase (EC 4.2.3.6) which catalyzes the first step of trichothecene biosynthesis. ∆*Fgtri5* (deletion) or *FgTR5* (insertion) will be blocked for trichothecene production and hence is useful as a marker strain apart from being a potential strain for developing antifungal drugs or fungicidal agents. Anamorphic (*F. graminaerum*) and teleomorphic (*Gibberella zeae*) ∆*Fgtri5* may be less virulent against wheat and barley (Maier et al., 2006). Likewise, the revertant or complemented isolates (strains containing the intact copy of the gene *FgTRI5*) of these strains exhibit restored wild type properties (Proctor et al., 1997), an indication that *FgTRI5* deletion is responsible for loss of function.

Boenisch and Schäfer (2011) successfully used ∆*Fgtri5* to investigate the role of trichotecenes in the initial infection stages. In this study, the different infection features of *F. graminearum*, such as the foot structures, infection cushion, infection hypha, lobate appressorium, papillae silica cell, and runner hyphae were defined for the first time. An intriguing observation here is that all the infection structures were also observed in the ∆*Fgtri5* strain, which suggested that DON production occurs distinctively during infection and may be unnecessary for the formation of such structures or necrotic lesions surrounding them. Overall, this analysis implies that trichotecenes are not crucial during the initial stages of *Fusarium* infection since infection structures and necrotic lesions develop independent of DON production (Boenisch and Schäfer, 2011). This in turn suggests that the initial stage of infection is associated with other virulence factors, apart from trichothecene biosynthesis, such as secretion of hydrolytic enzymes. It appears that trichotecenes at this stage might be crucial for suppressing host plant defense systems. These findings could suggest that trichothecene synthesis controls the biotrophic life of *F. graminearum* at the early infection phase and that it controls the necrotrophic life at later infection phases where the fungus extracts nutrients following plant cell death. Therefore, research efforts in finding pathways to manipulate *Fusarium* genome to

interrupting the trichothecene pathway can significantly reduce virulence and improve crop yields and quality. Alternatively, the host genome can be manipulated to permit tolerance to necrosis caused by mycotoxins with the overexpression of transport proteins (Walter et al., 2015).

1.3.3. Factors affecting F. graminearum virulence

Following characterization of Δ*Fgtri5* and other TRI-related strains, several studies enlightened that certain virulence factors determine a wide range of processes including sexual reproduction, filamentous growth, stress and infection. One of these genetic determinants is the mitogen activated protein (MAP) kinase, *FgGPMK1* (Jenczmionka et al., 2003). Independently constructed isolates of Δ*gpmk1* display defects in aerial mycelia, conidiation and sexual reproduction although they display wild type growth rate, culture color and morphology. Wheat spikelet infection tests demonstrated that these mutants were attenuated in comparison to the wild type strain (Jenczmionka et al., 2003). Sexual reproduction was usually linked to increased genetic diversity and adaptive response of the pathogen to its surroundings. Although Δ*Fggpmk1* was not tested against stress-inducing conditions (Jenczmionka et al., 2003), it is likely that the deleted kinase confers an adaptive response to stress. A study has shown that a kinase (Cek2p), orthologous to *Fggpmk1p*, which controls mating and filamentous growth in the major human fungal pathogen, *Candida albicans* (Table 1.2), plays a complimentary role with *Hog1p* (the high osmolarity glycerol kinase) during osmotic and cell wall stress (Eisman et al., 2006). Therefore, *GPMK1*, like MAP kinases in other fungal pathogens, controls interconnected cellular functions which lead to increased virulence.

An orthologue of the model yeast, *Saccharomyces cerevisiae* Hog1 was also implicated in sexual reproduction of *F. graminearum*, suggesting that it is also functional in *G. zeae*, the teleomorphic state (Nguyen et al., 2012). Deletion of the gene, *FgOS-2* (herein referred to as *FgHOG1*), caused a significant reduction in virulence in wheat heads and maize cobs, as well as reduced responses to osmotic stress relative to the wild type strain. This suggested that *FgHOG1*, like *HOG1* in *Candida albicans*, and *Saccharomyces cerevisiae*, as well as other fungal pathogens (Table 1.2), controls multiple cellular processes including adaptive responses, mating and infection. Therefore, several

important functions of *HOG1* are highly conserved across pathogenic yeasts and filamentous fungi (Table 1.2). Wang et al., (2011) provided a guide to the functional analysis of all the kinases of *F. graminearum*. A recent study by Gu et al., (2015) described the downstream transcription factor, *FgSte12*, a homologue of *S. cerevisiae* Ste12p and other pathogenic fungi (Table 1.2). This study found that the mutant, ∆*FgSte12*, like ∆*Fggpmk1*, is impaired for several important functions such as pathogenesis, penetration of cellophane sheet, secretion of protease, cellulose and perithecia development, except for conidiation, filamentous growth and DON production.

In addition, expression analysis indicated that *FgGpmk1p* is a positive regulator of *FgSTE12*. Completing this signaling cascade in *F. graminearum* is crucial since it is intertwined to other infection related pathways. Zhou et al., (2010) set out to characterize a well conserved cyclin C-like gene (*FgCID1)* (required for the phosphorylation of the carboxy-terminal domain of RNA polymerase II) orthologous to the *S. cerevisiae UME3* gene. A strain disrupted for this gene, ∆*Fgcid1*, displayed defects in important virulence factors such as filamentous growth, condition, differentiation of protoperithecia and sensitivity to heavy metal and oxidative stress. Furthermore, during maize stalk infection, the ∆*Fgcid1* strain caused 1.6 ± 0.2 cm stalk rot lesions compared to 6.3 ± 0.5 cm wild type lesions. Deletion of *FgCID1* also resulted in significantly reduced levels of *TRI5* gene and DON, suggesting that FgCid1p controls DON synthesis.

Mutagenic studies in *F. graminearum* offer important clues for linking gene function and virulence associated phenotypes. However, a number of constructed mutants, do not exhibit complete loss of virulence, implicating the roles of other proteins. Secreted proteins are known to play important roles in virulence across the fungal domain. In *F. graminearum*, the secretome has been examined by several authors which uncovered putative roles of cell wall degrading enzymes, effectors and lipases in virulence (Cuomo et al., 2007; Ji et al., 2013; Sperschneider et al., 2015). A margin of these secreted proteins has been directly linked to virulence, possibly due to redundancy. Despite this poor coverage, the secreted lipase, *FgFgl1p*, is responsible for causing infections on wheat and maize cobs (Voigt et al., 2005). A striking observation is that overexpression of *FgFGL1* in MAP kinase disruptants of ∆*Fggpmk1* restores virulence, providing

evidence that *FgFGL1* is a downstream component of FgGmk1p (Salomon et al., 2012). The possibility that *FgGmk1p* also regulates the expression of other hydrolytic enzymes should be carefully investigated. This could be strengthened by understanding of the *F. graminearum* secretome and its functional analysis.

1.4. Control strategies of FHB

1.4.1. Use of bio-control agents

The use of bio-control agents including bacteria and fungi results in reduction of *F. graminearum* and associated toxin production. *Brevibacillus* sp. (strain BRC263), *Streptomyces* sp. (BRC87B), and *Trichoderma gamsii* (6085) are recommended for testing as potential FHB bio-control agents (Matarese et al., 2012). *Pseudomonas flourescens* strains such as MKB 158 and MKB 249, and strain 202 of *P. frederiksbergensis* 202 reduced severities of FHB symptoms and mycotoxins contamination on wheat and barley by more than 23% (Khan and Doohan, 2009). The authors reported that strains of *P. flourescens* significantly reduced DON levels by 74 to 78% in wheat and barley. However, complete and timely eradication of FHB using biological agents has not yet been achieved. Until now, results obtained from a limited number of field experiments and the identified biological agents have not shown complete FHB control. Further studies are required to evaluate their effectiveness, survival and interaction with other organisms or bio-agents under varied agro-ecologies and environmental conditions.

1.4.2. Chemical control

Fungicides have been widely employed, providing limited disease protection, because even the best fungicides are not fully effective in controlling FHB. These factors that contribute to the effectiveness of fungicides need to be considered, (i) cultivar resistance, (ii) climate, (iii) economic returns or yield gain, (iv) fungicide type and dose and (vi) management inputs, which in turn include timing and frequency of application (Mesterházy et al., 2003). A number of fungicides including carbendazim, hexaconazole, mancozeb, benomyl, prochloraz, propiconazole, tebuconazole and triadimenol are useful for FHB control. However, none of these chemicals have resulted in complete FHB

control. Some countries like South Africa, have no chemicals registered for the control of FHB on wheat and barley. From recent reports, there is a high possibility of fungicide resistance development due to over-use of the same types of fungicides. This comes on the ground reports of a recently identified *F. graminearum* isolate that is resistant to the fungicide Tebuconazole in the USA (Spolti et al., 2014) and some strains that are resistant to benzimidazole based fungicide in China (Chen and Zhou, 2009). Hence some of the genes presented in Table 1.2 can be targets for development of improved fungicides.

In promoting adoption of fungicides, the chemical industry should manufacture products with minimal or no side effects to humans. Presently numerous anti-fungal targets are shared between these fungi and humans through some biosynthetic pathways which are missing in the latter. One such pathway is the branched-chained amino acid (BCAA) biosynthetic pathway. The first common enzyme, acetohydroxyacid synthase (AHAS) EC 2.2.1.6, has been one of the centre points for antimicrobial development for a number of major fungal pathogens such as *C. albicans* and *Magnaporthe oryzae*. Recently, the functions of the catalytic subunit, Ilv2p, and the regulatory subunit, Ilv6p of *F. graminearum* AHAS was investigated using targeted gene deletion (Liu et al., 2015). According to this analysis, AHAS subunit mutants, ∆*Fgilv-2* and ∆*Fgilv-6*, in comparison to the wild type and complement strains, displayed cidal phenotypes (auxotrophy) when cultured on medium deprived of amino acids. These mutants also displayed a significant reduction in mycelia, fungal biomass and conidiation when compared to the wild type strain. During infection, as well as during cultivation on sterilized wheat kernels, ∆*Fgilv-2* and ∆*Fgilv-6* severely reduce virulence and DON production, respectively, when compared to the wild type strain. Therefore, for the on-going search of effective fungicides to treat FHB, AHAS could be a potential for target-specific anti-fungal compounds. It would be of great interest to analyze this target in other major plant pathogenic fungi and attempt to develop a single anti-microbial compound which targets all major plant fugal pathogens.

1.4.3. Host plant resistance and genetic control of FHB

1.4.3.1. Types of FHB resistance

There are at least five types of resistance to FHB. Type I involves resistance of the plant to the initial fungal infection and type II occurs when the host plant prevents the spread of infection within the head. Type III involves resistance to the infection of the kernel and type IV is tolerance during which infection is present but without substantial effect on yield and quality losses in wheat. Type V is the ability of the host plant to degrade the mycotoxin that is responsible for virulence (Gilbert and Tekauz, 2000). The best known sources of resistance are those that confer Type I, which can be determined in the field following artificial inoculation by spray and inoculated grain/stable residue under favorable environmental conditions. Gilbert and Tekauz (2000) suggested that Type II resistance is more reliably assessed under controlled conditions because disease symptoms are restricted to the inoculated florets. Wheat cultivars that incorporate Type I and Type II resistance mechanisms are preferable as the resistance would be more stable and durable. It is often common to have cultivars containing more than one type of resistance because QTL for FHB generally confer a response on two or more types of FHB resistance though at varying levels.

1.4.3.2. Genetic improvement of FHB resistance in the host plant

Manipulation of genes conferring resistance to *F. graminearum* through various plant breeding and biotechnology techniques have been underway since the 1990s, with research currently focusing on identifying and cloning genes involved in plant defense responses. Yet there is no wheat cultivar that has been identified and released with complete resistance or immunity to the FHB pathogen. This is partly because resistance to FHB is a quantitative trait that is controlled by many genes with quantitative inheritance leading to limited genetic gain during breeding. Some of the factors that influence the long-term success of breeding resistant cultivars are; (i) the nature of the pathogen and diversity of virulence in the population (ii) availability and type of genetic resistance and (iii) the screening methodology and selection environments available for tracking resistance. Application of molecular markers compliments classical breeding; as supported by various studies on the development and application of molecular markers in wheat resistance improvement (Yang et al., 2003).

Over the last two decades, intensive research has been focusing on mapping QTL that influence various traits. Consequently, over 100 QTL have been identified impacting resistance to FHB with varying levels of significance. One of the major QTL is *Fhb1,* derived from the Chinese wheat cultivar 'Sumai 3' that is widely used as a source of resistance (Waldron et al., 1999). Cuthbert et al., (2006) mapped this QTL to the distal segment of chromosome 3BS of spring wheat using Sumai 3 as a resistant parent. A later study validated *Fhb1* as a major QTL in FHB resistance using the Chinese wheat line W14 (Chen et al., 2007). Liu et al., (2008) further delineated the *Fhb1* loci to a 261 kb region housing seven candidate genes. However, transgenic efforts to insert most of the identified genes into the genetic background of a susceptible wheat cultivar, Bobwhite, did not improve type II resistance, warranting further studies. Importantly, the study developed and confirmed the effectiveness of the DNA marker (UMN10) as diagnostic for *Fhb1*. Genes located on homologous chromosome 3 of the different wheat genomes, seem to be of great significance in the breeding for FHB resistance in wheat, because a major QTL, *Qfhs.ndsu-3*AS, that explained 37% and 55% of the phenotypic and genetic variation, respectively, in FHB resistance was identified on chromosome 3A (Otto et al., 2002).

A study conducted by Yang et al., (2003) showed that a microsatellite marker located on chromosome 6B explained approximately 21% of the phenotypic variation in one of the populations. A subsequent study to map *Fhb2*, which also controls FHB resistance, located this gene on chromosome 6B (Cuthbert et al., 2007). A novel FHB resistance gene, designated as *Fhb3*, was discovered in an alien species, *Leymus racemosus,* and wheat-*Leymus* introgression lines and was subsequently mapped to the distal region of the short arm of chromosome 7Lr#1 (Qi et al., 2008). Another resistance gene, *Fhb4* (*Qfhi.nau*-4B), was mapped to chromosome 4B in line Wangshuibai (Xue et al., 2010). Chromosome 5A was discovered to harbor another QTL that controls Type I resistance to FHB (Buerstmayr et al., 2002). This was further supported by a study on the FHB resistant wheat line, Wangshuibai, which showed the QTL *Qfhi.nau-5*A, later designated as *Fhb5* and mapped to chromosome 5A. A more recent development was the discovery of *Fhb6,* a novel FHB resistant gene that was initially identified and mapped on the sub-terminal region of the short arm of chromosome $1E^{IS}$#1S of a perennial grass *Elymus*

tsukushiensis (Cainong et al., 2015). The gene was subsequently transferred to chromosome 1AS of wheat resulting in 28% reduction in FHB severity among the progenies carrying this gene. Additionally chromosome 7A was shown to harbor another novel QTL for FHB, designated as *Fhb7AC,* explaining 22% phenotypic variation for type II resistance and 24% for type III resistance (Jayatilake et al., 2011). This QTL has an additive effect suggesting its potential to improve FHB resistance in wheat germplasm. Some other major QTL associated with FHB resistance included *QFHB.caas-2D, QFHB.caas-4B, QFHB.caas-4D, QFHB.caas-5B* and *QFHB.caas-5D* (Xue et al., 2010; Xue et al., 2011).

1.5. Next-generation-sequencing (NGS) in FHB research

Next-generation-sequencing (NGS) technologies are promising future genotyping strategies as they allow high-throughput sequencing of the entire genome leading to the generation of high density maps and discovery of millions of single nucleotide polymorphism (SNPs), some of which could possibly link with the gene of interest. The NGS could provide a platform for the discovery of major genes and QTL for resistance breeding of wheat to FHB. For instance, Xiao et al., (2013) used the Illumina sequencing technology for high-throughput RNA sequencing to discover pathways and genes involved in FHB resistance in wheat. These fast, cost effective and accurate technologies are applied on plants with or without sequenced genomes since they allow for vast amounts of sequence information to be generated.

Genotype by sequencing (GBS) was developed and applied to further expand the use of NGS on large crop genomes such as wheat. It is mostly used in sequencing multiplexed samples, combining molecular marker discovery and genotyping (He et al., 2014). A major advantage of GBS is that it uses data directly from the population being genotyped. The use of NGS allows for fast and precise trait mapping to identify genes and QTL of interest. NGS has allowed for the identification of FHB resistance genes and QTL, as well as for discovery of its complex pathways in wheat and barley (Xiao et al., 2013). More so, the development of high-throughput sequencing technologies are revolutionizing marker assisted selection for FHB towards effective genomic selection (Lorenz et al., 2012),

where large pools of molecular markers will be used to predict the genomic breeding value of populations based on models developed using training populations.

The availability of high throughput systems is enhancing studies on functional and comparative genomics which could possibly identify most of the FHB responsive genes on the crop's genome. This is because the whole genome and transcriptome can be precisely sequenced or profiled to allow comparison of the responses to stress of FHB susceptible and resistant plants, either using the same or different species or genera. Jia et al., (2009) reported high levels of conservation in the patterns of transcriptome accumulation from a comparative transcriptome analysis between wheat and barley infected with *F. graminearum*, however, with differential expression of some transcripts. Such findings show the existence of some levels of collinearity and syntheny on cereal genomes, and support the idea that comparative genomics using different cereal crops have prospects of unveiling complex disease resistance mechanisms. Coupled with various advanced expression analysis tools including transcript, proteome and metabolome profiling approaches, NGS will increase the traceability of the expression and co-expression of genes regulating FHB resistance. Hence, the complex genetic and biochemical responsive mechanisms associated with the interaction of the pathogen with the host could be elucidated (Jia et al., 2009; Xiao et al., 2013). This is rapidly allowing assignment of function to new genes and at the same time, discovering enzymes and other metabolites released by genotypes showing high levels of resistance to FHB.

A metabolomic study was undertaken to understand the resistance mechanism of *Fhb1* through mapping resistance-related metabolites and proteins to metabolic pathways, under FHB infection (Gunnaiah et al., (2012). This study revealed that phenylpropanoid biosynthesis enzymes and metabolites of the shunt phenylpropanoid pathway accumulated more in resistant than susceptible lines, and cell walls thicken due to hydroxycinnamic acid amides and flavonoids deposits. Further, Bernardo et al., (2007) conducted a transcriptomic analysis of transcripts expressed in FHB infected resistant and susceptible wheats and identified 44 differentially expressed genes, with three of the genes that were up-regulated in the resistant cultivar having unknown function. Uncovering such gene-to-transcript-metabolite networks will prospectively guide efforts

to enhance over-expression or introgression of newly discovered genes into elite germplasm, or may lead to the detection of genetic and biochemical markers for use in FHB resistance breeding (Kumaraswamy et al., 2011). Also, researchers need to look into the possibility of copying the chemical formula of over-expressed metabolites, to examine the possibility of developing effective fungicides at varying concentrations. Similarly, functional analysis can be done to trace all the genes and pathways involved in pathogen virulence using both virulent and avirulant strains to discover channels which can be blocked by certain fungicide formulations as discussed in section 6.2.6.

1.6. Prospects of transgenic FHB resistance in wheat

Significant levels of FHB resistance can be achieved through introducing foreign genes with major effects into elite genotypes. Several genes have either been shown or suggested to contribute to FHB resistance owing to their wide capacity to encode proteins involved in scab suppression as summarized in Table 1.3 (Xue et al., 2011). Some of these genes were incorporated from non-*Triticum* genomes, yielding non-negative physiological effects when expressed within the wheat genome (Han et al., 2012). However, some genes like the Arabidopsis *NPR1* gene caused FHB resistance but increased susceptibility to *Fusarium* seedling blight (Gao et al., 2013). Such growth stage specific differential effects should be confirmed before recommending alien genes. Target genes for genetic engineering include encoding enzymes which detoxify DON, and genes responsible for the biosynthesis of antifungal proteins that are fungistatic, fungicidal, or inhibitory to FHB pathogens (Ferrari et al., 2012; Hou et al., 2015).

Table 1.3: Target *trans*-genes influencing FHB resistance in wheat

Gene	Potential effect on FHB resistance in wheat.	Source	Reference
Taxi-III	Delays FHB symptoms in transgenic durum wheat by inhibiting enzymes that degrade xylans, a key cell wall component, to allow fungal to penetrate the host.	Chinese Spring wheat	Moscetti et al., (2013)
PvPGIP2	Confer FHB resistance in transgenic wheat by enhancing the expression of polygalacturonase-inhibiting proteins that inhibit fungal enzymes that digest pectin, a plant cell wall polymer during infection	Bean (*Phaseolus vulgaris* L.)	Ferrari et al., (2012)
Bovine lactoferrin (BLF) gene	Significantly increased FHB resistance in transgenic wheat by encoding broad-spectrum antimicrobial Lactoferrin	bovine	Han et al., (2012)
TaWRKY45 gene	Enhanced resistance against FHB in transgenic wheat	Chinese Spring Wheat	Bahrini et al., (2011)
Barley class II chitinase gene	Enhances Type II resistance to FHB in transgenic wheat by over-expressing the chitinase protein which digests the FHB fungi cell wall.	Barley (*Hordeum vulgare* L.)	Shin et al., (2008)
β-1,3-glucanase gene	Enhances FHB resistance of transgenic wheat through over-expression of *β*-1,3-Glucanases that are involved in host defense by cell wall fungal degradation.	Barley (*Hordeum vulgare* L.)	Mackintosh et al., (2007)
Arabidopsis NPR1 (*AtNPR1*) gene	Reduce severity of FHB by conferring a heritable, type II resistance.	*Arabidopsis thaliana*	Makandar et al., (2006)

Over-expression of some resistance genes existing within the wheat genome under pathogen attack including those encoding stress responsive hormones such as methyl jasmonate, ethylene and salicylic acid (Makandar et al., 2012) have the potential to improve FHB resistance. In addition, over-expression of genes encoding important transcription factors and signaling molecules in plants under FHB attack have been shown to enhance FHB resistance in wheat (Bahrini et al., 2011). Significant FHB resistance observed in various transgenic endeavors indicates that alien genes can potentially boost the genetic diversity and options of tackling the disease (Han et al., 2012). Researchers can take advantage of tools available for comparative genomics to track the genes accounting for differential responses of cereals to *Fusarium* attack, and to test the compatibility and influence of such genes in wheat.

1.7. Conclusions and future prospects

Fusarium head blight is a highly destructive disease of wheat, causing major yield and quality reductions across the world. The use of diagnostic or gene-derived markers is essential for the development of superior cultivars that can withstand fungal diseases. Host plant resistance integrated with other management practices will be the most promising and effective management strategy for FHB control and other known plant pathogenic fungi such as cereal rusts. However, this strategy does not guarantee high resistance to FHB since resistance will break down due to virulence shifts of *F. graminearum.* Therefore, integrated disease management strategy appears to be a suitable option.

In sub-Saharan Africa, research on FHB disease management and control is under-developed. There is a need for global partnerships and collaboration for effective disease control and to minimize yield and quality losses. Global partnerships will allow to tap genomic and genetic resources for FHB management. The global research on FHB was started by the International Maize and Wheat Improvement Centre (CIMMYT) in 2005, which facilitated global communication through the Japan-CIMMYT FHB project (Ban et al., 2006). Likewise the US Wheat and Barley Scab Initiative (USWBSI) also recognized the need of collaboration to combat FHB by developing an early warning or alert system which delivers short massage or email warnings to subscribers when conditions are favorable for scab development (http://www.wheatscab.psu.edu/riskTool.html). Such initiatives enhance exchange of novel FHB resistance sources from gene bank accessions and synthetic wheat derivatives for genetic characterization, gene or QTL mapping and genetic recombination through systematic breeding. Further, this will help to monitor the occurrences and distribution of the pathogen, at the same time allowing farmers to stay alert.

1.8. References

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CHAPTER 2:

2. Field response of newly developed recombinant inbred lines of wheat for *Fusarium* head blight resistance

Abstract

Fusarium Head Blight (FHB), also known as scab, is a devastating disease of wheat and barley. It is caused by *Fusarium graminearum* Species Complex (FGSC), predominantly by *F. graminearum*. The disease causes major quality and yield losses in wheat and poses health risks to humans and animals. Major FHB outbreaks were reported globally and various control strategies have been explored in an attempt to alleviate the occurrence and distribution of FHB. Breeding for FHB resistance is considered to be the most economic and environmentally friendly management approach. Consequently, a pre-breeding of wheat is actively undertaken at the Agricultural Research Council-Small Grain Institute (ARC-SGI) in collaboration with the International Centre for Maize and Wheat Improvement (CIMMYT) and regional collaborators. Thus far, the program has developed 778 recombinant inbred lines (RILs) of wheat for breeding through designed crosses and continuous selfing and selection. The objective of this study was to evaluate field response of the newly developed RILs and selected checks to FHB infection in order to identify sources of resistance for breeding or direct production. The 778 RILs, their eight parental lines (Baviaans, Buffels, Duzi, #910, #936, #937, #942 and #1036) and cultivars 'Sumai 3' and 'SST 806' as resistant and susceptible checks, respectively were field evaluated across four environments in South Africa. Test materials were artificially inoculated using five strains of *F. graminearum.* The percentage of heads showing FHB symptoms were rated using a scale of 1-100%. About 6% of the lines had <20% infection rate suggesting the presence of FHB resistance among tested lines. Analysis of variance showed significant differences among genotypes and testing environments. The heritability for FHB resistance was estimated at 64%, indicating the possibility of achieving considerable selection gains in the tested population and environments. Overall, the following five RILs were selected as new sources of resistance: 681 (Buff/1036/71), 134 (Duzi/910/8), 22 (Bav/910/22), 717 (Bav/937/8) and 133 (Duzi/910/7) with mean FHB scores of 6.8%, 7.8%, 9.5%, 9.8% and 10%, respectively. The selected lines are useful genetic resources for resistance breeding against FHB of wheat. Further phenotypic and molecular analyses are required to elucidate the numerous agronomic traits and resistance genes present in order to breed for elite well rounded lines.

Keywords: *Fusarium graminearum, Fusarium* head blight, phenotype, resistance, wheat

2.1. Introduction

Fusarium Head Blight (FHB) also known as scab is a devastating disease of wheat and barley. It is caused by the *Fusarium graminearum* species complex (FGSC), notably by *F. graminearum.* The disease causes considerable yield and quality reductions in wheat. FHB infected wheat grain possess mycotoxins such as deoxynivalenol (DON) and Zearalenone, produced by the pathogen, which are toxic to humans and animals (McMullen et al., 1997; Wegulo, 2012; Zain, 2012). The FHB causing pathogens occur mainly under humid and semi-humid conditions (Goswami and Kistler, 2004). Typically, FHB infection begins at the florets during anthesis stage that is, during anther retention/extrusion, and progresses to advanced symptoms expressing as premature bleaching of the heads (Leonard and Bushnell, 2003; Trail, 2009).

Bread wheat (*Triticum aestivum* L.), durum wheat (*T. durum* Desf.), barley (*Hordeum vulgare* L.) and oats (*Avena sativa* L.) are some of the most important hosts of FHB. In sub-Saharan Africa, FHB outbreaks would incite detrimental economic and social effects, given that these staple cereals are widely grown under low-input production systems. Elsewhere, major FHB outbreaks have been observed since the initial discovery of the disease in England in 1884 (Goswami and Kistler, 2004) resulting in economic losses of billions of dollars (Lilleboe, 2010; Lilleboe, 2011). However, the economic losses of FHB have not been well documented in South Africa. Given the exacerbating global warming, accompanied by increased humidity levels and temperatures, major epidemics of FHB are likely to occur in the near future (Shah et al., 2014). These require effective management and control of the disease in order to enhance productivity of wheat and for human wellbeing. Precise prediction of weather conditions is vital to assist farmers and researchers in putting strategic measures to minimize disease development and subsequently, yield and quality losses by FHB. Some models have been developed to assist wheat producers with FHB predictions and warning, including Boosted Regression Trees (BRTs) by Shah et al., (2014). More recently, a *Fusarium* head blight prediction center was initiated at the University of Delaware, (http://www.wheatscab.psu.edu/) providing prediction services of FHB epidemics in different regions.

Various FHB control strategies have been recommended, however, effective management is not achieved using a single control strategy. Synergistic use of various control strategies remains the best option. Therefore, use of the FHB resistant germplasm is regarded as an important component in the integrated management of FHB. This allows reduced inputs including labour, cost and fungicide use, making breeding resistant cultivars the main goal for FHB researchers. This approach is environmentally friendly and labour efficient when compared to other control strategies. However, breeding progress for FHB resistance has been hindered by the time and cost required to develop elite lines and the complex inheritance of the disease. The International Centre for Maize and Wheat Improvement (CIMMYT) initiated research on FHB in the early 1980s and since then created elite germplasm incorporating effective resistance genes (Osman et al., 2015).

Recently, Osman et al., (2015) conducted phenotypic and genotypic characterization of CIMMYT's international FHB screening nursery of wheat. This provided valuable information such as novel resistance sources that could be utilized by breeders for the creation of elite lines. There is a need to develop regionally adapted cultivars that combine high and stable yield and quality performance with resistance to FHB and other common diseases (Buerstmayr et al., 2009). A number of cultivars were reported to be useful sources of FHB resistance. The most widely used resistance source is the Chinese wheat cultivar 'Sumai 3'. This cultivar has been classified as highly resistant and therefore it has been extensively used in both spring and winter wheat breeding programs globally (Lui, 1984; Wilcoxson, 1993; Niwa et al., 2014). FHB resistance in Sumai 3 is reported to be the most heritable, stable and consistent across environments (Rudd et al., 2001).

The success factors of any resistance breeding program include: availability of efficient screening methodologies, proper selection environment, source and type of resistance, and the nature of the pathogen and its genetic diversity and virulence level (Singh and Rajaram, 2015). Various techniques are proposed for screening FHB resistance in wheat. The most common screening technique involves inoculation of the plant during the anthesis stage followed by provision of conducive environmental conditions. High temperatures (25 to 28°C) and relative humidity (>90%) are ideal conditions to initiate FHB infection and disease development (Rudd et al., 2001;

Goswami and Kistler, 2004). After successful disease development, each entry is evaluated using a rating system to record disease incidence and severity. The most commonly used rating system is the severity scale where the heads of the plants are assessed using severity levels varying between 0 to 100% (Engle et al. 2003). Mesterhazy (1995) described the five FHB resistance mechanisms (Types I to V) in wheat. Type I is associated with resistance to initial infection; Type II denotes the resistance to the spread of the fungus within the plant; Type III is the resistance to kernel infection (the rates of seed infection can differ at a given level of resistance); Type IV refers to tolerance to FHB (tolerant varieties maintain good yield levels despite disease development) and Type V involves resistance against toxin accumulation. Types I and II are the most common forms of resistance where type II is the focus of the present study. In an attempt to develop FHB resistant wheat cultivars, a pre-breeding research program was undertaken at the Agricultural Research Council-Small Grain Institute (ARC-SGI) in collaborations with global and regional wheat researchers to develop FHB resistant germplasm pool. A larger number of genetic resources was acquired from collaborators and about 778 new recombinant inbred lines (RILs) were developed through designed crosses and continuous selfing and selection. The objective of this study was to determine the field response of the newly developed RILs and standard check varieties of wheat for FHB resistance and to identify sources of resistance for breeding or direct production.

2.2. Material and methods

2.2.1. Plant materials and crosses

The study used five new sources of FHB resistance wheat lines obtained from CIMMYT to recombine their genes into local susceptible cultivars. The source parental lines and their pedigrees are designated as follows: BCHA/MILAN (no. 10 of the 9th scab resistance screening nursery [SRSN]), INIA CANURE/INIA TIJERETA (no. 36 of the 9th SRSN), INIA CABURE/LAJ3153 (no. 37 of the 9th SRSN), KAKATSI (no. 42 of the 9th SRSN) and IVAN/6/SABUF/5/SCN/4/RABI/GS/CRA/3/AE.SQUARROSA (190) (no. 36 of the 10th SRSN). Hereafter, the lines are referred to as #910; #936; #937; #942 and #1036, respectively. The five lines were crossed with three South African wheat cultivars (Baviaans [Bav], Buffels [Buff] and Duzi) known for their wide adaptation under local conditions but susceptible to FHB. The source lines and local parent materials were crossed using a bi-parental mating scheme providing 14

populations except the cross of Buff and #936 (Figure 2.1). From the 14 populations a total of 778 RILs were developed through the single seed descent selection method (Table 2.1). Progenies were continuously selfed, selected and advanced to $F_{6:7}$, providing homozygous RILs used in this study.

Table 2.1: Number of RILs of wheat developed in each population for the study

Cross	No. of RILs developed[a]	Cross	No. of RILs developed[a]
Bav/#910	70	Buff/#942	30
Bav/#936	12	Buff/#1036	100
Bav/#937	66	Duzi/#910	50
Bav/#942	67	Duzi/#936	82
Bav/#1036	56	Duzi/#937	56
Buff/#910	33	Duzi/#942	72
Buff/#937	43	Duzi/#1036	41
		Total	778

[a]RILs =Recombinant inbred lines

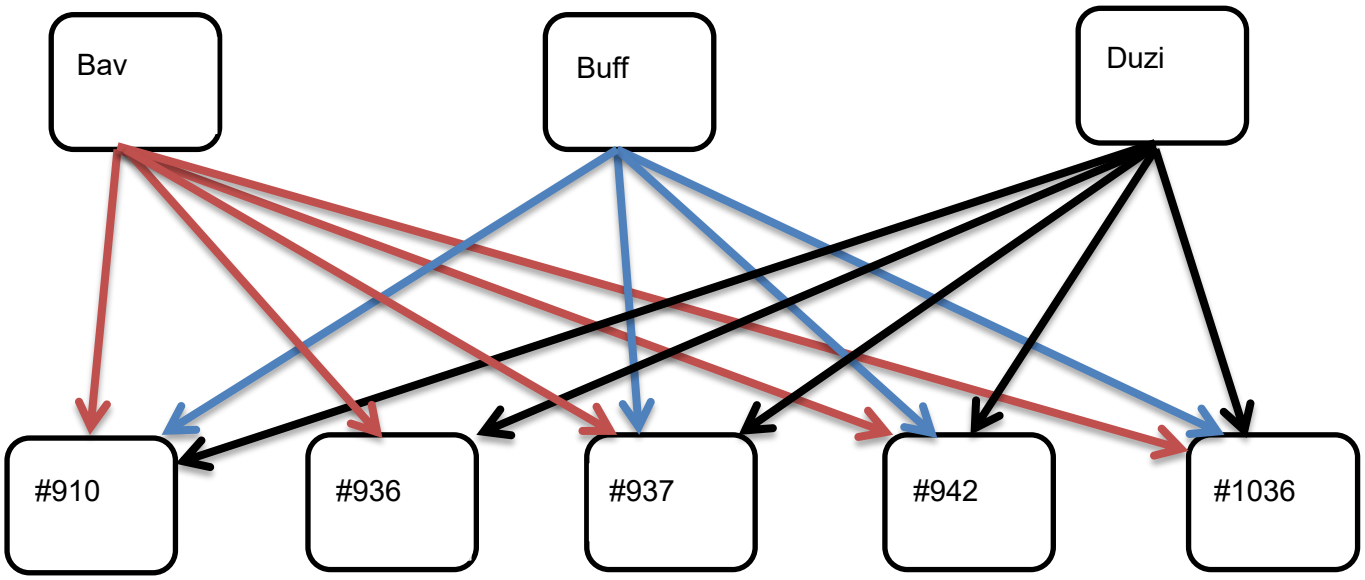

Figure 2.1: Bi-parental crossing scheme used to develop the initial 14 populations used to develop 778 RILs for the present study

2.2.2. Study sites and field establishment

The 778 RILs, two resistant and susceptible checks, cultivars Sumai 3 and SST 806, respectively along with the eight parental lines Bav, Buff, Duzi, #910, #936, #937, #942 and #1036 were evaluated across four environments. Briefly, the environments were as follows: Agricultural Research Council (ARC) Bethlehem Research Station situated in Free State Province during 2014 and 2015 (hereafter denoted as Beth 2014 and Beth 2015, in that order) and Cedara Research Station located in KwaZulu-Natal Province during 2014 and 2015 (Ced 2014 and Ced 2015, respectively). Hereafter the four environments are designated as Beth 2014, Beth 2015, Ced 2014 and Ced 2015.

The test materials were planted in hill plots under irrigation conditions using an augmented design. The parental lines, the susceptible and resistant checks and the 778 RILs were planted for FHB disease assessment. Cultivar Sumai 3, a Chinese wheat cultivar was used as a resistant control, while 'SST 806', a South African commercial wheat cultivar, highly susceptible to FHB served as susceptible control. Fertilizers [3:2:1 (32) + 0.5% Zn; with N 160 g/kg; P 107 g/kg; K 53 g/kg and Zn 5 g/kg, Sasol] were applied. Pre-emergent weeds were controlled using the herbicide Roundup (Pro® Concentrate, Monsanto), while post-emergent weeds were cleaned manually using hand hoes. Fields were irrigated immediately after planting and fortnightly afterwards. Daily average maximum temperatures during both growing seasons ranged between 24-27°C and 15-26°C at Cedara and Bethlehem, respectively. Minimum temperatures between 12-16°C and 0-12°C were recorded across the testing areas, in that order (www.worldweatheronline.com). Average humidity for the growing seasons at Bethlehem and Cedara ranged between 17-90% and 74-94%, respectively.

2.2.3. Inoculum preparation and inoculation

Five isolates of *F. graminearum* (F7.3, N22D, B7.3, F1.01 and B3.8) collected from plant protection laboratory of the ARC-SGI, originally isolated from wheat grown in the Prieska area, in the Northern Cape Province of South Africa were used for the study. The isolates are known for their high virulence and spore production. These isolates were grown on Potato dextrose agar (PDA) for 14-21 days (Figure 2.2A). Liquid inoculum was prepared following the method described by Dill-Macky (2003) with slight modification, where Mung-bean agar was used as a substrate to grow the

cultures. Upon production of fresh sufficient spores (Figure 2.2B), aliquots of the liquid inoculum were prepared and kept at -4ºC prior to inoculations.The inocula from various *F. graminearum* isolates were mixed prior to field inoculations.

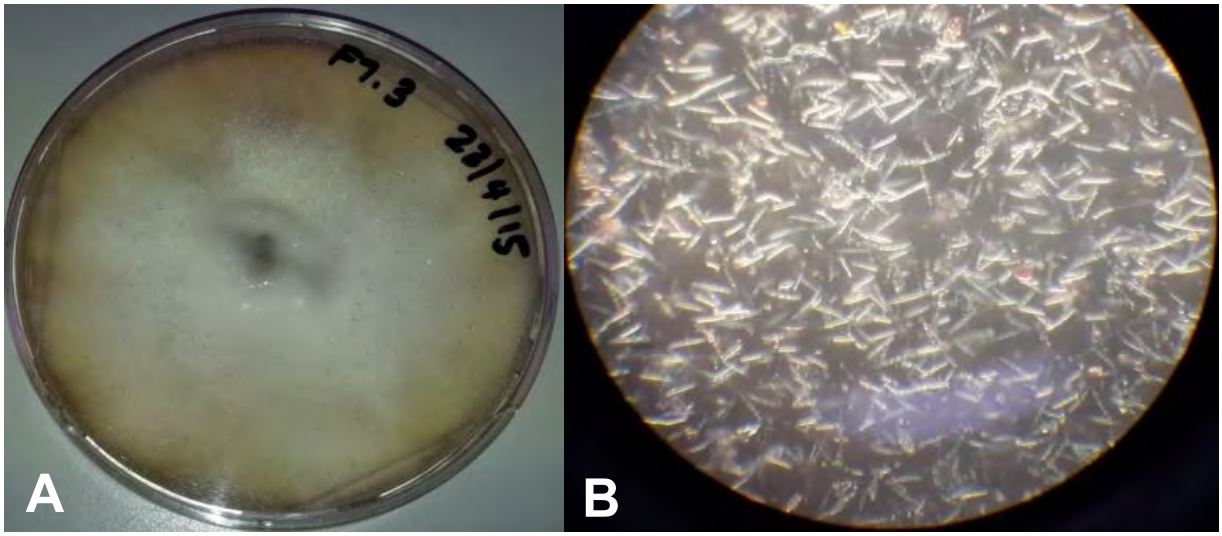

Figure 2.2: (A) *F. graminearium* isolate grown on PDA plate (B) *F. graminearium* spores viewed under microscope

Inoculum was applied to the spikes at the onset of anthesis using a high pressure 16 L capacity Kaufmann knapsack sprayer (Kaufmann), allowing for individual inoculation of plots. Inoculated spikes were kept under humid conditions by covering the inoculated plants with a plastic bag overnight to ensure optimum infection and disease development (Figure 2.3). A second inoculation was applied two days after initial inoculation to minimize escape and to facilitate infection. After inoculation, the field was irrigated 2-3 times a week to ensure humid conditions. Evaluations of the disease occurrence and severity were carried out 28 days post initial inoculation.

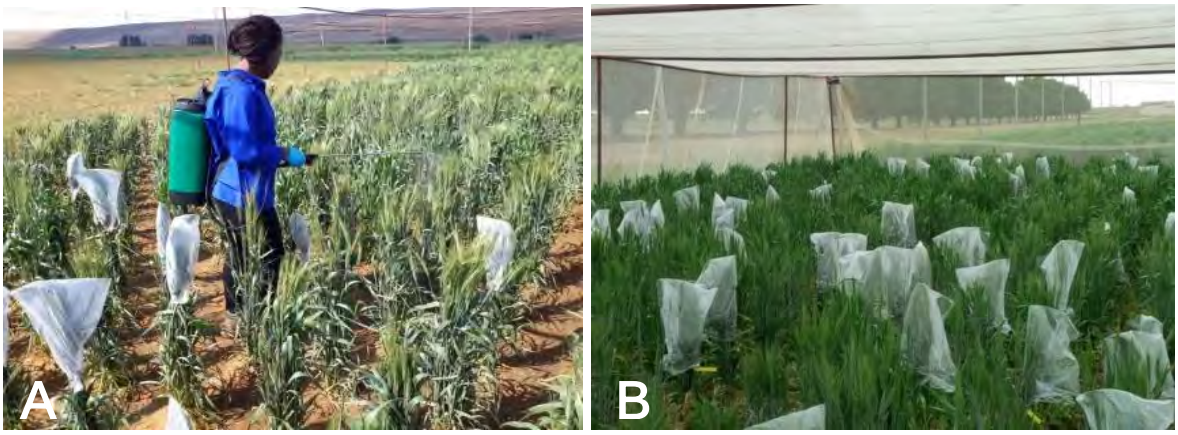

Figure 2.3: Photos showing (A) inoculation of plants with spore suspension of *F. graminearum* isolates and (B) plants covered with plastic bags to ensure high humidity levels after initial inoculation (Bethlehem, 2015)

Colonized grain inoculation method was carried out at the Cedara site. The site has significantly higher humidity level, allowing infection and disease development. Fuentes et al., (2005) suggested that both the colonized-grain and conidial-spray inoculation methods provided disease levels that are appropriate to differentiate resistant and susceptible cultivars. Briefly, dried maize (*Zea mays*) kernels prepared in the form of samp were autoclaved twice over two days and subsequently inoculated with *F. graminearum* following the Dill-Macky (2003) protocol. The colonized grain was incubated at 30°C to promote sufficient production of the fungi. The grain inoculum was then dried and stored at room temperature prior to use. The Cedara field was inoculated uniformly by spreading the *F. graminearum* colonized grains on the soil surface approximately 6 weeks post planting. Irrigations were maintained at 2-3 times a week to ensure humid conditions and provide conducive conditions for disease initiation, infection and development.

2.2.4. Data collection and analysis

FHB severity was scored approximately 4 weeks post heading. The response of sampled wheat lines against FHB were evaluated using the rating scale outlined in Figure 2.4 (Engle et al., 2003). FHB Severity (%) was recorded as the proportion of the heads showing FHB symptoms per RIL including those; with zero severity. FHB severity of the heads was also determined by additional symptoms including pink to salmon-orange spore masses on the infected spikelets and the dark brown/purple discolouration on the stem immediately below the head. To facilitate disease rating, resistant and susceptible checks were planted after every 10-12 RILs in the field. Four of the 778 RILs failed to germinate across all four environments and therefore,

analyses were carried out on 774 RILs. Figure 2.5 depicts the two standard checks: SST806 (A) and Sumai 3 (B) showing 100% and 0% FHB severity during the study, respectively.

Weather conditions were recorded for the four environments during the study period. This included mean temperatures, rainfall and relative humidity. This was intended to establish whether the weather conditions were conducive for infection and disease development.

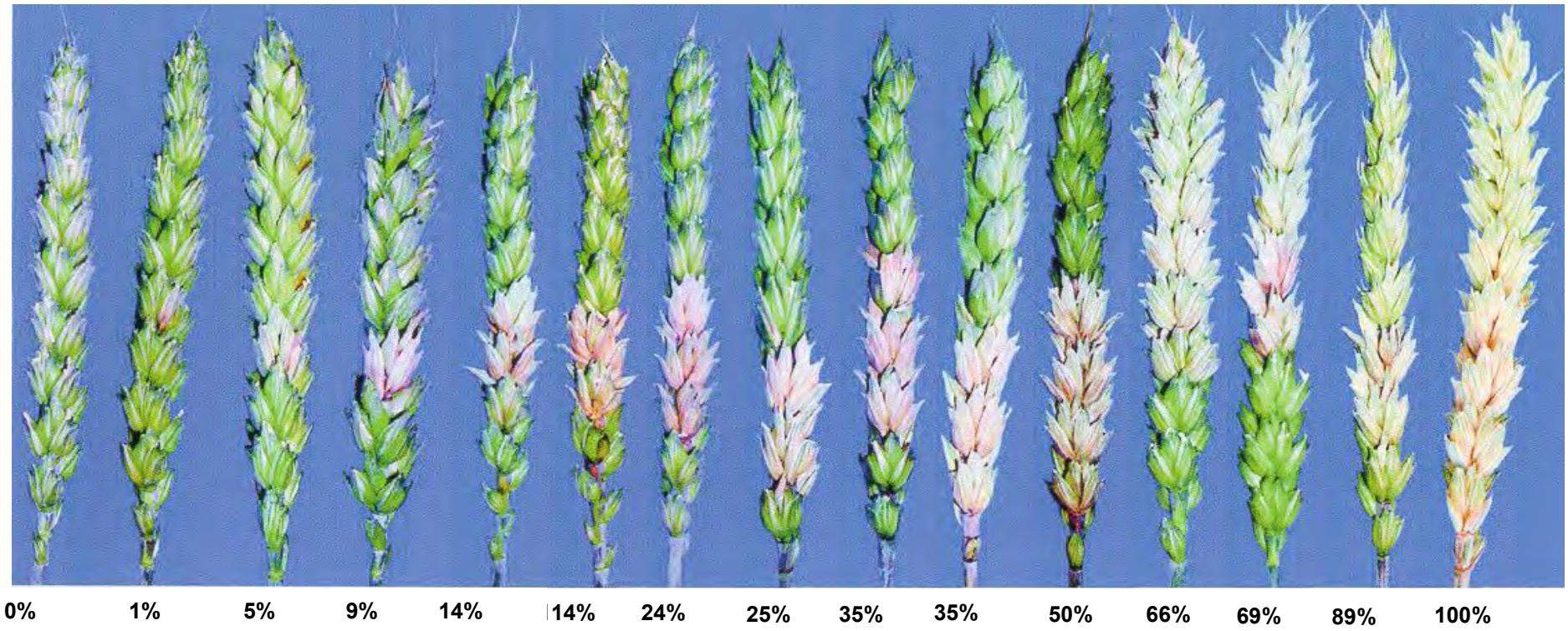

Figure 2.4: FHB severity scale of infected wheat heads, showing percentages of diseased spikelets (Engle et al., 2003)

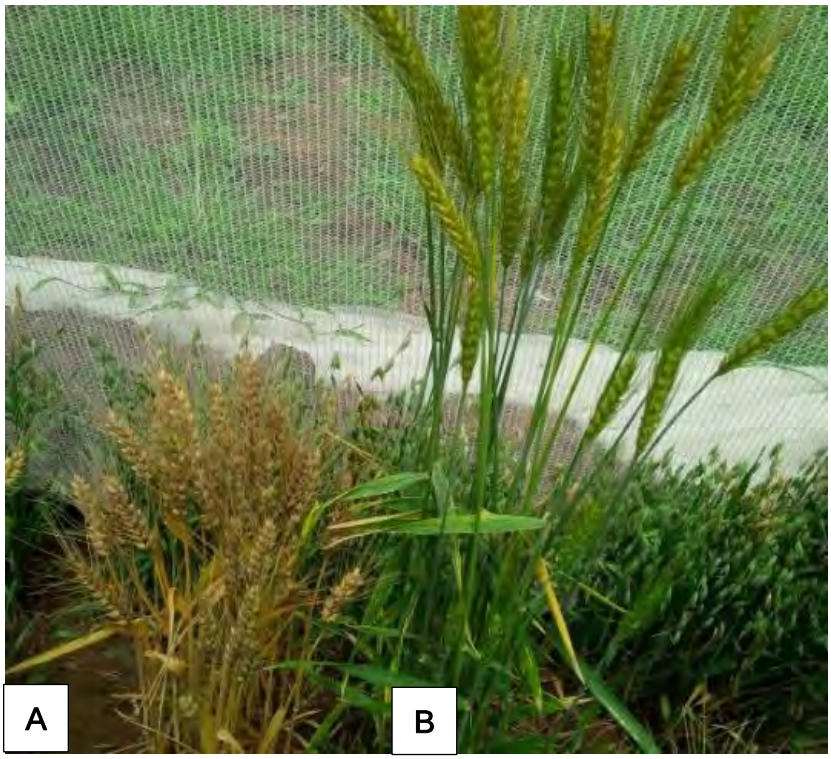

Figure 2.5: FHB susceptible check cultivar SST 806 (A) and resistant cultivar Sumai 3 (B) at Bethlehem site during 2014

Disease severity data was subjected to analysis of variance (ANOVA) using the general linear model procedure (PROC GLM) in SAS (version 9.1; SAS Institute In., Cary NC), with the genotype and environment as random effects. Mean comparisons were performed using the least significant difference (LSD) test procedure at 5% level of significance. The maximum, minimum, range, coefficient of variation (CV) expressed in percent were calculated. Correlation analysis was performed and Pearson's correlation coefficients were calculated to describe the association of disease severity among the four environments. Variance components (genotype and genotype-environment interactions) were used to estimate the broad-sense heritability and to explain the total proportion of the phenotypic variance that is attributed to the genetic variance based on diseases severity. The heritability estimate was calculated using the following formula:

$$h^2_B = 1 - MS_{ge}/MS_g$$

where h^2_B=heritability in the broad sense; MSge=Mean Square of genotype-environment interaction; MSg=Mean square of genotype.

2.3. Results and discussion

2.3.1. Analysis of variance of FHB severity

Two separate analysis of variance were conducted. The first involved the RILs across the four testing environments, and the second considered the 10 check entries (8 parents, 1 susceptible and 1 resistant) only. This allowed analysis of the unreplicated RILs within the environment (Federer, 1961). Both analyses revealed the presence of highly significant (P<0.001) effects of genotype, environment and genotype by environment on FHB severity (Table 2.2) implying differential performance of genotypes across the testing environments. The G x E interaction was tested for significance effect using the error mean square (MS_e) estimated from the check genotypes that were replicated within the environments as described in the augmented design. Further, the analysis suggested the possibility of discerning the most conducive environment for screening of FHB resistance. Heritability for FHB resistance was estimated at 64%, indicating moderate level of response to selection to FHB resistance in the tested populations. The coefficient of variation was 24.77%, suggesting considerable variation of the test lines for FHB disease reaction.

Table 2.2: Analysis of variance of FHB severity involving 774 RILs, eight parents and two check wheat lines evaluated across four testing environments

Source of variation	DF	Mean Square	F value
RILs			
Genotypes	773	1061	2.78**
Environments	3	237923	622.55**
Genotypes x Environments	1850	382.2	8.82**
Checks			
Genotypes	9	4272.82	98.67**
Error	81	43.3063	
CV (%)= 24.77			
LSD (5%)=3.46			

** significant at the 0.01 level of probability.

2.3.2. Field response of RILs, parents and checks for FHB resistance

Table 2.3 presented the field response of the RILs, parents and checks across the four testing environments. FHB severity scores of the RILs showed marked variation across all testing sites. The FHB reading varied from 3.5% to 99% with a mean of 46.65%. Results suggested differential responses of test genotypes for FHB severity. Approximately 10% of the RILs (75) were selected as the best performers showing the least FHB severity scores. About 6% of RILs had a FHB severity score of less than 20%. FHB severity score of <20% is regarded as a relatively resistant reaction type. The FHB reaction of the resistant check varied from 1% to 15% across the four environments, therefore, some of the newly developed RILs possessed comparatively high levels of FHB resistance for selection.

The mean FHB readings at Bethlehem were at 13.23% and 13.56% in 2014 and 2015, respectively. This is a relatively low reading compared to that of Cedara yielding mean scores of 32.25% and 31.61% during 2014 and 2015, in that order. The following five RILs: (Buff/1036/71), 134 (Duzi/910/8), 22 (Bav/910/22), 717 (Bav/937/8) and 133 (Duzi/910/7) had FHB severity scores of ≤10% across the four environments (Table 2.3). The resistant check, Sumai 3, had mean FHB severity scores of 5.96%, 5.46%, 10.43% and 8.28% at Beth 2014, Beth 2015, Ced 2014 and Ced 2015, respectively. Therefore, the five candidate RILs had comparatively good level of FHB resistance. These lines are valuable genetic resources for further breeding or large-scale production under FHB prone environments. The selected RILs expressed consistently low FHB rating across all four environments. The lowest FHB scoring environments were Beth 2014 and Beth 2015 for both the elite lines as well as the resistant checks, while infection levels at Cedara were relatively higher. The Cedara site is a hotspot area of various plant diseases in South Africa, this includes the *Stenocarpella* ear rot (diplodia) which was found to be more prevalent in Cedara compared to other testing sites (Moremoholo, 2012). A recent study revealed a much higher disease pressure of leaf rust in Cedara, compared to other testing sites across the country (Sandiswa Figlan, personal communication).

The parental lines showed significant variability for FHB reaction across the testing sites. The following parents: #1036, #937 and #910 had the lowest FHB scores with mean FHB reading of 17.8%, 30.2% and 35.8%, respectively (Table 2.3). The parents: #936 and #942 had mean FHB scores of 44.1% and 61.3%, respectively. As expected,

FHB susceptible South African wheat lines Baviaans, Buffels and Duzi, had relatively high mean severity scores of 44.83%, 51.4% and 55.7%, respectively (Table 2.3).

Some 29 RILs that were relatively FHB resistant in this study had the donor parent #910 in their parentage (Table 2.3). Whereas, 26 RILs had the donor parent #1036, and 10 RILs had the parent #937 in their pedigree. About 8% and 5% of the resistant RILs had donor parents #942 and #936, respectively. The significant occurrence of the donor parents #910, #1036 and #937 in the pedigree of the promising RILs selected in this study highlights their genetic worthiness for FHB resistance breeding in wheat. The susceptible check 'SST 806' had mean FHB scores of 68.84%, 65.38%, 80.38% and 84.11% at Beth 2014, Beth 2015, Ced 2014 and Ced 2015 testing sites, respectively. The following RILs had high FHB scores: 106 (Bav/1036/36), 367 (Duzi/937/32), 432 (Duzi/1036/41), 221 (Duzi/936/33) and 224 (Duzi/936/36) with mean scores of 95%, 90%, 90%, 89% and 88.3%, respectively. These values were comparable to the susceptible check (Table 2.3).

To successfully identify FHB resistant genotypes, effective phenotyping protocols and testing conditions are crucial (Osman et al., 2015). Populations that segregate for certain traits will often exhibit phenotypes that are on the extreme ends of the scale relative to parental lines (Rieseberg et al., 2003). This genetic phenomenon is referred to as transgressive segregation. Individuals with novel phenotypes better than the parents are often selected due to transgressive segregation for economic traits. Therefore, the RILs selected with FHB resistance or susceptibility comparatively better or worse than their respective donor parents are better explained by transgressive segregation. The segregants in this population have been stabilized through continuous selection and selfing. In addition, these lines may possess additive or dominance genes contributing to the high FHB resistance. Overall, the best performing RILs were derivatives of the best performing parents such as #1036, #937 and #910 reiterating their genetic value for breeding.

The Fusarium Head Blight Screening Nursery (FHBSN) was established in 1985 at CIMMYT, Mexico and has since released numerous FHB resistant candidate wheat lines after strict field screening (He at al. 2013). This strategy has provided for identification of new resistant sources with novel resistance. Similarly, the results from this study allowed for identification of new FHB resistant genetic stocks after successful gene introgression into the South African wheat gene pool. The five best performing genotypes developed in this study are valuable for FHB resistance breeding programs or the wheat industry in South Africa or similar agro-ecologies. There is limited information that reported successful selection or breeding for FHB resistance in South Africa.

Table 2.3: Field severity (%) of FHB among 774 RILs, eight parents and two checks of bread wheat genotypes evaluated across four testing environments in South Africa

		Environment							Environment				
Ser. No.	Genotype	Beth 2014	Beth 2015	Ced 2014	Ced 2015	Mean	Ser. No.	Genotype	Beth 2014	Beth 2015	Ced 2014	Ced 2015	Mean
RILs													
1	Bav/910/1	16	18	55	60	37.25	34	Bav/910/34	10	15	67	78	42.50
2	Bav/910/2	22	55	55	52	46.00	35	Bav/910/35	78	11	98	48	58.75
3	Bav/910/3	25	17	65	95	50.50	36	Bav/910/36	18	60	72	92	60.50
4	Bav/910/4	4	6	41	55	26.50	37	Bav/910/37	26	27	80	88	55.25
5	Bav/910/5	23	18	92	95	57.00	38	Bav/910/38	3	5	65	58	32.75
6	Bav/910/6	6	12	15	69	25.50	39	Bav/910/39	10	61	85	80	59.00
7	Bav/910/7	25	nd	80	nd	52.50	40	Bav/910/40	8	15	75	95	48.25
9	Bav/910/9	5	nd	38	62	35.00	41	Bav/910/41	50	35	nd	nd	42.50
10	Bav/910/10	2	2	94	92	47.50	42	Bav/910/42	15	15	60	nd	30.00
11	Bav/910/11	13	nd	50	nd	31.50	**43**	**Bav/910/43**	**4**	**13**	**23**	**28**	**17.00**
12	Bav/910/12	2	3	75	nd	26.67	44	Bav/910/44	45	7	69	33	38.50
13	Bav/910/13	80	15	43	60	49.50	45	Bav/910/45	12	10	32	nd	18.00
14	Bav/910/14	22	45	70	nd	45.67	46	Bav/910/46	13	47	83	nd	47.67
15	Bav/910/15	20	30	89	62	50.25	**47**	**Bav/910/47**	**10**	**15**	**60**	**60**	**36.25**
16	Bav/910/16	25	20	87	55	46.75	48	Bav/910/48	3	10	30	88	32.75
17	Bav/910/17	18	30	69	45	40.50	49	Bav/910/49	5	25	82	12	31.00
18	Bav/910/18	20	nd	22	nd	21.00	50	Bav/910/50	11	33	58	89	47.75
19	Bav/910/19	3	7	81	48	34.75	51	Bav/910/51	19	42	75	78	53.50
20	Bav/910/20	40	52	93	65	62.50	52	Bav/910/52	6	nd	18	nd	12.00
21	Bav/910/21	9	23	98	68	49.50	53	Bav/910/53	5	15	nd	nd	10.00
22	**Bav/910/22**	**8**	**8**	**10**	**12**	**9.50**	54	Bav/910/54	50	46	70	40	51.50
23	Bav/910/23	5	nd	37	92	44.67	55	Bav/910/55	8	5	50	82	36.25
24	Bav/910/24	45	32	98	90	66.25	56	Bav/910/56	8	8	35	98	37.25
25	Bav/910/25	5	nd	67	nd	36.00	57	Bav/910/57	5	11	82	8	26.50
26	Bav/910/26	12	nd	68	nd	40.00	58	Bav/910/58	17	nd	78	nd	47.50
27	**Bav/910/27**	**2**	**11**	**55**	**35**	**25.75**	**59**	**Bav/910/59**	**6**	**15**	**18**	**62**	**25.25**
28	Bav/910/28	28	nd	62	nd	45.00	**60**	**Bav/910/60**	**5**	**16**	**26**	**35**	**20.50**
29	Bav/910/29	5	47	62	80	48.50	61	Bav/910/61	8	68	79	nd	51.67
30	Bav/910/30	65	11	62	nd	46.00	62	Bav/910/62	18	12	89	85	51.00
31	Bav/910/31	5	5	80	60	37.50	63	Bav/910/63	2	17	40	nd	19.67
32	Bav/910/32	12	15	Nd	65	30.67	64	Bav/910/64	11	10	68	98	46.75
33	Bav/910/33	2	2	78	55	34.25	65	Bav/910/65	8	10	80	nd	32.67

Table 2.3. Continued

		Environment							Environment				
Ser. No.	Genotype	Beth 2014	Beth 2015	Ced 2014	Ced 2015	Mean	Ser. No.	Genotype	Beth 2014	Beth 2015	Ced 2014	Ced 2015	Mean
66	Bav/910/66	3	8	40	nd	17.00	99	Bav/1036/29	11	20	37	nd	22.67
67	Bav/910/67	20	32	53	25	32.50	**100**	**Bav/1036/30**	**17**	**20**	**60**	**71**	**42.00**
68	Bav/910/68	5	15	80	75	43.75	101	Bav/1036/31	20	12	70	nd	34.00
69	Bav/910/69	4	8	54	nd	22.00	102	Bav/1036/32	5	12	35	nd	17.33
70	Bav/910/70	2	5	72	80	39.75	103	Bav/1036/33	41	44	48	72	51.25
71	Bav/1036/1	65	25	55	80	56.25	104	Bav/1036/34	8	12	88	80	47.00
72	Bav/1036/2	62	10	65	nd	45.67	105	Bav/1036/35	11	21	nd	90	40.67
73	Bav/1036/3	50	55	32	85	55.50	106	Bav/1036/36	nd	nd	95	nd	95.00
74	Bav/1036/4	43	8	45	nd	32.00	107	Bav/1036/37	10	BD	45	35	30.00
75	Bav/1036/5	20	30	48	45	35.75	108	Bav/1036/38	50	48	55	80	58.25
76	**Bav/1036/6**	**8**	**2**	**59**	**8**	**19.25**	109	Bav/1036/39	11	20	78	nd	36.33
77	Bav/1036/7	50	45	99	62	64.00	110	Bav/1036/40	7	5	90	33	33.75
78	Bav/1036/8	80	72	70	nd	74.00	111	Bav/1036/41	65	52	70	nd	62.33
79	Bav/1036/9	8	9	52	92	40.25	112	Bav/1036/42	20	17	97	51	46.25
80	Bav/1036/10	8	7	81	80	44.00	113	Bav/1036/43	2	3	87	85	44.25
81	Bav/1036/11	5	5	80	nd	30.00	**114**	**Bav/1036/44**	**25**	**18**	**60**	**70**	**43.25**
82	Bav/1036/12	20	4	65	26	28.75	115	Bav/1036/45	55	nd	23	nd	39.00
83	Bav/1036/13	20	5	82	1	27.00	116	Bav/1036/46	18	18	55	85	44.00
84	Bav/1036/14	13	45	50	nd	36.00	117	Bav/1036/47	20	42	80	nd	47.33
85	Bav/1036/15	15	28	75	90	52.00	118	Bav/1036/48	35	58	nd	nd	46.50
86	Bav/1036/16	28	35	40	80	45.75	119	Bav/1036/49	10	37	75	nd	40.67
87	Bav/1036/17	15	38	58	nd	37.00	120	Bav/1036/50	4	nd	62	nd	33.00
88	Bav/1036/18	30	65	58	75	57.00	121	Bav/1036/51	50	58	99	82	72.25
89	**Bav/1036/19**	**12**	**7**	**49**	**59**	**31.75**	122	Bav/1036/52	45	30	70	42	46.75
90	Bav/1036/20	18	12	78	45	38.25	123	Bav/1036/53	28	37	65	60	47.50
91	Bav/1036/21	33	51	12	75	42.75	124	Bav/1036/54	19	22	98	42	45.25
92	Bav/1036/22	32	10	69	2	28.25	125	Bav/1036/55	30	nd	40		35.00
93	Bav/1036/23	32	28	51	85	49.00	126	Bav/1036/56	12	2	60	nd	24.67
94	Bav/1036/24	45	10	70	9	33.50	**127**	**Duzi/910/1**	**6**	**7**	**10**	**28**	**12.75**
95	Bav/1036/25	50	65	85	90	72.50	**128**	**Duzi/910/2**	**10**	**20**	**10**	**20**	**15.00**
96	Bav/1036/26	20	40	62	80	50.50	**129**	**Duzi/910/3**	**30**	**22**	**22**	**20**	**23.50**
97	**Bav/1036/27**	**12**	**12**	**23**	**1**	**12.00**	**130**	**Duzi/910/4**	**6**	**5**	**60**	**50**	**30.25**
98	Bav/1036/28	20	30	85	88	55.75	131	Duzi/910/5	28	30	28	nd	28.67

Table 2.3. Continued

		Environment							Environment				
Ser. No.	Genotype	Beth 2014	Beth 2015	Ced 2014	Ced 2015	Mean	Ser. No.	Genotype	Beth 2014	Beth 2015	Ced 2014	Ced 2015	Mean
132	Duzi/910/6	30	27	35	50	35.50	**164**	**Duzi/910/38**	**12**	**17**	**48**	**52**	**32.25**
133	**Duzi/910/7**	**2**	**5**	**15**	**18**	**10.00**	**165**	**Duzi/910/39**	**3**	**4**	**19**	**20**	**11.50**
134	**Duzi/910/8**	**5**	**5**	**10**	**11**	**7.75**	166	Duzi/910/40	nd	nd	75	nd	75.00
135	**Duzi/910/9**	**7**	**12**	**34**	**38**	**22.75**	**167**	**Duzi/910/41**	**9**	**35**	**15**	**30**	**22.25**
136	Duzi/910/10	7	15	68	80	42.50	168	Duzi/910/42	9	12	23	89	33.25
137	**Duzi/910/11**	**28**	**10**	**20**	**12**	**17.50**	**169**	**Duzi/910/43**	**18**	**11**	**15**	**18**	**15.50**
138	**Duzi/910/12**	**20**	**30**	**28**	**32**	**27.50**	**170**	**Duzi/910/44**	**23**	**5**	**60**	**52**	**35.00**
139	Duzi/910/13	42	55	95	58	62.50	171	Duzi/910/45	58	40	65	98	65.25
140	Duzi/910/14	40	19	90	89	59.50	172	Duzi/910/46	8	9	nd	nd	8.50
141	Duzi/910/15	70	62	97	nd	76.33	173	Duzi/910/47	37	nd	90	nd	63.50
142	Duzi/910/16	6	50	85	50	47.75	174	Duzi/910/48	17	27	70	nd	38.00
143	Duzi/910/17	63	72	15	32	45.50	**175**	**Duzi/910/49**	**12**	**18**	**19**	**20**	**17.25**
144	**Duzi/910/18**	**2**	**8**	**50**	**68**	**32.00**	176	Duzi/910/50	60	nd	82	nd	71.00
145	Duzi/910/19	15	25	82	77	49.75	177	Bav/936/1	68	50	nd	nd	59.00
146	Duzi/910/20	70	25	55	50	50.00	178	Bav/936/2	75	18	78	nd	57.00
147	Duzi/910/21	5	nd	82		43.50	179	Bav/936/3	55	25	99	nd	59.67
148	Duzi/910/22	25	50	70	82	56.75	180	Bav/936/4	50	10	85	nd	48.33
149	Duzi/910/23	6	nd	51	nd	28.50	181	Bav/936/5	50	23	59	92	56.00
150	**Duzi/910/24**	**5**	**5**	**19**	**15**	**11.00**	182	Bav/936/6	50	42	89	nd	60.33
151	Duzi/910/25	20	19	65	93	49.25	183	Bav/936/7	8	12	99	68	46.75
152	Duzi/910/26	24	50	75	nd	49.67	184	Bav/936/8	35	58	95	85	71.67
153	Duzi/910/27	10	nd	75		42.50	185	Bav/936/9	70	60	18	nd	49.33
154	**Duzi/910/28**	**7**	**15**	**28**	**32**	**20.50**	186	Bav/936/10	11	11	69	nd	30.33
155	Duzi/910/29	62	45	92	nd	66.33	187	Bav/936/11	10	6	62	75	38.25
156	Duzi/910/30	11	50	nd	nd	30.50	188	Bav/936/12	60	42	90	nd	64.00
157	Duzi/910/31	75	nd	92		83.50	189	Duzi/936/1	65	63	91	nd	73.00
158	Duzi/910/32	21	48	68	nd	45.67	190	Duzi/936/2	50	25	60	60	48.75
159	Duzi/910/33	5	5	76	78	41.00	191	Duzi/936/3	67	49	nd	nd	58.00
160	Duzi/910/34	5	2	nd	nd	3.50	192	Duzi/936/4	25	57	91	nd	57.67
161	**Duzi/910/35**	**12**	**28**	**15**	**25**	**20.00**	193	Duzi/936/5	50	nd	nd	nd	50.00
162	Duzi/910/36	50	37	80	nd	55.67	194	Duzi/936/6	56	50	69	nd	58.33
163	**Duzi/910/37**	**6**	**15**	**8**	**15**	**11.00**	195	Duzi/936/7	12	16	95	68	47.75

Table 2.3. Continued

		Environment							Environment				
Ser. No.	Genotype	Beth 2014	Beth 2015	Ced 2014	Ced 2015	Mean	Ser. No.	Genotype	Beth 2014	Beth 2015	Ced 2014	Ced 2015	Mean
196	Duzi/936/8	60	25	20	55	40.00	228	Duzi/936/40	30	36	88	44	49.50
197	Duzi/936/9	50	62	79	85	69.00	229	Duzi/936/41	60	70	nd	nd	65.00
198	Duzi/936/10	50	70	80	nd	65.00	230	Duzi/936/42	58	68	68	50	61.00
199	Duzi/936/11	68	54	87	88	74.25	231	Duzi/936/43	30	30	80	89	57.25
200	Duzi/936/12	73	49	89	nd	70.33	232	Duzi/936/44	45	50	97	nd	64.00
201	Duzi/936/13	37	15	nd	88	46.67	233	Duzi/936/45	65	51	93	68	69.25
202	Duzi/936/14	19	29	82	70	50.00	234	Duzi/936/46	25	21	78	nd	41.33
203	Duzi/936/15	45	39	78	75	59.25	235	Duzi/936/47	40	40	55	nd	45.00
204	Duzi/936/16	23	15	65	68	42.75	236	Duzi/936/48	70	71	69	85	73.75
205	Duzi/936/17	57	50	96	60	65.75	237	Duzi/936/49	60	20	51	75	51.50
206	Duzi/936/18	70	nd	80	nd	75.00	238	Duzi/936/50	40	60	88	78	66.50
207	Duzi/936/19	47	30	60	52	47.25	239	Duzi/936/51	9	8	85	nd	34.00
208	Duzi/936/20	6	15	nd	73	31.33	**240**	**Duzi/936/52**	**6**	**5**	**35**	**28**	**18.50**
209	Duzi/936/21	50	28	89	80	61.75	241	Duzi/936/53	20	nd	89	nd	54.50
210	**Duzi/936/22**	**10**	**5**	**35**	**39**	**22.25**	242	Duzi/936/54	45	18	75	nd	46.00
211	Duzi/936/23	58	28	75	nd	53.67	243	Duzi/936/55	15	54	70	nd	46.33
212	**Duzi/936/24**	**3**	**12**	**60**	**69**	**36.00**	244	Duzi/936/56	12	28	67	90	49.25
213	Duzi/936/25	70	79	79	20	62.00	245	Duzi/936/57	45	22	55	nd	40.67
214	Duzi/936/26	66	39	71	30	51.50	246	Duzi/936/58	17	15	90	nd	40.67
215	Duzi/936/27	52	52	nd	nd	52.00	247	Duzi/936/59	55	48	61	nd	54.67
216	Duzi/936/28	12	27	78	nd	39.00	248	Duzi/936/60	15	3	92	nd	36.67
217	Duzi/936/29	75	57	65	nd	65.67	249	Duzi/936/61	28	30	85	82	56.25
218	Duzi/936/30	68	81	41	80	63.00	250	Duzi/936/62	20	10	75	nd	35.00
219	Duzi/936/31	30	45	nd	nd	37.50	251	Duzi/936/63	20	11	69	50	37.50
220	Duzi/936/32	18	27	54	nd	33.00	252	Duzi/936/64	70	62	nd	nd	66.00
221	Duzi/936/33	nd	nd	89		89.00	253	Duzi/936/65	60	69	90	nd	73.00
222	Duzi/936/34	38	23	37	nd	32.67	254	Duzi/936/66	50	33	82	80	61.25
223	Duzi/936/35	nd	nd	69	nd	69.00	255	Duzi/936/67	50	nd	65	nd	57.50
224	Duzi/936/36	99	68	nd	98	88.33	256	Duzi/936/68	40	43	87	nd	56.67
225	Duzi/936/37	23	10	59	90	45.50	257	Duzi/936/69	50	58	32	85	56.25
226	Duzi/936/38	30	22	87	90	57.25	258	Duzi/936/70	30	19	91	nd	46.67
227	Duzi/936/39	45	54	78	nd	59.00	259	Duzi/936/71	15	22	92	98	56.75

Table 2.3. Continued

		Environment							Environment				
Ser. No.	Genotype	Beth 2014	Beth 2015	Ced 2014	Ced 2015	Mean	Ser. No.	Genotype	Beth 2014	Beth 2015	Ced 2014	Ced 2015	Mean
260	Duzi/936/72	50	59	68	nd	59.00	293	Bav/942/24	45	11	92	nd	49.33
261	Duzi/936/73	75	63	55	75	67.00	294	Bav/942/25	80	58	69	65	68.00
262	Duzi/936/74	62	59	76	nd	65.67	**295**	**Bav/942/26**	**6**	**10**	**60**	**40**	**29.00**
263	Duzi/936/75	5	nd	72	nd	38.50	296	Bav/942/27	72	34	78	nd	61.33
264	Duzi/936/76	62	50	72	88	68.00	297	Bav/942/28	61	42	48	32	45.75
265	Duzi/936/77	20	23	80	nd	41.00	298	Bav/942/29	40	10	30	60	35.00
266	Duzi/936/78	10	32	12	nd	18.00	299	Bav/942/30	45	19	75	68	51.75
267	Duzi/936/79	52	58	nd	80	63.33	300	Bav/942/31	65	65	80	6	54.00
268	**Duzi/936/80**	**19**	**10**	**38**	**35**	**25.50**	301	Bav/942/32	20	46	59	nd	41.67
269	Duzi/936/81	nd	nd	55		55.00	302	Bav/942/33	73	50	45	nd	56.00
270	Bav/942/1	50	40	nd	65	51.67	303	Bav/942/34	50	10	nd	70	43.33
271	Bav/942/2	68	65	57	nd	63.33	**304**	**Bav/942/35**	**3**	**10**	**39**	**35**	**21.75**
272	Bav/942/3	28	15	nd	61	34.67	305	Bav/942/36	90	82	42	nd	71.33
273	Bav/942/4	75	75	88	60	74.50	306	Bav/942/37	10	51	80	90	57.75
274	Bav/942/5	48	49	75	90	65.50	307	Bav/942/38	15	34	70	88	51.75
275	**Bav/942/6**	**23**	**21**	**30**	**40**	**28.50**	308	Bav/942/39	52	68	nd	nd	60.00
276	Bav/942/7	55	47	37	nd	46.33	309	Bav/942/40	63	13	96	nd	57.33
277	**Bav/942/8**	**15**	**6**	**40**	**42**	**25.75**	310	Bav/942/41	40	51	nd	80	57.00
278	Bav/942/9	36	50	55	90	57.75	311	Bav/942/42	72	60	85	nd	72.33
279	Bav/942/10	27	45	nd	85	52.33	312	Bav/942/43	50	51	50	95	61.50
280	Bav/942/11	60	25	nd	80	55.00	313	Bav/942/44	40	99	nd	35	58.00
281	Bav/942/12	58	68	nd	80	68.67	314	Bav/942/45	87	11	69	78	61.25
282	Bav/942/13	42	38	95	30	51.25	315	Bav/942/46	65	58	nd	nd	61.50
283	Bav/942/14	72	50	70	nd	64.00	316	Bav/942/47	65	52	88	89	73.50
284	Bav/942/15	65	48	40	nd	51.00	317	Bav/942/48	30	51	95	nd	58.67
285	**Bav/942/16**	**6**	**5**	**40**	**40**	**22.75**	318	Bav/942/49	51	38	72	90	62.75
286	Bav/942/17	60	38	nd	nd	49.00	319	Bav/942/50	nd	nd	68	nd	68.00
287	Bav/942/18	3	10	85	nd	32.67	320	Bav/942/51	28	15	99	nd	47.33
288	Bav/942/19	67	47	71	90	68.75	321	Bav/942/52	65	56	89	nd	70.00
289	Bav/942/20	80	25	90	90	71.25	322	Bav/942/53	69	29	65	65	57.00
290	Bav/942/21	90	49	89	nd	76.00	323	Bav/942/54	nd	nd	60	nd	60.00
291	Bav/942/22	45	60	65	nd	56.67	324	Bav/942/55	12	4	40	nd	18.67
292	Bav/942/23	50	10	69	10	34.75	325	Bav/942/56	58	63	65	nd	62.00

Table 2.3. Continued

		Environment							Environment				
Ser. No.	Genotype	Beth 2014	Beth 2015	Ced 2014	Ced 2015	Mean	Ser. No.	Genotype	Beth 2014	Beth 2015	Ced 2014	Ced 2015	Mean
326	Bav/942/57	70	50	59	nd	59.67	359	Duzi/937/24	74	40	75	95	71.00
327	**Bav/942/58**	**20**	**15**	**50**	**45**	**32.50**	360	Duzi/937/25	25	63	88	70	61.50
328	Bav/942/59	62	58	80	nd	66.67	361	Duzi/937/26	20	58	85	nd	54.33
329	Bav/942/60	35	25	70	85	53.75	362	Duzi/937/27	60	40	46	70	54.00
330	Bav/942/61	70	22	68	nd	53.33	363	Duzi/937/28	63	27	59	42	47.75
331	Bav/942/62	nd	nd	78	nd	78.00	364	Duzi/937/29	52	55	89	80	69.00
332	Bav/942/63	45	65	75	90	68.75	365	Duzi/937/30	27	37	75	60	49.75
333	Bav/942/64	10	40	79	90	54.75	366	Duzi/937/31	58	20	65	nd	47.67
334	Bav/942/65	63	45	85	nd	64.33	367	Duzi/937/32	nd	nd	90	nd	90.00
335	Bav/942/66	90	75	78	nd	81.00	368	Duzi/937/33	45	57	88	90	70.00
336	Duzi/937/1	60	45	96	17	54.50	369	Duzi/937/34	55	58	85	70	67.00
337	Duzi/937/2	12	16	80	90	49.50	370	Duzi/937/35	50	42	75	8	43.75
338	Duzi/937/3	30	25	98	77	57.50	371	Duzi/937/36	61	10	nd	60	43.67
339	Duzi/937/4	40	60	92	nd	64.00	372	Duzi/937/37	48	nd	88	nd	68.00
340	Duzi/937/5	45	47	90	nd	60.67	373	Duzi/937/38	60	39	85	nd	61.33
341	Duzi/937/6	52	50	88	nd	63.33	374	Duzi/937/39	60	58	90	nd	69.33
342	Duzi/937/7	10	58	93	68	57.25	375	Duzi/937/40	72	60	69	nd	67.00
343	Duzi/937/8	61	42	65	55	55.75	376	Duzi/937/41	50	55	79	nd	61.33
344	Duzi/937/9	28	42	87	nd	52.33	377	Duzi/937/42	21	21	90	50	45.50
345	Duzi/937/10	60	56	69	65	62.50	378	Duzi/937/43	50	48	58	nd	52.00
346	Duzi/937/11	68	55	88	15	56.50	379	Duzi/937/44	45	29	89	89	63.00
347	Duzi/937/12	65	nd	72	nd	68.50	380	Duzi/937/45	60	40	87	75	65.50
348	Duzi/937/13	50	25	88	51	53.50	381	Duzi/937/46	60	45	60	nd	55.00
349	Duzi/937/14	10	20	85	nd	38.33	382	Duzi/937/47	5	19	76	nd	33.33
350	Duzi/937/15	45	33	88	nd	55.33	383	Duzi/937/48	21	25	58	85	47.25
351	Duzi/937/16	20	35	62	95	53.00	384	Duzi/937/49	60	58	60	95	68.25
352	Duzi/937/17	17	10	92	nd	39.67	385	Duzi/937/50	58	47	90	nd	65.00
353	Duzi/937/18	68	11	92	90	65.25	386	Duzi/937/51	67	18	67	nd	50.67
354	Duzi/937/19	65	19	nd	42	42.00	387	Duzi/937/52	24	6	nd	nd	15.00
355	Duzi/937/20	65	31	85	nd	60.33	388	Duzi/937/53	50	30	70	76	56.50
356	Duzi/937/21	57	62	43	nd	54.00	389	Duzi/937/54	4	25	91	nd	40.00
357	Duzi/937/22	62	20	92	65	59.75	390	Duzi/937/55	50	28	82	nd	53.33
358	Duzi/937/23	65	15	80	5	41.25	392	Duzi/1036/1	57	21	45	80	50.75

Table 2.3. Continued

		Environment							Environment				
Ser. No.	Genotype	Beth 2014	Beth 2015	Ced 2014	Ced 2015	Mean	Ser. No.	Genotype	Beth 2014	Beth 2015	Ced 2014	Ced 2015	Mean
393	Duzi/1036/2	50	nd	55	79	61.33	**424**	**Duzi/1036/33**	**35**	**3**	**18**	**19**	**18.75**
394	**Duzi/1036/3**	**18**	**13**	**12**	**18**	**15.25**	425	Duzi/1036/34	30	30	55	80	48.75
395	Duzi/1036/4	45	48	40	81	53.50	426	Duzi/1036/35	37	28	75	90	57.50
396	Duzi/1036/5	65	22	60	86	58.25	427	Duzi/1036/36	88	49	67	nd	68.00
397	Duzi/1036/6	45	8	37	80	42.50	428	Duzi/1036/37	70	nd	60	nd	65.00
398	**Duzi/1036/7**	**6**	**15**	**20**	**35**	**19.00**	429	Duzi/1036/38	52	15	40	nd	35.67
399	Duzi/1036/8	23	25	63	42	38.25	430	Duzi/1036/39	50	2	18	22	23.00
400	Duzi/1036/9	40	5	60	76	45.25	431	Duzi/1036/40	32	52	39	11	33.50
401	Duzi/1036/10	62	nd	71	3	45.33	432	Duzi/1036/41	nd	nd	90	nd	90.00
402	Duzi/1036/11	25	57	50	nd	44.00	433	Buff/1036/1	45	12	38	65	40.00
403	**Duzi/1036/12**	**6**	**10**	**37**	**1**	**13.50**	434	Buff/1036/2	53	10	50	83	49.00
404	Duzi/1036/13	62	21	75	nd	52.67	435	Buff/1036/3	7	3	60	85	38.75
405	Duzi/1036/14	25	4	50	80	39.75	436	Buff/1036/4	15	15	58	nd	29.33
406	Duzi/1036/15	30	40	78	70	54.50	**437**	**Buff/1036/5**	**11**	**10**	**48**	**45**	**28.50**
407	**Duzi/1036/16**	**25**	**27**	**15**	**16**	**20.75**	438	Buff/1036/6	15	nd	65	nd	40.00
408	Duzi/1036/17	40	5	45	85	43.75	**439**	**Buff/1036/7**	**13**	**5**	**58**	**12**	**22.00**
409	**Duzi/1036/18**	**10**	**15**	**35**	**15**	**18.75**	440	Buff/1036/8	95	32	90	90	76.75
410	**Duzi/1036/19**	**25**	**8**	**40**	**35**	**27.00**	441	Buff/1036/9	10	25	97	8	35.00
411	Duzi/1036/20	27	21	nd	75	41.00	442	Buff/1036/10	30	15	45	65	38.75
412	Duzi/1036/21	13	8	50	nd	23.67	443	Buff/1036/11	78	80	40	85	70.75
413	Duzi/1036/22	10	12	82	88	48.00	444	Buff/1036/12	nd	nd	58	nd	58.00
414	**Duzi/1036/23**	**16**	**5**	**40**	**41**	**25.50**	445	Buff/1036/13	45	2	70	50	41.75
415	Duzi/1036/24	21	48	40	8	29.25	446	Buff/1036/14	21	25	48	81	43.75
416	Duzi/1036/25	55	6	61	80	50.50	447	Buff/1036/15	55	10	80	32	44.25
417	Duzi/1036/26	28	nd	52	31	37.00	448	Buff/1036/16	70	15	15	nd	33.33
418	Duzi/1036/27	60	55	90	90	73.75	449	Buff/1036/17	62	10	40	79	47.75
419	Duzi/1036/28	5	nd	89	35	43.00	450	Buff/1036/18	30	nd	88	nd	59.00
420	Duzi/1036/29	27	12	52	90	45.25	**451**	**Buff/1036/19**	**2**	**5**	**50**	**55**	**28.00**
421	Duzi/1036/30	12	18	80	nd	36.67	452	Buff/1036/20	30	23	54	78	46.25
422	Duzi/1036/31	20	35	62	9	31.50	453	Buff/1036/21	15	8	69	7	24.75
423	Duzi/1036/32	62	55	50	nd	55.67	454	Buff/1036/22	20	nd	90	99	69.67

Table 2.3. Continued

		Environment							Environment				
Ser. No.	Genotype	Beth 2014	Beth 2015	Ced 2014	Ced 2015	Mean	Ser. No.	Genotype	Beth 2014	Beth 2015	Ced 2014	Ced 2015	Mean
455	**Buff/1036/23**	**30**	**18**	**20**	**8**	**19.00**	488	Buff/1036/56	52	36	15	45	37.00
456	Buff/1036/24	31	nd	50	nd	40.50	489	Buff/1036/57	12	57	60	85	53.50
457	Buff/1036/25	25	28	78	nd	43.67	490	Buff/1036/58	50	37	30	75	48.00
458	Buff/1036/26	12	28	80	nd	40.00	491	Buff/1036/59	28	12	85	nd	41.67
459	Buff/1036/27	50	30	30	88	49.50	**492**	**Buff/1036/60**	**8**	**18**	**19**	**7**	**13.00**
460	Buff/1036/28	58	5	35	nd	32.67	493	Buff/1036/61	67	65	68	39	59.75
461	Buff/1036/29	18	35	55	nd	36.00	494	Buff/1036/62	36	52	48	25	40.25
462	Buff/1036/30	57	24	62	nd	47.67	495	Buff/1036/63	20	15	68	35	34.50
463	Buff/1036/31	35	40	87	85	61.75	496	Buff/1036/64	50	5	68	nd	41.00
464	Buff/1036/32	10	2	89	nd	33.67	497	Buff/1036/65	20	36	50	5	27.75
465	Buff/1036/33	18	12	48	nd	26.00	498	Buff/1036/66	15	10	70	81	44.00
466	Buff/1036/34	35	7	35	nd	25.67	**499**	**Buff/1036/67**	**30**	**35**	**30**	**35**	**32.50**
467	Buff/1036/35	85	nd	5	80	56.67	500	Buff/937/1	75	nd	55	nd	65.00
468	Buff/1036/36	22	5	60	88	43.75	501	Buff/937/2	37	49	75	nd	53.67
469	Buff/1036/37	51	8	78	60	49.25	502	Buff/937/3	29	11	36	nd	25.33
470	Buff/1036/38	10	8	75	85	44.50	503	Buff/937/4	10	nd	62	nd	36.00
471	Buff/1036/39	32	13	nd	nd	22.50	504	Buff/937/5	27	27	68	nd	40.67
472	Buff/1036/40	40	35	48	90	53.25	505	Buff/937/6	60	50	62	nd	57.33
473	Buff/1036/41	60	45	45	85	58.75	506	Buff/937/7	55	30	40	80	51.25
474	Buff/1036/42	23	10	80	nd	37.67	507	Buff/937/8	55	20	48	39	40.50
475	Buff/1036/43	12	15	57	nd	28.00	508	Buff/937/9	30	10	53	19	28.00
476	**Buff/1036/44**	**18**	**10**	**30**	**30**	**22.00**	509	Buff/937/10	15	50	65	nd	43.33
477	Buff/1036/45	22	9	95	nd	42.00	510	Buff/937/11	15	29	48	40	33.00
478	Buff/1036/46	37	35	nd	60	44.00	**511**	**Buff/937/12**	**25**	**18**	**55**	**60**	**39.50**
479	**Buff/1036/47**	**21**	**16**	**23**	**68**	**32.00**	512	Buff/937/13	69	37	63	45	53.50
480	Buff/1036/48	55	18	42	48	40.75	513	Buff/937/14	42	30	86	nd	52.67
481	Buff/1036/49	35	5	60	78	44.50	514	Buff/937/15	65	35	55	77	58.00
482	Buff/1036/50	46	15	80	80	55.25	515	Buff/937/16	37	45	65	40	46.75
483	Buff/1036/51	70	70	50	nd	63.33	516	Buff/937/17	30	nd	54		42.00
484	Buff/1036/52	35	33	40	nd	36.00	517	Buff/937/18	11	18	62	nd	30.33
485	Buff/1036/53	51	43	54	80	57.00	518	Buff/937/19	35	60	54	nd	49.67
486	Buff/1036/54	30	28	40	10	27.00	**519**	**Buff/937/20**	**25**	**20**	**45**	**19**	**27.25**
487	**Buff/1036/55**	**10**	**25**	**32**	**30**	**24.25**	520	Buff/937/21	40	nd	54	nd	47.00

Table 2.3. Continued

		Environment							Environment				
Ser. No.	Genotype	Beth 2014	Beth 2015	Ced 2014	Ced 2015	Mean	Ser. No.	Genotype	Beth 2014	Beth 2015	Ced 2014	Ced 2015	Mean
521	**Buff/937/22**	**17**	**12**	**25**	**37**	**22.75**	553	Buff/942/11	70	55	89	nd	71.33
522	Buff/937/23	42	8	30	nd	26.67	554	Buff/942/12	60	30	53	nd	47.67
523	Buff/937/24	4	40	87	nd	43.67	555	Buff/942/13	73	45	60	nd	59.33
524	Buff/937/25	60	28	50	79	54.25	556	Buff/942/14	57	40	99	90	71.50
525	Buff/937/26	65	65	73	nd	67.67	557	Buff/942/15	45	69	82	88	71.00
526	Buff/937/27	40	35	55	nd	43.33	558	Buff/942/16	15	29	85	90	54.75
527	Buff/937/28	32	65	72	60	57.25	559	Buff/942/17	58	60	90	nd	69.33
528	Buff/937/29	58	25	75	88.79	61.70	560	Buff/942/18	58	58	60	nd	58.67
529	Buff/937/30	72	40	83	nd	65.00	561	Buff/942/19	25	27	80	nd	44.00
530	Buff/937/31	11	25	35	35	26.50	562	Buff/942/20	58	55	97	5	53.75
531	Buff/937/32	20	35	60	nd	38.33	563	Buff/942/21	15	47	65	85	53.00
532	Buff/937/33	23	5	54	nd	27.33	564	Buff/942/22	57	35	99	nd	63.67
533	**Buff/937/34**	**23**	**18**	**26**	**25**	**23.00**	565	Buff/942/23	50	21	95	nd	55.33
534	Buff/937/35	55	27	30	nd	37.33	566	Buff/942/24	65	51	77	90	70.75
535	Buff/937/36	10	nd	56	nd	33.00	567	Buff/942/25	58	nd	45	90	64.33
536	Buff/937/37	35	15	79	nd	43.00	568	Buff/942/26	60	47	69	nd	58.67
537	Buff/937/38	67	50	60	85	65.50	569	Buff/942/27	75	51	65	nd	63.67
538	Buff/937/39	72	65	69	nd	68.67	570	Buff/942/28	20	7	80	85	48.00
539	Buff/937/40	40	38	89	60	56.75	571	Buff/942/29	60	70	78	nd	69.33
540	Buff/937/41	60	65	65	nd	63.33	572	Buff/942/30	70	60	69	nd	66.33
541	Buff/937/42	35	22	78	86	55.25	573	Duzi/942/1	70	40	72	nd	60.67
542	Buff/937/43	12	35	nd	78	41.67	574	Duzi/942/2	37	48	99	90	68.50
543	Buff/942/1	30	18	88	95	57.75	575	Duzi/942/3	60	57	25	85	56.75
544	Buff/942/2	35	51	85	90	65.25	576	Duzi/942/4	28	20	70	nd	39.33
545	Buff/942/3	47	68	80	nd	65.00	577	Duzi/942/5	77	21	88	80	66.50
546	Buff/942/4	65	72	90	nd	75.67	578	Duzi/942/6	5	nd	nd	nd	5.00
547	Buff/942/5	70	68	91	nd	76.33	579	Duzi/942/7	60	nd	65	88	71.00
548	Buff/942/6	35	21	nd	nd	28.00	580	Duzi/942/8	77	60	48	87	68.00
549	Buff/942/7	68	65	95	80	77.00	581	Duzi/942/9	73	65	nd	90	76.00
550	Buff/942/8	60	45	90	nd	65.00	582	Duzi/942/10	32	10	60	88	47.50
551	Buff/942/9	22	29	90	60	50.25	583	Duzi/942/11	68	67	61	nd	65.33
552	Buff/942/10	52	57	88	nd	65.67	584	Duzi/942/12	20	10	81	40	37.75

Table 2.3. Continued

		Environment							Environment				
Ser. No.	Genotype	Beth 2014	Beth 2015	Ced 2014	Ced 2015	Mean	Ser. No.	Genotype	Beth 2014	Beth 2015	Ced 2014	Ced 2015	Mean
585	Duzi/942/13	96	30	nd	10	45.33	617	Duzi/942/45	55	40	92	nd	62.33
586	Duzi/942/14	45	53	80	61	59.75	618	Duzi/942/46	55	35	57	90	59.25
587	Duzi/942/15	26	15	87	nd	42.67	619	Duzi/942/47	nd	nd	69	nd	69.00
588	Duzi/942/16	nd	nd	83	nd	83.00	620	Duzi/942/48	11	12	58	nd	27.00
589	Duzi/942/17	63	44	85	1	48.25	621	Duzi/942/49	2	nd	59	nd	30.50
590	Duzi/942/18	8	5	92	nd	35.00	622	Duzi/942/50	16	15	nd	90	40.33
591	Duzi/942/19	27	23	30	68	37.00	623	Duzi/942/51	40	42	46	25	38.25
592	Duzi/942/20	60	37	61	78	59.00	624	Duzi/942/52	75	32	43	nd	50.00
593	Duzi/942/21	11	40	50	58	39.75	625	Duzi/942/53	65	10	90	90	63.75
594	Duzi/942/22	50	15	62	nd	42.33	626	Duzi/942/54	50	45	79	75	62.25
595	Duzi/942/23	15	25	nd	72	37.33	627	Duzi/942/55	73	78	80	80	77.75
596	Duzi/942/24	18	20	90	65	48.25	628	Duzi/942/56	45	42	92	88	66.75
597	Duzi/942/25	50	48	78	85	65.25	629	Duzi/942/57	27	35	58	65	46.25
598	Duzi/942/26	67	48	57	88	65.00	630	Duzi/942/58	40	30	47	12	32.25
599	Duzi/942/27	42	53	80	nd	58.33	631	Duzi/942/59	57	49	15	99	55.00
600	Duzi/942/28	80	62	nd	90	77.33	632	Duzi/942/60	30	25	95	35	46.25
601	Duzi/942/29	46	10	65	63	46.00	633	Duzi/942/61	32	70	45	nd	49.00
602	Duzi/942/30	15	8	77	32	33.00	634	Duzi/942/62	27	12	nd	90	43.00
603	Duzi/942/31	40	54	79	nd	57.67	635	Duzi/942/63	50	40	49	58	49.25
604	Duzi/942/32	35	28	90	nd	51.00	636	Duzi/942/64	30	58	42	nd	43.33
605	Duzi/942/33	5	8	70	nd	27.67	637	Duzi/942/65	80	38	20	86	56.00
606	Duzi/942/34	65	48	55	89	64.25	638	Duzi/942/66	50	49	37	nd	45.33
607	Duzi/942/35	78	68	89	70	76.25	639	Duzi/942/67	60	55	50	62	56.75
608	Duzi/942/36	70	60	nd	85	71.67	640	Duzi/942/68	35	45	70	89	59.75
609	Duzi/942/37	18	57	46	nd	40.33	641	Duzi/942/69	65	58	15	98	59.00
610	Duzi/942/38	10	8	72	88	44.50	642	Duzi/942/70	45	40	nd	nd	42.50
611	Duzi/942/39	60	31	75	88	63.50	643	Duzi/942/71	10	48	20	12	22.50
612	Duzi/942/40	65	32	90	55	60.50	644	Duzi/942/72	55	33	20	59	41.75
613	Duzi/942/41	nd	nd	52	nd	52.00	645	Buff/910/1	6	18	40	nd	21.33
614	Duzi/942/42	12	8	95	10	31.25	646	Buff/910/2	3	8	25	nd	12.00
615	Duzi/942/43	65	62	62	50	59.75	647	Buff/910/3	19	9	65	nd	31.00
616	Duzi/942/44	12	nd	95	nd	53.50	648	Buff/910/4	5	3	18	nd	8.67

Table 2.3. Continued

		Environment							Environment				
Ser. No.	Genotype	Beth 2014	Beth 2015	Ced 2014	Ced 2015	Mean	Ser. No.	Genotype	Beth 2014	Beth 2015	Ced 2014	Ced 2015	Mean
649	Buff/910/5	45	42	78	nd	55.00	**681**	**Buff/1036/71**	**4**	**1**	**12**	**10**	**6.75**
650	Buff/910/6	28	25	30	nd	27.67	682	Buff/1036/72	20	18	22	nd	20.00
651	Buff/910/7	14	18	67	nd	33.00	683	Buff/1036/73	47	35	nd	60	47.33
652	**Buff/910/8**	**9**	**11**	**58**	**41**	**29.75**	684	Buff/1036/74	48	32	89	23	48.00
653	**Buff/910/9**	**24**	**32**	**30**	**33**	**29.75**	685	Buff/1036/75	nd	nd	58	nd	58.00
654	**Buff/910/10**	**5**	**5**	**32**	**35**	**19.25**	686	Buff/1036/76	27	30	55	nd	37.33
655	Buff/910/11	15	20	55	88	44.50	687	Buff/1036/77	12	13	78	5	27.00
656	Buff/910/12	8	12	28	nd	16.00	688	Buff/1036/78	55	nd	61	nd	58.00
657	Buff/910/13	8	11	35	95	37.25	689	Buff/1036/79	52	75	85	95	76.75
658	Buff/910/14	15	22	58	nd	31.67	690	Buff/1036/80	25	nd	12	nd	18.50
659	Buff/910/15	45	32	65	80	55.50	691	Buff/1036/81	15	10	28	nd	17.67
660	Buff/910/16	35	35	35	95	50.00	692	Buff/1036/82	59	35	nd	90	61.33
661	Buff/910/17	26	27	88	90	57.75	693	Buff/1036/83	58	45	12	89	51.00
662	Buff/910/18	20	nd	57	35	37.33	**694**	**Buff/1036/84**	**10**	**17**	**15**	**50**	**23.00**
663	Buff/910/19	22	15	50	nd	29.00	695	Buff/1036/85	42	20	63	40	41.25
664	Buff/910/20	58	47	60	nd	55.00	696	Buff/1036/86	7	nd	60	nd	33.50
665	Buff/910/21	38	10	52	58	39.50	697	Buff/1036/87	10	10	65	nd	28.33
666	Buff/910/22	12	12	71	71	41.50	698	Buff/1036/88	5	21	50	68	36.00
667	Buff/910/23	7	8	50	90	38.75	699	Buff/1036/89	10	17	28	nd	18.33
668	Buff/910/24	19	17	55	85	44.00	**700**	**Buff/1036/90**	**12**	**15**	**32**	**10**	**17.25**
669	Buff/910/25	6	4	43	85	34.50	701	Buff/1036/91	20	30	55	5	27.50
670	Buff/910/26	17	7	nd	35	19.67	702	Buff/1036/92	61	41	42	80	56.00
671	Buff/910/27	4	5	nd	75	28.00	703	Buff/1036/93	20	nd	24	nd	22.00
672	Buff/910/28	7	25	38	80	37.50	704	Buff/1036/94	59	25	50	55	47.25
673	Buff/910/29	48	28	38	nd	38.00	705	Buff/1036/95	50	nd	48	nd	49.00
674	Buff/910/30	20	12	nd	80	37.33	706	Buff/1036/96	40	15	48	88	47.75
675	Buff/910/31	35	35	35	40	36.25	707	Buff/1036/97	63	45	49	50	51.75
676	Buff/910/32	22	28	50	79	44.75	708	Buff/1036/98	38	38	41	65	45.50
677	Buff/910/33	20	15	49	53	34.25	709	Buff/1036/99	48	30	51	nd	43.00
678	**Buff/1036/68**	**18**	**18**	**48**	**41**	**31.25**	710	Bav/937/1	20	10	49	nd	26.33
679	Buff/1036/69	32	13	38	68	37.75	711	Bav/937/2	47	55	68	70	60.00
680	Buff/1036/70	52	10	70	nd	44.00	712	Bav/937/3	11	8	75	60	38.50

Table 2.3. Continued

		Environment							Environment				
Ser. No.	Genotype	Beth 2014	Beth 2015	Ced 2014	Ced 2015	Mean	Ser. No.	Genotype	Beth 2014	Beth 2015	Ced 2014	Ced 2015	Mean
713	Bav/937/4	16	8	54	85	40.75	745	Bav/937/36	25	25	55	88	48.25
714	Bav/937/5	18	12	35	nd	21.67	746	Bav/937/37	47	11	18	42	29.50
715	Bav/937/6	28	nd	42	nd	35.00	747	Bav/937/38	10	10	25	10	13.75
716	Bav/937/7	62	45	59	10	44.00	748	Bav/937/39	15	nd	65	60	46.67
717	**Bav/937/8**	**5**	**9**	**10**	**15**	**9.75**	749	Bav/937/40	45	82	54	nd	60.33
718	Bav/937/9	5	nd	28	nd	16.50	750	Bav/937/41	60	nd	nd	nd	60.00
719	Bav/937/10	50	48	38	75	52.75	751	Bav/937/42	10	12	55	80	39.25
720	Bav/937/11	31	28	48	65	43.00	752	Bav/937/43	58	11	17	78	41.00
721	Bav/937/12	20	15	61	38	33.50	753	Bav/937/44	37	35	50	nd	40.67
722	**Bav/937/13**	**28**	**15**	**26**	**27**	**24.00**	754	Bav/937/45	23	11	68	77	44.75
723	Bav/937/14	35	13	49	68	41.25	755	Bav/937/46	27	18	45	60	37.50
724	Bav/937/15	42	20	61	75	49.50	756	Bav/937/47	11	8	20	nd	13.00
725	Bav/937/16	5	10	67	nd	27.33	757	Bav/937/48	15	3	nd	65	27.67
726	Bav/937/17	30	12	54	28	31.00	758	Bav/937/49	12	19	30	68	32.25
727	Bav/937/18	18	10	39	61	32.00	759	Bav/937/50	13	nd	56	nd	34.50
728	Bav/937/19	35	35	50	83	50.75	760	Bav/937/51	5	10	35	63	28.25
729	Bav/937/20	51	42	10	nd	34.33	761	Bav/937/52	7	7	65	65	36.00
730	**Bav/937/21**	**12**	**10**	**15**	**20**	**14.25**	762	Bav/937/53	13	nd	60	nd	36.50
731	**Bav/937/22**	**15**	**13**	**35**	**28**	**22.75**	763	Bav/937/54	21	nd	68	nd	44.50
732	Bav/937/23	40	20	35	65	40.00	764	Bav/937/55	4	nd	51	nd	27.50
733	**Bav/937/24**	**14**	**15**	**33**	**10**	**18.00**	765	Bav/937/56	60	42	nd	88	63.33
734	Bav/937/25	12	nd	54	nd	33.00	766	Bav/937/57	5	nd	nd	nd	5.00
735	Bav/937/26	71	55	20	1	36.75	767	Bav/937/58	5	10	nd	87	34.00
736	**Bav/937/27**	**19**	**27**	**20**	**19**	**21.25**	768	Bav/937/59	25	30	nd	87	47.33
737	Bav/937/28	50	45	12	nd	35.67	769	Bav/937/60	5	12	nd	67	28.00
738	Bav/937/29	7	8	44	65	31.00	770	Bav/937/61	12	7	nd	65	28.00
739	Bav/937/30	35	25	8	68	34.00	771	Bav/937/62	45	38	nd	nd	41.50
740	Bav/937/31	52	15	72	65	51.00	773	Bav/937/64	37	20	nd	20	25.67
741	Bav/937/32	5	12	25	nd	14.00	774	Bav/937/65	7	8	nd	95	36.67
742	Bav/937/33	30	45	68	19	40.50	775	Bav/937/66	4	8	nd	95	35.67
743	Bav/937/34	48	50	72	13	45.75	776	Bav/937/67	nd	8	nd	nd	8.00
744	Bav/937/35	28	6	54	nd	29.33	778	Bav/937/69	12	7	nd	65	28.00

Table 2.3. Continued

Parents

Ser. No.	Genotype	Environment: Beth 2014	Beth 2015	Ced 2014	Ced 2015	Mean
1	#910[a] (BCHA/MILAN)	13.5	18.5	70.5	40.5	35.75
2	#936[a](INIACAB URE/INATIJERE TA)	42.5	48	44	42	44.13
3	#937[a](INIACAB URE/LAJ3153)	29	30	32	30	30.25
4	#942[a](KAKATSI)	68	65	57	55	61.25
5	#1036(IVAN/6/ SABUF/5/SCN/ 4/RABI/GS/CRA /3/AE.SQUARR OSA (190))	15.67	11	25	19.5	17.8
6	Baviaans[b]	37.3	30	60	52	44.8
7	Buffels[b]	45	50	65.6	45	51.4
8	Duzi[b]	57	31	69.8	65	55.7

Checks

Ser. No.	Genotype	Beth 2014	Beth 2015	Ced 2014	Ced 2014	Mean
1	Sumai 3[c]	5.96	5.46	10.43	8.28	7.53
2	SST 806[d]	68.84	65.38	80.38	84.11	74.68

[a] Source parental lines obtained from Scab Resistant Screening Nursery (SRSN) of the International Center for Wheat and Maize Improvement (CIMMYT)

[b] South African irrigation cultivars used in the crosses

[c] Resistant check

[d] Susceptible check

Bold text: 10% of the best performing lines

Highlighted text: Best five RILs across all environments

nd: No data available

Beth2014, Beth2015, Ced2014, Ced2015 denote testing sites at Bethlehem (Beth) and Cedara (Ced) during 2014 and 2015, in that order

Ser. No: Serial Number

2.3.3. Correlation of FHB severity across testing environments

There were significant correlations (P<0.01) of FHB severity scores of genotypes across the four testing environments suggesting similar trend of response to selection. Beth 2014 and Beth 2015 had significantly higher correlation coefficient of 0.579 followed by Ced 2014 and Ced 2015 with a correlation coefficient of 0.294 (Table 2.4). Therefore, a relatively similar pattern of FHB responses was noted on testing the genotypes across the four environments. Correlations of FHB severity between the Bethlehem and Cedara sites were relatively low but positive and significant. Environmental effects play a significant role in the infection process and disease development and ultimate response to FHB in wheat. This was evident from the correlation coefficients recorded using 774 RILs across four testing environments. The Cedara site had higher humidity levels. As such, development of FHB was highly favoured in this site when compared to the Bethlehem site. In addition, experimental trials at Cedara were inoculated with colonized samp kernels which resulted in a reliable disease infection. The Bethlehem site was sprayed with inoculum that had a standardized spore count of $1X10^5$ macroconidia ml^{-1}. Fields inoculated with the same procedure may show a stronger correlation than with the different inoculation methods. However, both inoculation methods provided disease levels that were appropriate to differentiate between resistant and susceptible genotypes. The stronger correlation values signalled the importance of the different environments. Cedara proved to be the best environment for FHB resistance screening as it has environmental conditions conducive for initial infection and high disease development and consequently high levels of infection pressure for accurate selection of promising RILs.

Considerable variability was detected among the RILs, within and across the testing environments. This could be contributed by genotypic (g), environmental (e) and g x e interaction effects. Field management and weather conditions are some aspects influencing the g x e effect. The Cedara site had higher temperatures and humidity conditions (Table 2.5) compared to the Bethlehem site. These conditions favoured high levels of FHB development for efficient selection of wheat genotypes with high level of resistance. These findings concurred with Osman et al., (2015) who reported that high precipitation during the epidemic season showed a higher FHB disease pressure.

Table 2.4: Pearson's correlation coefficients across the four environments

Testing sites	Beth2014	Beth2015	Ced2014	Ced2015
Beth2014	1			
Beth2015	0.579**	1		
Ced2014	0.189**	0.233**	1	
Ced2015	0.184**	0.193**	0.294**	1

**. Correlation is significant at the 0.01 level (2-tailed).

Beth2014, Beth2015, Ced2014, Ced2015 denote testing sites at Bethelem (Beth) and Cedara (Ced) during 2014 and 2015, in that order.

Table 2.5: Weather conditions during the study period across the four testing environments

			Variables				
			Temperature (°C)		Relative Humdity (%)		Rainfall (mm)
Testing sites	Year	Month	Min	Max	Min	Max	
Bethlehem	2014	July	-3.77	16.66	19.66	85.2	0
		August	1.38	19.12	22.56	84.26	10.67
		September	5.17	25.34	14.98	79.01	4.57
		October	8.04	25.59	19.33	86.69	12.7
		November	10.5	22.94	35.75	91	186.18
		December	13.7	26.26	35.06	90.51	102.87
	2015	July	-0.02	16.68	17.78	88.29	17.78
		August	2.1	23.11	0	81.57	0
		September	6.89	23.31	24.89	86.21	24.89
		October	10.49	28.04	32.77	82.25	32.77
		November	9.6	27.66	52.58	82.86	52.58
		December	14.32	31.44	39.37	87.08	39.37
Cedara	2014	August	6.51	23.33	26.24	91.18	2.29
		September	8.34	26.98	26.51	98.76	3.56
		October	10.22	21.66	52.12	99.39	49.79
		November	12.02	22.79	56.22	100	87.89
		December	14.24	25.14	56.34	100	132.58
		January	15.22	27.37	52.53	100	118.61
	2015	August	7.29	24.3	30.14	94.05	2.79
		September	10.17	23.18	43.38	97.38	42.67
		October	11.65	27.19	35.47	98.37	24.89
		November	11.17	24.79	41.08	98.24	54.1
		December	15.65	27.94	46.27	99.65	84.59
		January	15.86	26.54	54.76	100	17.17

2.4. Conclusions

The results of this study indicated the presence of considerable level of variation among the tested RILs for FHB resistance. Only 2% of the RILs had FHB scores greater than the susceptible check, while 60% of the lines scored less than 50%. The study selected promising lines along with the parental lines obtained from CIMMYT. The selected lines are excellent sources of resistance for FHB resistance breeding. Of the 10% selected lines with the least FHB severity score, five lines performed comparable to the resistant check with FHB scores less than 10% averaged across all the four environments. The five best genotypes were 681 (Buff/1036/71), 134 (Duzi/910/8), 22 (Bav/910/22), 717 (Bav/937/8) and 133 (Duzi/910/7). The best parental lines with relatively promising FHB resistance were #910 (BCHA/MILAN), #937 (INIA CABURE/LAJ3153) and #1036 [IVAN/6/SABUF/5/SCN/4/RABI/GS/CRA/3/AE.SQUARROSA (190)]. The testing environment providing high level of FHB infection and development with the higher potential for FHB screening is the Cedara site, attributable to high humidity levels and temperatures compared to the Bethlehem site. The selected wheat lines are useful for wheat growers, researchers and breeders. Although FHB research in South Africa is still in its infancy, this study will contribute to the screening of ARC-SGI wheat germplasm for FHB resistance. However, the selected promising lines need to be further phenotyped for important agro-morphological traits such as grain yield response, nitrogen use efficiency, enhanced water use efficiency and enhanced tolerance to drought and cold stresses and resistance to wheat rust diseases. Furthermore, genotyping of the parental lines is required in order to fully elucidate the FHB resistance genes present with their mode of gene actions as well as identify both the predominant sources of resistance as well as lines that possess novel resistance. This allows for the development of diagnostic markers linked to resistance QTL/genes while identifying possible novel genes of FHB resistance. Pyramiding of resistance genes controlling various pathogens in various selected genetic background is crucial for the development and deployment of elite lines to enhance wheat productivity.

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CHAPTER 3

3. Haplotype comparison of new sources of *Fusarium* head blight resistance in wheat

Abstract

Fusarium head blight (FHB), predominantly caused by the fungus *F. graminearum*, is a global threat to wheat production. The disease causes critical yield and quality losses in wheat and related cereals. In South Africa, FHB causes estimated wheat yield losses of up to 70%. Host plant resistance is currently the most effective and environmentally friendly approach to control FHB. However, there are currently limited FHB resistant wheat genetic resources available. Sumai 3 is the global standard wheat cultivar with significant FHB resistance, owing to its highly effective *Fhb1* gene. There is a critical need of identification of novel FHB resistance sources with desirable genetic backgrounds. In this study, a total of 76 wheat lines were phenotypically characterized in the field; 11 resistant lines were selected and genotyped to identify possible novel resistance sources. Lines were phenotyped across four environments and haplotyped using 24 diagnostic microsatellite markers. Entry #937 showed similar allelic patterns to Sumai 3 at chromosome 3B, presumably containing the prevalent resistance gene, *Fhb1*. Entry #936 showed allele banding patterns similar to Sumai 3 for markers linked to resistance gene, *Fhb5*. Entry #930 showed the presence of three QTL/genes as it had allelic similarities to Sumai 3 for *Fhb2*, 7A-*Fhb7AC* and the 2D/*Rht8* QTL.The rest of the test lines with FHB resistance showed non-significant allelic similarities to the resistant checks, suggesting that the FHB resistance in the new lines is novel. Many genes or blocks of QTL are responsible for controlling FHB resistance; therefore, presence of one allelic form of the same gene does not necessarily translate to resistance. Therefore, there is a need to employ more diagnostic markers that will allow for accurate distinction of the FHB resistant lines. Pyramiding candidate resistance genes is vital in the development of FHB resistant wheat germplasm in a suitable genetic background.

Keywords: *Fusarium* head blight; haplotype; host plant resistance; novel resistance; quantitative trait loci

3.1. Introduction

Fusarium head blight (FHB), predominantly caused by the fungus *Fusarium graminearium*, is the most common and widespread disease of wheat (*Triticum aestivum* L.) worldwide. It is ranked fourth among the top ten important fungal pathogens (Dean et al., 2012). In South Africa, FHB is one of the most prevalent and economically significant diseases under irrigated spring-wheat production systems (Kriel and Pretorius, 2008). Significant yield losses occur under severe disease pressure in combination with growing of highly susceptible cultivars. Furthermore, high moisture conditions during anthesis, maize-wheat crop rotation, no-till and conservation agriculture practices promote FHB infection and disease development (Parry et al., 1995). The past decade has witnessed major FHB outbreaks, leading to significant economic losses in cereal crops globally (Kriel and Pretorius, 2008; Lilleboe, 2011; Salgado et al., 2015). Economic losses of $13.6 million to $2.5 billion have been reported during the past decade due to FHB outbreaks in wheat fields (Nganje et al., 2004; Cowger and Sutton, 2005; Mengistu et al., 2007). However, the economic impact of FHB on wheat in South Africa is not sufficiently documented.

Typically, FHB symptoms are associated with development of white bleached spikes and curled-out awns after flowering. Apart from yield losses, FHB infected grains carry harmful mycotoxins such as Deoxynivalenol (DON) and Zearalenone (ZEA). Infected grains appear shriveled and bleached and are harmful to humans and animals after consumption (Ruckenbauer et al., 2001; Stein et al., 2009).

With the currently increasing global temperatures and relative humidity, FHB epidemics are predicted to increase (Shah et al., 2014). Therefore, breeding for FHB resistance is required to combat this important fungal disease. Gilbert and Tekauz (2000) suggested that although there are other control options, genetic resistance is the most desirable and economical. This has led to the search and discovery of genes that confer FHB resistance that can be exploited in breeding programs to improve elite lines. Several genes have been found to confer resistance to FHB. Furthermore, the development and availability of gene-derived DNA markers make it possible to track these genes. However, breeding progress is hindered because the inheritance of FHB resistance is highly complex and expression is influenced by various factors including multiple genes and the environment and their interaction (Gilbert and Tekauz, 2000).

Host plant resistance (HPR) is the ability of the host to inhibit infection development. Plants have developed a wide range of defense systems such as physical barriers denying the pathogen entry (e.g. development of thick leaf cuticles) (Freeman and Beattie, 2008). HPR is long lasting and more durable, and serves as a corner stone of an integrated disease management (IDM) strategy that will potentially reduce use of fungicides. Deployment of host plant resistance as part of IDM is the most economic and environmentally sound method to control FHB. Different forms of FHB host plant resistance are documented including Type I-resistance to initial infection, Type II-resistance to the spread of disease symptoms within the spike, Type III-resistance to kernel infection, Type IV- tolerance to FHB and Type V- resistance against mycotoxin accumulation (Mesterhazy, 1995).

There are a number of sources of FHB resistance reported in various mapping populations (Niwa et al., 2014; Yu et al., 2008a). The most frequently used and global standard source of FHB resistance is the Chinese wheat cultivar Sumai 3. This cultivar was developed from a cross between two moderately susceptible cultivars Funo and Taiwanxiaomai (Bai and Shaner, 1994). The transgressive segregation patterns observed in the development of the highly resistant Sumai 3 is attributed to the presence of multiple genes controlling FHB resistance. Niwa et al., (2014) identified genetic variations, using SSR markers, among Sumai 3 derivatives collected from six different countries. These results concluded that genetic diversity plays a significant role in FHB resistance response of the various Sumai 3 accessions as well as other wheat cultivars.

For a number of years, FHB resistance QTL in cultivar Sumai 3 have been well documented and utilized globally in the development of FHB resistant varieties. The major QTL that conditions FHB resistance in Sumai 3 are located on chromosomes 3BS (*Fhb1)* providing Type II resistance, 5AS (*Fhb5)* mostly conferring Type I resistance, 6B (*Fhb2*) and 7A (*Fhb7AC)* (Anderson et al., 2001; Buerstmayr et al., 2002; Cuthbert et al., 2007; Jayatilake et al., 2011).

Another well-known FHB resistance source is the Brazilian wheat cultivar Frontana, developed from a cross between Fronteira and Mentana. Frontana has Type I QTL located on chromosome 3A (Steiner et al., 2004). In recent years, other FHB sources with various QTL have been characterized and documented such as: Asozaira (ASO),

Baisanyuehuang (BAI) (Zhang et al., 2012), Catbird (CBRD) (*QFhs.inta-7D*) (Cativelli et al., 2013), Huangcandou (HCD) (*Fhb1, 3BSc QTL, 2D QTL*) (Cai and Bai, 2011), Huangfangzhu (HFZ) (*Fhb1* and *7A QTL*) (Li et al., 2012), Haiyanzhong (HYZ) (*7D QTL*) (Li et al., 2011) and Wangshuibai (WSB) (*Fhb4* and *Fhb5)* (Xue et al., 2010, 2011). These FHB resistant QTL have provided good sources of resistance worldwide. To date, there are no FHB tolerant wheat cultivars available in South Africa. Furthermore, there are no officially approved and registered chemicals or fungicide(s) for the control of FHB on wheat in South Africa (Croplife, 2016).

To broaden the genetic basis of FHB resistance on desired genetic backgrounds and to limit a total dependence on the same sources of FHB resistance, there is a need to search for novel FHB resistant sources for breeding. Several efforts are underway by various research programs around the world to find and locate new genes that confer resistance to FHB. A recent study by De Villiers (2014) identified promising FHB resistant sources from CIMMYT scab resistance screening nursery (SRSN) during greenhouse screening. Field trials were required to confirm and compare the observed resistance with several entries from other SRSNs. Also there is a need to determine if there is any kinship to any of the nine known FHB resistance sources based on pedigree analysis. To effectively conclude on the presence of novel FHB resistance sources, DNA analysis based on SSR marker haplotypes from well-characterized sources is required. Therefore, the objectives of this study were to determine FHB resistance among 76 wheat lines using field based phenotyping and to determine the genetic background of 11 selected most resistant lines and four susceptible checks using SSR markers in order to identify possible novel FHB resistance sources.

3.2. Material and methods

3.2.1. Phenotypic screening for FHB resistance

3.2.1.1. Plant materials and study sites

A total of 76 wheat lines were evaluated in this study. The genotypes included 73 test lines acquired from the 9th and 10th CIMMYT Scab Resistance Screening Nursery (SRSN) along with two resistance checks (Sumai 3 and Marico) and one susceptible check (SST 806). The CIMMYT test lines were developed and integrated into the respective SRSNs following extensive field phenotypic studies by CIMMYT- Mexico. The entry names and pedigree of the 76 wheat lines used in the present study are summarized in Table 3.1.

This study was carried out across four environments. Briefly, the environments were as follows: Agricultural Research Council-Small Grain Institute (ARC-SGI) Bethlehem Research Station in the Free State Province during 2008 and 2009 (hereafter denoted as Beth 2008 and Beth 2009, in that order) and Douglas Research Station situated in the Northern Cape Province during 2008 and 2009 (hereafter denoted as Doug 2008 and Doug 2009, respectively). Resistant and susceptible checks were replicated throughout the trials. A honeycomb planting design was used across all testing sites with 15 seeds planted per entry. Fertilizers [3:2:1 (32) + 0.5% Zn; with N 160 g/kg; P 107 g/kg; K 53 g/kg and Zn 5 g/kg, Sasol] were applied. Pre-emergent weeds were controlled using the herbicide Roundup (Pro® Concentrate, Monsanto), while post-emergent weeds were cleaned manually using hand hoes. Fields were irrigated immediately after planting and fortnightly afterwards.

3.2.1.2. Inoculations and evaluations

F. graminearum isolates were used for artificial inoculation at the Bethlehem trials. A cocktail of *F. graminearium* isolates originally collected from wheat in the Prieska region was used to develop a liquid inoculum that was subsequently sprayed using a mist blower during the flag leaf stage. The Douglas testing site allowed for natural infection. This site is a hotspot area for FHB disease. Evaluations of the entries were carried out three weeks after inoculation and disease severity was recorded as a percentage of the bleached head florets in relation to the total number of florets (see Figure 2.4). As a guide of FHB resistance, the study took into consideration the mean FHB response of each of the 76 wheat lines across the four environments and thereafter best performing wheat lines showing low levels of FHB severity scores (%) were selected.

Table 3.1: Name and pedigree of the 76 wheat lines used in the study.

Ser. No.	Entry	Pedigree	Ser. No.	Entry	Pedigree
1	#969	THB/KEA//PF85487/3/RIVADENEIRA 4	39	#942	KAKATSI
2	#930	Gondo	40	#974	TURACO/5/CHIR3/4/SIREN//ALTAR 84/AE.SQUARROSA
3	#960	SHA4/3/2*CHUM18//JUP/BJY	41	#938	IRENA/CETTIA/5/ND/VG9144//KAL/BB/3/YACO/4/CHIL
4	#975	WUHAN #3	42	#955	PRINIA
5	#947	MILAN/AMSEL//CBRD	43	#961	SURUTU-CIAT
6	#972	TRAP#1/BOW//TAIGU DERIVATIVE	44	#963	SW89-5124*2/FASAN
7	#953	PF8944/BBGL//BR23/EMB27	45	#933	ESDA/WEAVER
8	#91	605-87/SNB	46	#934	HEILO
9	#956	PROINTA GRANAR	47	#916	CATBIRD
10	#931	GONDO//BAU/MILAN	48	#922	CNO79/IAC24
11	#968	THB/KEA/PF85487/3/RIVADENEIRA 4	49	#957	R37/GHL 121//KAL/BB/3/JUP/MUS/4/2*YMI #6/5/CBRD
12	#98	BAU/MILAN//CBRD	50	#920	CHIL/IANB
13	#99	BAU/MILAN//CBRD	51	#970	TNMU//LIRA/VEE#7
14	#95	BAU/MILAN//CBRD	52	#913	BOW//BUC/BUL/3/KAUZ/4/CHOIX
15	#944	KAUZ/4/GOV/AZ//MUS/3/KEA	53	#965	SWM7079/KLSL//KLCH
16	#917	CATBIRD	54	#925	EHAL//CHUM18/BAU
17	#96	BAU/MILAN//CBRD	55	#946	MILAN/AMSEL//CBRD
18	#943	KAUZ//TRAP#1/BOW	56	#926	EHAL//CHUM18/BAU
19	#952	PF85235/SA8615/5/CEP8879/4/KLAT/SOREN//PSN/3/BOW	57	#929	FILIN/MILAN
20	#973	TRAP#1/BOW//TAIGU DERIVATIVE	58	#950	MURGA
21	#912	BNDU/CONA/3/9.72/BNAP//COCA	59	#966	TAM200/TUI
22	#959	SHA3/SERI//YANG87-142	60	#923	COOPERACION MAIPUN
23	#971	TNMU//LIRA/VEE#7	61	#927	EMB27/CEP8825//MILAN
24	#92	80456/YANGMAI 5	62	#936	INIA CABURE/INIA TIJERETA
25	#93	ALBERT/FDRC	63	#918	CHAT/CEP7780//PRL/BOW
26	#910	BCHA/MILAN	64	#932	GRANERO INTA
27	#915	BR 23	65	#939	ITAPU 45- DON PANI
28	#914	BPAL/3/COCA/BCEN//BNAM	66	#924	ESTANZUELA COLIBRI
29	#937	INIA CAURE/LAJ3153	67	#964	SW89.-5124*2/FASAN
30	#951	PROINTA ALAZAN	68	#941	KLCAR/SNB
31	#919	CHIL/CHUM18	69	#945	LFN/1158.57//PRL/3/HAHN
32	#967	THB/KEA//PF85487/3/MILAN	70	#94	ATTILA/5/FURY-KEN/SLM//ALDAN/4/PAT10ALD//PAT72300/3/PVN/6/DUCULA
33	#97	BAU/MILAN//CBRD	71	#954	PGO/SARA
34	#928	EMB27/CEP8825//MILAN	72	#911	BJY/COC//PRL/BOW
35	#958	SHA3/SERI//SHA4/LIRA	73	#935	IAN 8-PIRAPO
36	#962	SW89.5277/BORL95///SKAUZ	74	SST 806	SST 806
37	#921	CHIL/URES	75	Marico	MARICO
38	#940	ITAPUA 50- AMISTAD	76	Sumai 3	SUMAI 3

3.2.2. Haplotyping

3.2.2.1. Plant materials

Haplotyping was conducted using simple sequence repeat (SSR) molecular markers involving nine known FHB resistance sources, 11 selected CIMMYT lines with high FHB resistance and four susceptible cultivars. The highly FHB resistant and four susceptible cultivars were obtained from the ARC-SGI germplasm seed bank. This was aiming at comparison of the haplotype profile of the test lines against the resistant and susceptible checks in order to identify potential novel sources of FHB resistance. The 13 cultivars and 11 breeding lines used for this study show variable responses to FHB infection as summarized in Table 3.2.

The study used 24 SSR markers linked to known and validated FHB resistance QTL/genes (Table 3.3). These markers have been characterized and well documented for their diagnostic power of FHB resistance sources.

3.2.2.2. DNA extraction

Five seeds were taken from each line in the study and soaked in distilled water (dH_2O) overnight to soften them and were thereafter homogenized in a tissue lyser (Qiagen Tissue Lyser II). DNA was extracted from seeds using the DArT DNA extraction protocol (Diversity Array Technology, Pty Ltd) and treated with 2µl of RNAse. DNA concentration was determined by the Thermo Scientific Nanodrop 2000 spectrophotometer, diluted to 50ng/µl with TE (Tris-hydrochloride, 1.0mM EDTA, pH 8.0) buffer before PCR analysis.

Table 3.2: Description of the 11 breeding lines[a] and 13 cultivars haplotyped for their reaction to FHB infection

Ser. No.	Name	FHB reaction	Ser. No.	Name	FHB reaction
1	#910	Resistant	13	Frontana	Resistant
2	#930	Resistant	14	Asozaira	Resistant
3	#936	Resistant	15	Baisanyuehuang	Resistant
4	#937	Resistant	16	Catbird	Resistant
5	#942	Resistant	17	Huangcandou	Resistant
6	#947	Resistant	18	Huangfangzhu	Resistant
7	#953	Resistant	19	Haiyanzhong	Resistant
8	#960	Resistant	20	Wangshuibai	Resistant
9	#969	Resistant	21	Baviaans	Susceptible
10	#972	Resistant	22	Buffels	Susceptible
11	#1036	Resistant	23	Duzi	Susceptible
12	Sumai 3	Resistant	24	SST 806	Susceptible

[a]See Table 3.1 for pedigree of entries

Ser. No: Serial Number

3.2.2.3. Polymerase chain reaction, data collection and analysis

Polymerase chain reaction (PCR) was carried out in a MyCycler™ Thermal Cycler (Bio-Rad, Johannesburg, South Africa) with a final volume of 20 µl consisting of 4 µl template DNA, 10 µl 2X KAPA Taq ReadyMix (KAPA BIOSYSTEMS, Lasec SA, Cape Town, South Africa), 0.5 µl of 10 µm per primer and 5 µl PCR water. PCR conditions were as follows: 1 Cycle at 95 ˚C for 3 min; 35 Cycles at 95˚C for 30 sec; 50/51/55/60/61˚C for 30 sec; 72˚C for 30 sec; 1 Cycle at 72˚C or 5 min, with different annealing temperatures being used for the different primers as shown in Table 3.3. PCR products were ran on 3% high resolution agarose gel (MetaPhor™ Agarose, Lonza Rockland Inc, USA) stained with GRGreen (Inqaba Biotechnical Industries (Pty) Ltd). DNA fragments were sized by comparison with a 100 bp and 50 bp DNA ladders (SimplyLoad Lonza Rockland, Inc). PCR reactions that gave null alleles were repeated two/three times to confirm the null allele status of the respective genotype. PCR fragments were analysed and scored from digital gel photos taken with the MiniBis Pro *DNR* Gel documentation system (Bio-Imaging Systems, Lasec SA).

Table 3.3: Description of polymorphic SSR markers linked to FHB resistance QTL used to characterize the haplotype profile of 11 breeding lines and 13 cultivars

Ser. No.	SSR marker	Annealing temperature (˚C)	Targeted gene/QTL	Resistance source	Resistance Type	Reference
1	*Xgwm389*	60				
2	*Xgwm533*	60				
3	*UMN-10*	60	*3B QTL/Fhb1*	Sumai 3	II	Anderson et al., (2001); Buerstmayr et al., (2002)
4	*Xbarc133*	50				
5	*Xgwm493*	60				
6	*Xgwm156*	60				Buerstmayr et al., 2003, 2009; Xue et al., 2011
7	*Xbarc197*	60				
8	*Xgwm304*	60	*5A QTL/Fhb5*	Wangshuibai	I	
9	*Xgwm415*	50				
10	*Xgwm293*	60				
11	*Xgwm133*	60	*6B QTL/Fhb2*	Sumai 3	II	Cuthbert et al., 2007
12	*Xgwm644*	60				
13	*Dupw227*	60	3A QTL	Frontana	I	Steiner et al., 2004
14	*Xcfd14*	60	*QFhs.inta-7D*	Catbird	II	Cativelli et al., 2013
15	*Xbarc128*	52				
16	*Xwmc17*	51	*7A-Fhb7AC*	Sumai 3	II	Jayatilake et al., 2011
17	*Xbarc121*	50	*7A-QTL*	Huangfangzhu	II	Li et al., 2012
18	*Xgwm276*	55				
19	*Xgwm261*	55	*2D-QTL/Rht8*	Huangcandou	II	Cai and Bai, 2011
20	*Xhbg226*	60	*4B-Fhb4*	Wangshuibai	I	Xue et al., 2010
21	*Xgwm149*	55				
22	*Xwmc702*	61				
23	*Xwmc121*	61	*7D QTL*	Haiyanzhong	II	Li et al., 2011
24	*Xcfd46*	60				

3.3. Results and discussion

3.3.1. Phenotyping for FHB resistance across four environments

The FHB severity data of 73 SRSN entries and three checks is shown in Table 3.4. Twenty-eight percent (21 lines) of tested entries had better levels of FHB resistance, ranging from 2.0-9.5%. Seven lines had severity scores less than that of the most resistant check Sumai 3 (FHB severity = 5.08%), while 14 lines had significantly less resistance than the moderately resistant check Marico. Fourteen percent (11 lines) of the best performing lines selected based on extensive pedigree analysis and various agronomic traits were targeted for haplotyping. These lines included #910, #930, #936, #937, #942, #947, #953, #960, #969, #972 and #1036 (Table 3.1). Phenotypic characterization for FHB resistance is a crucial step in the development of elite germplasm though the procedure is labour intensive and expensive. Seventy-six CIMMYT SRSN entries, resistant and susceptible checks were previously phenotyped (Table 3.4); this allowed for the identification and selection of most resistant lines to be utilized in further studies.

There are various sources of FHB resistance that have been identified (Niwa et al., 2014; Yu et al., 2008b) with varying degrees of resistance. The ultimate goal of various research groups, including the ARC-SGI wheat germplasm development group, is to develop new lines with novel FHB resistance to diversify the FHB resistant genepool for effective breeding. The FHB severity results showed a considerable number of resistant lines. These lines were comparable to both the highly and moderately resistant checks, Sumai 3 and Marico, respectively. The following entries: #969, #930, #960, #975, #947, #972 and #953 showed significantly high levels of resistance to FHB across all four testing environments, performing comparable to the highly resistant check Sumai 3 (Table 3.4). The present findings suggested the presence of great potential of new sources of FHB resistance in the 9th CIMMYT SRSN. It is noteworthy that some genes conferring FHB resistance are linked in a repulsion phase with other undesirable genes. For instance, the favorable dwarfing genes *Rht-B1b* and *Rht-D1b* in wheat are associated with FHB susceptibility (Hilton and Hollins, 1999; Schmolke et al., 2005, Holzapfel et al., 2008; Yan et al., 2011). Therefore, some FHB resistant lines may have undesirable genes for plant height. Furthermore, FHB resistance gene *Fhb1* and stem rust resistance gene *Sr2* were discovered to be linked in a repulsion phase suggesting that deployment of one gene may result in the elimination of another gene (Flemming, 2012). Thus, various factors need to be considered when selecting FHB resistant lines for breeding.

Table 3.4: FHB severity (%) scores of 76 wheat lines [a] across four environments

		FHB severity (%)							FHB severity (%)				
Ser. No.	Entry	Beth 2008	Beth 2009	Doug 2008	Doug 2009	Mean	Ser. No.	Entry	Beth 2008	Beth 2009	Doug 2008	Doug 2009	Mean
1	#969	-	2	2	-	2,00	39	#942	15	20	20	10	16,25
2	#930	5	3	3	4	3,75	40	#974	15	10	10	30	16,25
3	#960	1	2	2	15	5,00	41	#938	8	3	3	55	17,25
4	#975	1	2	2	15	5,00	42	#955	5	2	2	60	17,25
5	#947	1	-	-	10	5,50	43	#961	15	3	3	50	17,75
6	#972	2	1	1	18	5,50	44	#963	60	2	2	8	18,00
7	#953	2	8	8	5	5,75	45	#933	10	3	3	58	18,50
8	#91	5	2	2	15	6,00	46	#934	15	7	7	45	18,50
9	#956	2	2	2	18	6,00	47	#916	30	5	5	38	19,50
10	#931	8	1	1	15	6,25	48	#922	40	2	2	35	19,75
11	#968	15	2	2	6	6,25	49	#957	10	12	12	45	19,75
12	#98	4	2	2	18	6,50	50	#920	10	3	3	64	20,00
13	#99	2	2	2	20	6,50	51	#970	20	5	5	50	20,00
14	#95	10	5	5	-	6,67	52	#913	60	3	3	15	20,25
15	#944	10	2	2	15	7,25	53	#965	5	10	10	60	21,25
16	#917	1	2	2	25	7,50	54	#925	-	3	3	58	21,33
17	#96	20	2	2	8	8,00	55	#946	30	8	8	40	21,50
18	#943	5	4	4	20	8,25	56	#926	-	3	3	60	22,00
19	#952	10	2	2	22	9,00	57	#929	45	2	2	40	22,25
20	#973	-	5	5	18	9,33	58	#950	20	7	7	55	22,25
21	#912	2	2	2	32	9,50	59	#966	70	2	2	15	22,25
22	#959	1	2	2	35	10,00	60	#923	30	10	10	40	22,50
23	#971	8	2	2	30	10,50	61	#927	35	10	10	35	22,50
24	#92	2	2	2	38	11,00	62	#936	1	-	-	45	23,00
25	#93	15	2	2	25	11,00	63	#918	10	10	10	65	23,75
26	#910	10	10	10	15	11,25	64	#932	60	1	1	35	24,25
27	#915	-	5	5	25	11,67	65	#939	20	7	7	65	24,75
28	#914	5	7	7	28	11,75	66	#924	5	-		45	25,00
29	#937	5	1	1	45	13,00	67	#964	35	8	8	55	26,50
30	#951	-	2	2	35	13,00	68	#941	50	10	10	45	28,75
31	#919	8	10	10	25	13,25	69	#945	55	12	12	40	29,75
32	#967	5	7	7	35	13,50	70	#94	80	2	2	38	30,50
33	#97	10	15	15	15	13,75	71	#954	20	20	20	68	32,00
34	#928	15	10	10	20	13,75	72	#911	75	10	10	45	35,00
35	#958	15	5	5	30	13,75	73	#935	50	20	20	68	39,50
36	#962	5	10	10	30	13,75	74	SST 806					51,85
37	#921	5	15	15	28	15,75	75	Marico					12,94
38	#940	25	2	2	35	16,00	76	Sumai 3					5,08

[a]Entries 1 to 73 are CIMMYT test lines and 74 to 76 are resistant checks

A wide array of diseases affect wheat. Therefore, elite lines should be selected for their resistance to FHB and other diseases including the wheat rusts. In addition, these lines should possess good agronomic traits. Entries #910, #936, #937, #942 and #1036 were among the CIMMYT lines phenotyped in the present study. Although the FHB resistance of these lines was not comparable to the resistant checks, they still showed considerably high FHB resistance with FHB severity scores ranging between 11.25 to 23.00%, implying their potential as new sources of FHB resistance. These lines have reduced plant height and useful agronomic attributes. Therefore, the CIMMYT lines were selected for being parental lines in creating a mapping population developed and phenotyped as presented in Chapter 2. However, the genetic basis of their FHB resistance is unknown and therefore, genotyping studies that included haplotype analysis were conducted as presented below.

3.3.2. Molecular analysis using SSR markers linked to known FHB resistant QTL

Four of the markers used for screening (*Xgwm276, Xbarc121, Xhbg226* and *Xwmc121*) were not significantly polymorphic and thus were not included in the analysis. The allele sizes of the 20 polymorphic markers are summarized in Table 3.5. In this study, 3B-*Fhb1* seemed to be the most prevalent FHB resistance gene. Alleles linked to this gene were identified in the test genotype #937, where alleles similar to those of *UMN10, Xgwm493* and *Xgwm533* were identified. However, none of the other test lines seemed to have similar alleles with the *Fhb1* locus (Figure 3.1). Test line #936 showed similar alleles to Sumai 3 for the markers *Xgwm156, Xbarc 197, Xgwm415* and *Xgwm293* linked to 5A-QTL/5A-*Fhb5.* Test line #930 contained similar alleles to those of Frontana for the markers *Xgwm133* and *Xgwm644* linked to the 6B-*Fhb2* gene. Both test lines #936 and #930 showed allelic similarities with Sumai 3 for the markers *Xwmc17* and *Xgwm261* linked to the 7A-*Fhb7AC* and 2D-QTL, respectively.

Marker *Xwmc17* linked to *7A-Fhb7AC* amplified well in most of test lines, which was similar to Sumai 3. As the markers for the *7A QTL* from HFZ were not polymorphic, no significant information could be obtained. Marker *Xgwm261,* which is closely linked to a *2D QTL* from HCD and a gene controlling plant height *Rht8,* showed the same allele in the resistant checks ASO, BAI, HYZ and WSB, suggesting possession of *Rht8* height gene by these lines. None of the test lines had the same allele for marker *Xgwm261.* Marker *Xgwm149* specifically linked to the *Fhb4* gene was identified in WSB. This marker was not present in any of the test lines, but similar size alleles were present in the susceptible lines Buffels and Duzi. The marker alleles specific for the *7D QTL* from HYZ were difficult to score using a high resolution agarose gel due to the close nature of the allele sizes. The nine resistant varieties had similar alleles especially for the *Fhb1, 5A/Fhb5,* and *2D QTL/Rht8* QTL regions.

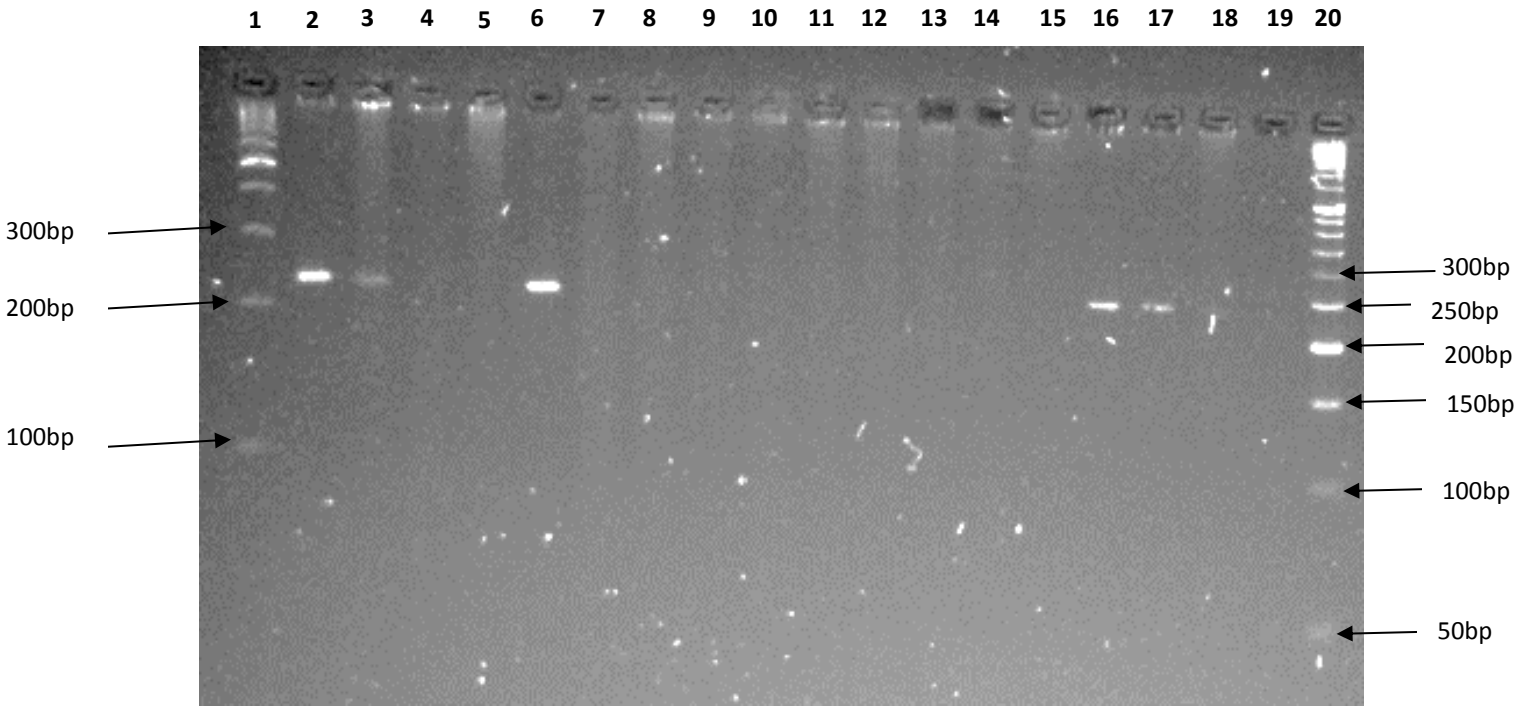

Figure 3.1: PCR amplification products of marker *UMN10* on 11 CIMMYT test lines, 2 resistant checks and 4 susceptible checks on high-resolution agarose with Sumai 3, Wangshuibai and entry #937 shown on lanes 2, 3 and 6, respectively showing a positive 240bp allele for marker *UMN10*. Lanes 1: 100bp DNA ladder, 2: Sumai 3, 3: Wangshuibai, 4: #910, 5: #936, 6: #937, 7: #942, 8: #1036, 9: #930, 10: #947, 11: #953, 12: #960, 13: #969, 14: #972, 15: Bav, 16: Buff, 17: Duzi, 18: SST 806, 19: Negative Control (dH_2O), 20: 50bp DNA ladder

McCartney et al., (2004) stated that: (i) wheat lines with the same haplotype spanning an FHB resistant QTL may likely carry FHB resistant QTL, and (ii) wheat lines with a number of alleles in common with an FHB resistant haplotype may have a similar FHB resistance gene. From these scenarios it can be noted that a number of resistant checks carry the same genes observed to have common alleles when compared to each other. This level of similarity can be expected as a result of a number of similar QTL regions identified in different resistant varieties and mapping populations. There are a number of markers that are tightly linked to QTL, making them useful diagnostic markers. *UMN10* is a widely used diagnostic marker to detect the *Fhb1* gene that was reported and validated in wheat lines such as BAI, Sumai 3, HFZ, HCD and WSB (Yu et al., 2008b; Cai and Bai, 2011 Li et al., 2012; Zhang et al., 2012) with a positive allele of 240bp. Jayalitake et al., (2011) reported a novel QTL in chromosome 7A of Sumai 3 and the closest marker that was found to be diagnostic for this QTL was *Xwmc17* with a positive allele of 170bp.

The results of the present study showed that *Fhb1* is the most common FHB resistance gene. This is expected because the gene was derived from Sumai 3, which is a widely used source of FHB resistance globally. This supports Liu et al., (2008) who reported that Chinese cultivar Sumai 3 and its derivatives were the most popular FHB resistance sources, providing major resistance QTL *Fhb1*. The CIMMYT test line #937 showed allelic similarities to the resistant checks, predominantly Sumai 3 and Frontana. This entry shows allelic similarity to Sumai 3 for the *Fhb1* gene. This is supported by the presence of a positive 240bp for the diagnostic marker *UMN10*. Also present in the entry are the alleles for the 3A-QTL and 7A-*Fhb7AC* suggesting the genetic potential of this line as an FHB resistance source containing multiple resistance genes. Entry #936 showed a haplotype profile similar to that of Sumai 3 for *Fhb5* as well as for alleles present on the 3A-QTL, 7A-*Fhb7AC* and 2D-QTL/*Rht8*. This suggests that this entry is of great significance as it contains all these resistance QTL/genes while also possessing good agronomic attributes. Entry #930 showed the presence of three QTL/genes as it had allelic similarities to Sumai 3 for *Fhb2*, 7A-*Fhb7AC* and the 2D/*Rht8* QTL. None of the test lines seemed to carry the 7D QTL.

Table 3.5: Allele sizes (bp) of each of the amplified polymorphic FHB specific DNA markers screened on eight resistance sources, 11 CIMMYT test selection and four susceptible lines for haplotype comparison

QTL/gene	Marker	FHB resistant varieties								CIMMYT selections/ Test lines											South African cultivars			
		SUM3	FRON	ASO	BAI	HCD	HFZ	HYZ	WSB	#910	#936	#937	#942	#1036	#930	#947	#953	#960	#969	#972	SST806	BAV	BUFF	DUZ
	Xgwm389	140	120	140	140	140	140	140	140	150	120	120	120	Null	null	null	null	null	null	120	null	130	120	120
	Xgwm533	120	120	150	150	150	140	150	150	120	130	120	120	Null	null	null	null	null	null	null	140	130	130	130
	UMN-10	240	260	260	240	240	240	260	240	260	260	240	260	260	null	null	null	null	null	null	260	270	260	270
	Xbarc133	140	110	140	100	140	100	100	100	Null	120	120	120	Null	null	110	null	null	null	null	null	100	120	120
3B-Fhb1	*Xgwm493*	150	null	180	220	220	220	220	220	150	150	150	null	Null	null	null	null	null	null	null	null	180	180	180
	Xgwm156	290	310	320	320	320	320	320	340	290	290	290	320	Null	null	null	null	null	null	null	null	300	280	280
	Xbarc197	185	170	185	185	185	185	185	185	Null	185	185	185	Null	180	null	null	null	null	null	null	175	170	170
	Xgwm415	130	130	140	140	140	140	140	140	130	130	120	130	Null	120	null	null	null	null	null	null	130	130	130
	Xgwm304	200	200	220	220	220	220	220	230	190	190	190	null	Null	190	null	null	null	null	null	null	190	190	190
5A-QTL/5A-Fhb5	*Xgwm293*	190	200	null	170	200	200	200	200	190	190	180	null	Null	180	null	null	null	null	null	null	210	210	200
	Xgwm133	100	120	130	130	110	110	130	130	110	110	130	110	Null	120	null	null	null	null	null	null	110	110	120
6B-Fhb2	*Xgwm644*	150	160	180	180	180	180	180	180	180	160	150	160	null	160	null	null	null	null	null	160	160	160	160
3A-QTL	*Dupw227*	190	175	190	190	190	190	190	190	190	190	190	190	null	null	null	null	null	null	null	null	190	175	190
7A-Fhb7AC	*Xwmc17*	170	170	180	180	180	180	null	180	170	170	170	170	170	170	null	null	null	null	180	null	180	180	180
2D-QTL/Rht8	*Xgwm261*	175	150	192	192	192	175	192	192	Null	175	150	150	null	175	null	null	null	null	null	null	175	175	175
4B-Fhb4	*Xgwm149*	165	165	175	175	175	175	175	155	165	175	null	175	null	null	null	null	null	null	null	null	165	155	155
	Xwmc702	200	200	200	200	200	180	180	180	180	180	180	180	null	180	null	null	null	null	null	190	180	170	180
7D QTL	*Xcfd46*	180	170	180	180	180	190	180	180	180	180	170	170	null	null	null	null	180	null	null	null	170	180	170
	Xcfd14	150	160	160	160	165	150	150	150	Null	null	140	null	null	null	null	null	null	null	null	130	null	110	120
QFhs.inta-7D	*Xbarc128*	180	160	180	190	20	null	220	210	Null	null	160	null	null	null	null	null	null	null	null	null	null	130	140

ASO- Asozaira, **BAI-** Baisanyuehuang, **BAV-** Baviaans, **BUFF-** Buffels, **DUZ-** Duzi, **FRON-** Frontana, **HCD-** Huangcandou, **HFZ –** Huangfangzhu, **HYZ-** Haiyanzhong, **WSB-** Wangshuibai**, SUM3**- Sumai 3

The test lines including #910, #942, #1036, #947, #953, #960, #969 and #972 showed non-allelic similarities for the various QTL and therefore, showed no haplotype patterns similar to any of the eight FHB resistance sources. Some of the individual test lines had one or two alleles in common with a resistance source. However, entire haplotype blocks per QTL loci were not common. It is noteworthy that a single marker represented some QTL, such that the 3A-QTL and 7A-*Fhb7AC* gene appear in most of the test lines than the other QTL. The rest of the test lines (#947, #953, #960, #969 and #972) did not show any allelic similarities to any of the resistant checks but rather had null alleles without detectable resistance genes. Though these test lines carry no detected resistance genes, they have significant FHB resistance expression when phenotyped; this implies they may have novel resistance genes. This is further confirmed by the entry #947, which showed null alleles for all three SSR markers (*Xwmc702, Xbarc128* and *Xcfd14*) linked to the *QFhs.inta-7D* QTL that was identified in CBRD, a cultivar that is included in the pedigree of entry #947, suggesting that the resistance observed in this entry is new.

Some of the known susceptible lines were observed displaying allelic similarities with the resistant checks, such as Buffels and Duzi showing the *Fhb4* allele and Baviaans, Buffels and Duzi displaying allele sizes similar to the 2D-QTL/*Rht8.* This can be explained by the phenomenon that FHB resistance is a quantitative trait (Buerstmayr et al., 2009) controlled by the synergy of many genes; thus the presence of one gene in a wheat cultivar does not necessarily confer resistance. This reiterates the fact that the pyramiding of various resistance genes into one line should be the ultimate goal in breeding for FHB resistance.

3.4. Conclusions

There were a number of FHB resistance sources previously identified. However, there is need for a continued search for additional sources of resistance. This will enable diversification of the genetic basis of FHB resistance in elite wheat germplasm and subsequently increasing the level of FHB resistance. Effective control of FHB is possible by stacking multiple resistance genes into a wheat line (McCartney et al., 2004). Therefore, it would be advantageous for breeders to know which resistance sources carry different resistance genes as well as which new sources of resistance carry novel resistance genes. The current study, therefore, shed some light on the

resistance present in the CIMMYT SRSN while also identifying potential novel sources of FHB resistance.

Haplotyping is an ideal starting point for the strategic planning of directed marker-assisted crossing of new FHB QTL/gene sources. This will improve the genetic base of FHB resistant germplasm available to wheat breeders. However, based on the phenotyping data which included FHB severity scoring the most useful lines to be used for further studies are those that show significant FHB resistance. Entries #910, #936, #937, #942 and #1036 were used in the previous study to develop a mapping population that was further phenotyped and characterized to validate the resistance of both the resistance source parents as well as their progenies. Identification and characterization of QTL/genes present in the CIMMYT SRSN lines that are now incorporated into the South African germplasm will aid in the enrichment of this germplasm and also will allow for the identification of novel FHB resistance genes which leads to the exploration and possible diversification of the current resistant genepool. However, the identification of novel QTL/genes requires extensive mapping studies. Although there are novel sources of resistance that were identified in this study, subsequent designed crosses are required to develop new populations from these resistant parents and thereafter to phenotype the progeny to identify resistant recombinant inbred lines and to establish FHB resistant germplasm which contains elite lines that carry useful attributes for large-scale production. The use of these new novel sources of resistance will enhance the diversity of FHB resistance sources and avoid complete dependence on limited sources of resistance across different breeding programs.

3.5. References

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Introduction and objectives of the study

Wheat (*Triticum* spp.) is one of the most important commodity crops widely grown globally. With the rapidly growing world population, increased quality and quantity of wheat production is expected to come from integrated wheat breeding programs. South Africa is the major wheat producing country in sub-Saharan Africa (SSA). However, wheat production in the country declined over the past years due to various abiotic, biotic and socio-economic constraints. *Fusarium* head blight (FHB) caused by the members of the *F. graminearum* species complex is the most devastating fungal disease affecting wheat, barley and maize. The disease causes considerable yield and quality losses. Also, FHB infected wheat grains possess mycotoxins produced by the pathogen, posing health risks to both humans and animals. The past decade has witnessed major FHB outbreaks causing significant economic losses in cereal crops globally. Given the current global warming associated with increased temperatures, major epidemics of FHB are likely to occur in the near future particularly under high humidity conditions.

Various control strategies have been recommended to manage FHB losses. However, effective control was not achieved by using a single control strategy needing synergistic use of various control strategies. A major problem in South Africa is that there are currently no registered fungicides to control FHB epidemics. Therefore, the use of the FHB resistant germplasm is regarded as an important component in the integrated management of FHB. There are numerous studies that track genes responsible for conferring resistance to FHB. Some the resistant genes have been well characterized and mapped on wheat chromosomes. Further, there is need to identify novel resistance genes in order to diversify the FHB resistant genepool. Consequently, a pre-breeding of wheat is actively undertaken at the Agricultural Research Council-Small Grain Institute (ARC-SGI) in collaboration with the International Centre for Maize and Wheat Improvement (CIMMYT) and regional collaborators. Thus far, the program has developed 778 recombinant inbred lines (RILs) of wheat for breeding through systematic crosses and continuous selfing and selection. Thus, the objectives of this study were:

- To determine the field response of the 778 newly developed RILs and standard check varieties of wheat for FHB resistance and to identify sources of resistance for breeding or direct production and
- To determine FHB resistance among 76 wheat lines using field based phenotyping and to determine the genetic background of the 11 selected most resistant lines and four susceptible checks using SSR markers in order to identify possible novel FHB resistance sources.

Research findings in brief

Field response of newly developed recombinant inbred lines of wheat for *Fusarium* head blight resistance

This study phenotyped 778 recombinant inbred lines (RILs) and resistant check (Sumai 3) and susceptible check (SST 806) varieties across four environments. The RILs were developed from crossing five resistant CIMMYT lines with three South African irrigation cultivars with known FHB susceptibility. A total of fourteen populations were developed using a bi-parental mating scheme. The core findings of this chapter are indicated below:

- Six percent of the lines had <20% infection rate suggesting the presence of FHB resistance among tested lines.
- Analysis of variance showed significant differences among genotypes, testing environments and genotypes x testing environments affecting FHB severity.
- The heritability for FHB resistance was estimated at 64%, indicating the possibility of achieving considerable selection gains in the tested population and environments.
- Five RILs were identified as significantly resistant, scoring comparable to the resistant check, Sumai 3.
- Cedara was the best environment for germplasm screening against FHB.
- Overall, the following five RILs were selected as new sources of resistance: 681 (Buff/1036/71), 134 (Duzi/910/8), 22 (Bav/910/22), 717 (Bav/937/8) and 133 (Duzi/910/7) with mean FHB scores of 6.8%, 7.8%, 9.5%, 9.8% and 10%, respectively.

Haplotype comparison of new sources of *Fusarium* head blight resistance in wheat

This study conducted field phenotypic analysis on a total of 76 wheat lines. These lines included 73 lines with known FHB resistance acquired from the International Maize and Wheat Improvement Centre (CIMMYT), two resistant checks (Sumai 3 and Marico) and one susceptible check (SST 806). Lines were phenotyped across four environments. Eleven lines were selected and haplotyped using 24 diagnostic microsatellite markers. The main findings of this chapter are indicated below:

- Current sources of resistance share similar haplotype profiles, implicating that they shared similar resistance genes
- CIMMYT entries #937, #936 and #930 showed allelic similarities with some of the known resistant checks.
- Entry #937 showed similar allelic patterns to Sumai 3 at chromosome 3B, presumably containing the prevalent resistance gene, *Fhb1*.
- Entry #936 showed allele banding patterns similar to Sumai 3 for markers linked to resistance gene, *Fhb5.*
- Entry #930 showed the presence of three QTL showing allelic similarities to Sumai 3 for *Fhb2*, 7A-*Fhb7AC* and the 2D/*Rht8* QTL.
- The rest of the test lines with FHB resistance showed non-significant allelic similarities to the resistant checks, suggesting that the FHB resistance in the new lines is novel.

Implications of research findings to *Fusarium* head blight research in South Africa

- Five wheat genotypes were identified to have extremely good resistance to FHB. The five genotypes identified performed comparable to the resistant check. The selected lines are useful genetic resources for resistance breeding against FHB of wheat, thus these genotypes can be recommended to wheat breeders and farmers for breeding and/or direct production.
- Many of the CIMMYT resistant lines do not have similar haplotype profiles which implies that they have genes that are different from the known resistance sources.
- The apparent novelty of the resistance in the CIMMYT test lines is highly significant as it will allow for the diversification of the FHB resistant genepool.

- Future studies should focus on further characterizing these identified lines for important agro-morphological traits such as grain yield response, nitrogen use efficiency, enhanced water use efficiency and enhanced tolerance to drought and cold stresses and resistance to wheat rust diseases.
- Further phenotyping across diverse testing environments and mapping of the candidate genes are essential for marker-assisted breeding and introgression of novel genes with FHB resistance.