MOLECULAR MAPPING OF QUANTITATIVE TRAIT LOCI CONTROLLING FUSARIUM HEAD BLIGHT RESISTANCE AND DEOXYNIVALENOL ACCUMULATION IN TWO WINTER WHEAT DOUBLE HAPLOID POPULATIONS

BY

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ABSTRACT

Fusarium head blight (FHB) caused by *Fusarium graminearum* leads to severe yield and quality losses in wheat, but the most serious concern is the accumulation of mycotoxins such as deoxynivalenol in food and feed. Development of resistant varieties is an important component of integrated control of this fungal disease. Two large double haploid (DH) populations were developed using CDC Buteo as the moderately resistant parent. The objective was to detect (quantitative trait loci) QTL for resistance against FHB. The 19H*16/CDC Buteo cross (9HBT, N=228) produced six DH lines with <10% Fusarium head blight index (FHB index) and 56 lines with FHB index of 10-20%. The cross 22A*13/CDC Buteo (2ABT, N=218) produced 93 lines with an FHB index of 10-20%. For all FHB traits investigated, FHB resistance QTL were identified on 13 chromosomes with stable QTL on 2B, 4A, 4B, 4D, 6B and 7A for the 9HBT population. The 4B and 4D QTL mapped on the exact positions for the *Rht-B1* and *Rht-D1* dwarfing genes, respectively, and they explained the largest proportion of phenotypic variation. CDC Buteo contains allele combination *Rht-B1b/Rht-D1a* while 19H*16 contains *Rht-B1a/Rht-D1b*. Anther retention (AR) results for the cross 19H*16/CDC Buteo, showed that low AR was correlated with increased FHB resistance. Anther retention is a stable trait which can be used for indirect selection in FHB resistance. Classification of the 9HBT population into genotypic groups based on *Rht* genes identified double wild-type (*Rht-B1a/Rht-D1a)* that were the tallest with low anther retention (AR) and reduced FHB infection compared to double dwarf mutants (*Rht-B1b/Rht-D1b)* that were very short with high AR and high FHB infection. The allele combination *Rht-B1b/Rht-D1a* had lower AR and lower disease symptoms than *Rht-B1a/Rht-*

D1b, but almost the same plant height. Nineteen QTL were reported for 2ABT with stable QTL expressed on chromosomes 2B, 3B, 4B, 6A and 6B. Stable QTL reduced multiple FHB-related traits and could be used for improving FHB resistance in wheat. The two DH populations showed somewhat different results in terms of QTL detected.

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DEDICATION

To my family

Faith, James, and Dr. Elijah Gituanjah

My parents

Elizabeth Wambui and Alfred Mwaniki

My brothers, sisters and Joseph Kagwa

Thank you for your love, patience, motivation and prayers. To God be the glory, blessed be the name of God.

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15-ADON	15-O-acetyl deoxynivalenol
3-ADON	3-O-acetyl deoxynivalenol
2ABT	22A*13/CDC Buteo
3Siteyear	Three site years
6Siteyear	Six site years
9HBT	19H*16/CDC Buteo
AE	Anther extrusion
AR	Anther retention
BLAST	Basic Local Alignment Search Tool
CDC	Crop Development Centre
CIM	Composite Interval Mapping
CIMMYT	International Maize and Wheat Improvement Center
CRM	Carman
cM	Centimorgan
D3G	Deoxynivalenol-3-*O*-glucoside
DELLA	Transcription regulators (growth repressors) which inhibit gibberelin signalling
DH	Double haploid
DNA	Deoxyribonucleic acid
DON	Deoxynivalenol
ELISA	Enzyme Linked Immunosorbent Assay
FAO	Food Agricultural Organization
FDK	Fusarium damaged kernel

FHB	Fusarium head blight
FHBI	Fusarium head blight index
ISK index	Incidence, Severity and kernel damage index
KASP	Kompetitive Allele Specific PCR
LOD	Logarithm of odds
MAS	Marker Assisted Selection
MIM	Multiple Interval Mapping
MSTMap	Minimum Spanning Tree of a Graph
ppm	Parts per million
PTMDI	Provisional maximum tolerable daily intake
PV	Phenotypic variation
QTL	Quantitative trait loci
RF	Recombination fraction
Rht	Reduced height
RIL	Recombinant inbred lines
SIM	Simple Interval Mapping
SNP	Single nucleotide polymorphism
STS	Sequence tagged sites
SSR	Simple sequence repeats
U o M	University of Manitoba
USDA	United States Department of Agriculture
WHO	World health Organization
WNP	Winnipeg

FOREWORD

This thesis is written in manuscript style following the guidelines set by the University of Manitoba. A general introduction about the research project and literature review precedes the six chapters and three manuscripts that comprise the main part of the thesis. Each manuscript has an abstract, introduction, materials and methods, results and discussion. The manuscripts are formatted according to the journal of Theoretical and Applied Genetics (TAG), and they are followed by general discussion and conclusions, literature cited and appendices sections.

CHAPTER 1

GENERAL INTRODUCTION

Fusarium head blight (FHB) is one of the most devastating diseases of wheat and other small grain cereals worldwide (Nicholson et al. 2003; Osborne and Stein 2007). *Fusarium graminearum* Schwabe (teleomorph *Gibberela zeae* (Schwein and Petch) is the primary causal pathogen of FHB in Eastern Canada, Saskatchewan, Manitoba, the United States (Tekauz et al. 2000; Goswami and Kistler 2005) and many other countries (Bai and Shaner 1994; McMullen et al. 1997).This pathogen belongs to the *F. graminearum* species complex (Fg complex), which consists of at least 16 phylogenetically distinct species (O'Donnell et al. 2000; O'Donnell et al. 2004; O'Donnell et al. 2008; Starkey et al. 2007; Yli-Mattila et al. 2009; Sarver et al. 2011).

Fusarium graminearum represents over 97% of Fusarium isolates found in Manitoba (Gilbert et al. 2010). The fungus reduces yield and end-use grain quality and contaminates the grain with trichothecene mycotoxins, mainly deoxynivalenol (DON), limiting its use for food or feed (Gilbert and Fernando 2004; Pestka and Smolinski 2005). Maximum allowed levels for the most prevalent Fusarium mycotoxins in cereals and cereal products (van Egmond 2004), and animal feeds have been established in many countries to protect the consumers and livestock from mycotoxicosis. According to the joint Food and Agriculture Organization /World Health Organization expert committee on food additives , the provisional maximum tolerable daily intake (PMTDI) for DON was converted to a group PTMDI of 1 $\mu g/kg^{-1}$ body weight for DON and its acetylated derivatives (3-ADON and 15-ADON) (FAO/WHO, 2010). There are also limits set in grain that are in parts per million (ppm) of the product to be consumed. This is different from the daily intake and is what is measured when grain is traded around the world.

Various methods are used for FHB control, namely use of resistant cultivars, chemical control, biological control, and agronomic practices such as crop rotation and tillage (Paul et al. 2005). Genetic resistance to FHB is considered the best and most environmentally sound strategy to control the disease (McMullen et al. 1997; Zhang et al. 2008). Access to genetically diverse germplasm with broad FHB resistance is crucial to the success of wheat breeding programs. Breeding for FHB resistance must be accompanied by selection for desirable agronomic traits. Since many resistance sources are associated with undesirable traits, breeding for resistance is complex. In the last two decades, the major focus has been breeding for resistance to improve the level of FHB resistance in new and existing elite cultivars. No complete resistance or immunity to FHB has been observed, but wide genotypic variation has been documented in wheat and its close relatives.

Fusarium head blight resistance is quantitatively inherited and many quantitative trait loci (QTL) for FHB resistance have been identified from different genetic backgrounds (Buerstmayr et al. 2009). Markers within QTL regions can be used for marker assisted selection (MAS) to select for DNA segments that are genetically linked to genes that provide incremental resistance to FHB. Accumulation of these resistant alleles at QTL is the most practical approach for enhancing FHB resistance in locally adapted cultivars.

A major QTL on chromosome 3BS, designated *Fhb1*, has been consistently detected across multiple environments and populations, explaining 20-40% of phenotypic variation (Buerstmayr et al. 2009; Anderson 2007; Jiang et al. 2007), and many breeders are using it to develop new FHB resistance cultivars. Stacking of multiple QTL using MAS has also been used to improve FHB resistance (Miedaner et al. 2006; McCartney et al. 2007). For example, Miedaner et al. (2006) used MAS to introgress and stack two donor QTL from CM82036

(pedigree: Sumai 3/Thornbird) located on chromosomes 3B and 5A, and one donor QTL from Frontana on chromosome 3A into the susceptible elite German spring wheat varieties Nandu and Munk using two to three closely linked DNA markers per QTL. FHB resistance was highest in recombinant lines with multiple FHB resistance QTL, especially for those that combined QTL from chromosomes 3B and 5A. Although QTL from different sources have been mapped and, in some cases, successfully used in wheat breeding programs, finding new sources of resistance is paramount to avoid complete dependence on limited sources of resistance such as Sumai 3, because this may lead to breakdown of resistance (McCartney et al. 2004). A wide variety of genetic backgrounds, including elite cultivars, need to be evaluated for FHB resistance to reduce the losses incurred due to FHB.

Currently, wheat cultivars that are highly resistant to FHB and DON accumulation are not available in Canada and North America. Repeated testing (Seed Manitoba, 2017) has shown that CDC Buteo (Fowler 2010), a Canadian elite winter wheat cultivar is moderately resistant to FHB. Exploitation of elite resistance sources may speed up resistant cultivar development. The objectives of this study were to evaluate the value of CDC Buteo as an alternative source of resistance to FHB in two winter wheat crosses with CDC Buteo as the common parent. Their different genetic backgrounds could reveal more different FHB loci due to different polymorphic loci among the parents. The crosses were 19H*16/CDC Buteo (susceptible/moderately resistant and 22A*16/CDC Buteo (moderately resistant/moderately resistant)

The specific objectives were:

(a) To study the inheritance of FHB in two double haploid populations developed with CDC Buteo as the resistant parent

(b) To evaluate the relationship between FHB resistance, anther morphology, and spike maturation patterns in one double haploid population using CDC Buteo as the resistant parent.

(c) To quantify the phenotypic variation for FHB resistance and map QTL associated with FHB resistance in two double haploid populations using CDC Buteo as the resistant parent.

CHAPTER 2

LITERATURE REVIEW

2.1 Wheat

Wheat belongs to the Gramineae family consisting of cultivated wheat, rye, barley, oat, triticale and important forage grass species. Within the Gramineae family, the genus *Triticum* includes a number of species that form a series based on multiples of seven related chromosomes (diploids = 7, tetraploids = 14, hexaploids = 21).The hexaploid wheat genome consists of three homeologous groups of chromosomes belonging to the A, B, and D genomes (Moolhuijzen et al. 2007). Hexaploid wheat (*T. aestivum*) has a chromosome number of 2*n* = 6x = 42, AABBDD and has the largest cereal genome at 16 000 Mb (Moolhuijzen et al. 2007) compared to the maize (2500 Mb) and rice (389 Mb) genomes. The A sub-genome originated from *Triticum urartu*, the B sub-genome originated from an unknown species related to *Aegilops speltoides*, and the D sub-genome from *Aegilops tauschii*. AABB tetraploids (*Triticum turgidum*) appeared less than 0.5 million years ago, and bread wheat from a further hybridization with the D genome 10,000 years ago (Marcussen et al. 2014). About 95% of the wheat grown worldwide is hexaploid wheat, with most of the remaining 5% being tetraploid durum wheat.

Hexaploid wheat is one of the most important food crops worldwide (Curtis et al. 2002; Shewry 2009), providing 20% of humanity's dietary energy supply, and serving as the main source of protein in developing nations. Approximately 65% of wheat is used directly as human food, 21% to feed livestock, 8% as seed, and the remaining 6% for other uses (Gooding and Davies 1997; Shewry 2009). Wheat is the second most produced cereal after maize in terms of tonnes of grain produced (FAOSTAT 2012) in the world. Over 220 million hectares of wheat are planted annually with a global production average of around 670 million metric tonnes, making

it one of the most widely cultivated cereals in the world (Shiferaw et al. 2013). For the 2013-2014 season, global wheat production was 717.2 million metric tons (FAO 2014). Wheat is the major crop grown in Canada with 95% of wheat produced in the Prairie Provinces of Saskatchewan, Alberta and Manitoba (Curtis et al. 2002).

Winter wheat is known to yield higher than spring wheat in North America due to its ability to take advantage of the spring moisture, longer growing season, and its high tillering capacity. However, the protein levels of winter wheat are lower than those of spring wheat (McCallum and DePauw 2008). Winter wheat in western Canada is segregated into two classes for marketing purposes: Canadian Western Red Winter (CWRW) and Canada Western Special Purpose (CWSP). Wheat yield can exceed 10 T ha^{-1}, although the worldwide average is much lower at 2.8 T ha^{-1} due to several factors including nutrient deficiencies, drought and pests (Shewry 2009). Worldwide, it is estimated that 25-30% of the wheat crop is lost to biotic and/or abiotic stresses (Bockus et al. 2010).

2.2 Fusarium head blight of wheat

Fusarium head blight is a disease of wheat, corn, barley and other small grains in the world caused primarily by *Fusarium graminearum* Schwabe (teleomorph *Gibberela zeae* (Schwein and Petch; Nicholson et al. 2003; Osborne and Stein 2007). It is one of the most important diseases of wheat in Eastern Canada, Saskatchewan, Manitoba and the United States (Gilbert et al. 2000; Gilbert and Tekauz 2000; Goswami and Kistler 2004). It can cause bleached spikes, spikelet sterility, poor seed filling, low test weight and tombstone seeds, and in epidemic years, the yield losses can be high. Canadian wheat losses related to FHB in the last 30 years were estimated to be one billion dollars (Clear and Nowicki 2009).

Fusarium head blight is a monocyclic disease occurring only once during a cropping season such that after the initial or primary infection there is little or no secondary infection occurring from conidia formed on infected spikes. The fungus causes severe losses in seed yield and end-use grain quality. In addition, contamination of grain with mycotoxins, predominantly the trichothecene, deoxynivalenol (DON), limits the marketability of the grain for food or feed (Gilbert and Fernando 2004; Pestka and Smolinski 2005).

Maximum allowed levels for the most prevalent Fusarium mycotoxins in cereals and cereal products (van Egmond 2004) and animal feeds have been established in many countries to protect the consumers and livestock from mycotoxicosis. Health Canada has set maximum DON concentrations at 2 ppm for un-cleaned soft wheat used in non-staple foods and 1.0 ppm for un-cleaned soft wheat used in baby food; however, both of these limits are currently under review (Health Canada 2012).

2.3 *Fusarium graminearum*

2.3.1 Taxonomy of *Fusarium graminearum*

The genus *Fusarium* comprises of a high number of fungal species that can be pathogenic to a number of economically important crops. The genus *Fusarium* belongs to the phylum *Ascomycota*, class *Ascomycetes*, order *Hypocreales*, while the teleomorphs of *Fusarium* species are mostly classified in the genus *Gibberella*, and for a smaller number of species, *Hemanectria* and *Albonectria* genera (Moretti 2009).The Fusarium species fall into four sections namely Discolor, to which *F. graminearum* belongs, Roseum, Gibbosum and Sporotrichiella which share several characteristics, although each section is biologically distinct (Liddell 2003).

Understanding the population structure of *F. graminearum* is complicated by differences in many factors including species groupings, mating types, and chemotypes (Guo et al. 2008;

Miedaner et al. 2008). There are differences in conclusions on the nature of genetic diversity within *F. graminearum* based on types, origin of isolates, and evaluation method used (Xu et al. 2004; Gale et al. 2007; Starkey et al. 2007; John Leslie 2008). Pathogenicity and selection for complex chemotypes have been suggested as major factors in spread of FHB in North America (Ward et al. 2008).

Fusarium graminearum is the primary causal pathogen of FHB in the Canada, United States and many other countries (Bai and Shaner 1994; Wagacha and Muthomi 2007). The *Fusarium graminearum* complex (Fg) consists of 16 phylogenetically distinct species which have been recognised using multi-locus sequence typing, some of which cannot be discriminated based on their morphology (O'Donnell et al. 2000, 2004, 2008; Starkey et al. 2007; Yli-Mattila et al. 2009; Sarver et al. 2011).The species in the *Fg* complex differ in chemotype, geographic distribution, and ability to cause disease and produce trichothecenes on particular crops (Xu and Nicholson 2009; Malihipour et al. 2012), highlighting the difficulties that FHB poses to wheat breeders. The *Fg* complex includes: *F. graminearum, F. gerlachii, F. louisianense, F. asiaticum, F. ussurianum, F. nepalense, F. vorosii, F. acaciae-mearnsii, F. aethiopicum, F. boothii, F. mesoamericanum, F. austroamericanum, F. cortaderiae, F. brasilicum, F. meridionale* and *F. pseudograminearum (*O'Donnell et al. 2000, 2004, 2008; Starkey et al. 2007; Yli-Mattila et al. 2009; Sarver et al. 2011; Qiu et al. 2014). The *Fg* complex produces type B trichothecenes (Goswami and Kistler 2004; Starkey et al. 2007; O'Donnell et al. 2008; Yli-Mattila et al. 2009; Sarver et al. 2011). In N. America and Europe, *F. graminearum sensu stricto* is the most common species (O'Donell 2000, 2004; Starkey et al. 2007). *Fusarium asiaticum* and *F. graminearum sensu stricto* were the predominant species in Asia, Japan and Korea (Suga et al. 2008; Lee et al. 2012). However, in China, most *F. graminearum sensu stricto* isolates were

from cooler northern regions, while *F. asiaticum* was mainly found in the warmer wheat growing regions where FHB epidemics are more frequent (Qu et al. 2008).

2.3.2 Fusarium head blight chemotypes and deoxynivalenol production

The *Fusarium* species are capable of producing mycotoxins called trichothecenes, which are sesquiterpenoid molecules, of which many variants are known. The type B trichothecenes are the most important. Among the type B trichothecene-producing *Fusarium* species, three main chemotypes of *F. graminearum,* based on differences in secondary trichothecene metabolites produced by different fungal strains within the species, are used to describe chemical phenotypes or the "toxigenic potential" of an isolate (Goswami and Kistler 2005; Osborne and Stein 2007; Ward et al. 2008). Strains producing mainly nivalenol (NIV) are referred to as the NIV chemotype, while those producing 15-acetyl deoxynivalenol (15-ADON) and DON are the 15-ADON chemotype and those producing 3-acetyl deoxynivalenol (3-ADON) and DON are the 3-ADON chemotype (Miller et al. 1991; O'Donnell et al. 2000). Although slight differences exist between these compounds in the pattern of hydroxylation and acetylation, the biological activities of these compounds can be different (Kimura et al. 2007).

Three *F. graminearum* chemotypes are present in Canada. In North America, the 15-ADON chemotype was the primary chemotype (Mirocha et al. 1989), whereas in Asia, the 3-ADON chemotype is more prevalent (Miller et al. 1991). Recently, the first evidence of the presence of NIV-producing *F. cerealis* isolates in winter wheat fields in Carman, Manitoba, Canada was reported (Amarasinghe et al. 2015). Presence of nivalenol was previously reported on barley samples in China (Zhang et al. 2011) and Argentina (Castañares et al. 2013), but the discovery of nivalenol producing *F. cerealis* species in Canadian winter wheat samples poses a serious threat to the Canadian wheat industry in the future. Research has shown that there has

been a rapid chemotype shift in Eastern and Central Canada and North Central USA from the 15-ADON chemotype to the 3-ADON chemotype (Guo et al. 2008; Ward et al. 2008; Puri and Zhong 2010). In 2004, over 90% of the *Fusarium* species isolated in Manitoba from Fusarium damaged kernels were *F. graminearum* (Gilbert et al. 2010); over 30% represented the 3-ADON chemotype (Ward et al. 2008). Now the 3-ADON chemotype isolates are more prevalent than the 15-ADON chemotype isolates in many regions of Manitoba (Guo et al. 2008; Ward et al. 2008). These conclusions have been confirmed in several field studies in wheat in Canada (Tamburic-Ilincic et al. 2009; Gilbert et al. 2010; von der Ohe et al. 2010b) and in the USA (Schmale et al. 2011). The basis for this chemotype shift is unclear, but Ward et al. (2008) believe there must be a large selection advantage for the 3-ADON chemotype, since its incidence was very low prior to 1998 in Western Canada. The 3-ADON chemotype appears to be more aggressive and produces nearly two times more mycotoxin in infected grains compared with 15-ADON chemotype (Ward et al. 2008; Gilbert et al. 2010; Puri and Zhong 2010; von der Ohe et al. 2010b) which leads to greater disease severity on wheat than 15-ADON isolates (Puri and Zhong 2010). In contrast, Ward et al. (2008) found no difference in pathogenicity between 15-ADON and 3-ADON isolates of *F. graminearum* when tested individually and under controlled conditions against a susceptible and a resistant wheat cultivar, despite the fact that 3-ADON isolates produced significantly more trichothecenes *in vivo*.

2.3.3 Types of resistance to FHB in wheat

There are two broad types of resistance to FHB, namely active and passive resistance. Five mechanisms of resistance to FHB have been defined. Type 1 resistance has been defined as resistance to initial infection and is thought to be primarily passive in nature. In contrast, Type 2 resistance reduces fungal spread from infected florets to other florets along the rachis and is

considered to be an active form of resistance. Type 1 and Type 2 resistance were first described by Schroeder and Christensen (1963) and are widely accepted and extensively studied. Mesterházy (1995) described Type 3 resistance as reduction in kernel infection; Type 4 as tolerance where yield and quality are maintained despite disease pressure; and Type 5 resistance as the ability of the host to degrade mycotoxins. The Type 3 resistance, Type 4 resistance and Type 5 resistance are not well understood and there is a lack of consistency among researchers on the definition and measurements of these types of resistance and their underlying mechanisms (Shaner et al. 2003).

The different types of resistance should be thoroughly considered to evaluate the FHB resistance of a given accession. Environmental effects on FHB development further complicate the expression of resistance, requiring screening over several environments to characterize the true FHB resistance potential of a particular genotype (Fuentes et al. 2005). Type 1 and Type 2 resistance can be assessed by either spray inoculation with *Fusarium* macroconidia or spreading *Fusarium* infected grain or plant debris directly on the soil followed by evaluation of the proportion of infected spikes (Type 1) and proportion of spikelets infected within a spike (Type 2). Disease incidence (Type 1 resistance) is easy to measure. The difficulty in assessing Type 2 resistance is that multiple infections in the same spike can be confused as spread within the spike and make Type 2 reaction higher than it should be. Type 2 resistance is reliably assessed by single or dual point inoculation of spikelet(s) in the top third of the spike and evaluating the extent of disease symptoms spread from the inoculation point in each spike (Mesterházy 1995; Hall 2002).The Type 2 resistance test is commonly done in controlled environment experiments and measured by scoring the number of infected spikelets 7-21 days post-inoculation (Lin et al. 2006).Type 1 and 2 are the two major forms of resistance to FHB studied (Foroud 2011).

Differences between Type 1 and 2 resistance are based on symptom development only and do not imply specific physiological or morphological resistance reactions.

Type 3 resistance is measured by counting the proportion of visibly Fusarium damaged kernels (FDK) as a percent of the total number of kernels, or by weight of FDK over the total weight. The measurement of kernel weight, test weight, or visual estimates of FDK are common assessment methods for Type 3 resistance (Mesterházy 1995; Verges 2004). Type 4 resistance is tolerance to *Fusarium* (Mesterházy 1995). Tolerance to disease means that genotypes continue to yield well despite significant FHB infection (Mesterházy 1995). It is the plant's ability to endure the impact of pathogen infection levels. Tolerance is assessed by calculating and comparing plot yields that are affected by FHB with similar plots without FHB symptoms (Rudd et al. 2001). Type 5 resistance is evaluated by mycotoxin analysis in grain using either ELISA (Enzyme Linked Immuno-sorbent Assay) DON test kits (Hall and Van Sanford 2003), near infrared reflectance (NIR) (Balut et al. 2013), or gas chromatography.

2.3.4 Fusarium head blight epidemiology

2.3.4.1 The life cycle of *F. graminearum*

A number of *Fusarium* species are known to cause symptoms of FHB in wheat. The most common species include *Fusarium avenaceum*, *Fusarium culmorum*, and *Fusarium poae* and *F. graminearum*. *Fusarium graminearum sensu stricto*, is the predominant causal agent of FHB in most areas of the world. The fungus has several hosts including wheat (*Triticum aestivum*), durum wheat (*Triticum turgidum spp.durum*), barley (*Hordeum vulgare*) and oat (*Avena sativa*). *Fusarium graminearum* is also known to parasitize/infect roots, stems, leaves, and reproductive tissues of many species of cereals and grasses and crop debris such as soybeans and canola residues. Sampling of roots of canola, flax, lentil and pea crops, normally grown in rotation with

wheat and barley in the Canadian-prairies revealed presence of *F. graminearum* from roots of field-grown pulse and oilseed crops in western Canada (Fernandez 2007).

Fusarium head blight severity and DON contamination significantly increase with the density of residues left from the preceding crop (Blandino et al. 2010). *Fusarium graminearum* overwinters on infested crop residues like corn stalks, wheat straw, and other host plants. The fungus produces asexual spores (macroconidia) from infested residues which are dispersed to wheat plants by rain-splash or wind. This is not the primary mode of infection. Ascospores are the primary inoculums in the field. When conditions are favorable (warm, humid, and wet) the sexual stage of the fungus (*Gibberella zeae*) develops on the infested plant debris. Bluish-black perithecia form on the surface of these residues, and forcibly discharge sexual spores (ascospores) into the air. The ascospores are picked up by turbulent wind currents and may travel great distances in the air.

The head blight disease is initiated by airborne spores landing on flowering spikelets in the wheat field, germinating, and entering the plant through natural openings like the base of the lemma and palea, or stomata, or through degenerating anther tissues (Bushnell et al. 2003). At the infection area, the fungus grows intercellularly and asymptomatically (Bushnell et al. 2003; Guenther and Trail 2005), spreading through the xylem and pith. The fungus also spreads radially and necrosis begins as the fungus grows intracellularly and rapidly colonizes the tissue. Symptoms at this stage include water soaking, especially of the chlorenchyma and the colonized tissue becomes bleached. Premature bleaching of spike tissue is a characteristic of infected spikes in the field, and bleached tissue may form on a band of several florets in the center of the spike, a typical symptom of head blight of wheat. Following infection of wheat florets, the fungus expresses genes for DON biosynthesis almost immediately (Jansen et al. 2005).

Deoxynivalenol is a virulence factor in wheat, causing tissue necrosis (Desjardins et al. 1996) and allows the fungus to spread into the rachis from florets in wheat (Jansen et al. 2005).

Fusarium graminearum can complete its life cycle in association with its host. *Fusarium graminearum* is haploid for most of its life cycle. The sexual development of this fungus begins with the formation of hyphae with binucleate cells. *Fusarium graminearum* belongs to phylum Ascomycota which is exclusively known for an extended binucleate phase known as dikaryotic phase when two genetically distinct nuclei remain paired as new cells form. This phase is the initial step in sexual development of Ascomycota. *Fusarium graminearum* is homothallic, and it does not require a sexually distinct partner to develop sexual spores (ascospores). Homothallism in *F. graminearum* is due to the presence of genes associated with both mating types (*Mat1-1* and *Mat1-2*) in the haploid genome (Yun et al. 2000). The binucleate cells of *F. graminearum* develop small coiled cells, which are the fruiting body structures (Trail et al. 2002). Asci are tubular sacs containing the ascospores, which are the products of meiosis. Asci extend up to the mouth of the perithecium and forcibly discharge their ascospores into the air due to turgor pressure. In the laboratory, the entire life cycle takes about two weeks, with asci maturing and firing sequentially for about the last four days (Trail et al. 2002).

Sexual development is a critical part of the disease cycle. In infected wheat, perithecium structures develop in association with the plant's stomates and silica cells, and, together with the binucleate hyphae from which they arise, are the overwintering structures (Guenther and Trail 2005). In the field, the perithecia are ephemeral. The airborne ascospores are the primary inoculum of the disease, which is considered to be a monocyclic disease. In one study, elimination of the sexual stage by deletion of the mating-type locus resulted in substantial

disease reduction in field trials compared with plots inoculated with a strain complemented back to wild-type ascospore production (Desjardins et al. 2006).

The macroconidia (asexual spores) are produced on the surface of infected plants or on crop residue during damp periods. Macroconidia are produced in slimy masses borne on sporodochia (cushion-shaped hyphal structures). The fusi-form shape of the macroconidia and their formation in slimy masses has been associated with rain-splash dispersal (Deacon and Deacon 2005). Macroconidia are known to be short-distance dispersed (Shaner et al. 2003).

Infection occurs when the ascospores (and also macroconidia) land on susceptible wheat spikes. Florets that are infected will produce diseased kernels that are shriveled and wilted, or "tombstone" in appearance. Kernels that are colonized by the pathogen during late kernel development may not appear to be affected, but may still be contaminated with the mycotoxin DON. Viable propagules (ascospores and macroconidia) of *F. graminearum* exist in the air before, during, and after wheat flowering. The majority of spores dispersed from crop residues generally travel only short distances, but given appropriate weather conditions and wind, spores may spread over long distances (Fernando 2000). Fusarium head blight infection is favored by extended periods of high moisture or relative humidity (>90%) and moderately warm temperatures (between 15 to 30°C). These conditions occurring before, during, and after flowering favor inoculum production, floret infection, and colonization of developing grains.

2.3.4.2 *Fusarium graminearum* infection process

Fusarium graminearum can survive in the soil and on crop residues as mycelia, ascospores, macroconidia, chlamydospores, and perithicia (Dill-Macky 2010). Corn and wheat residues are very suitable for survival of the fungus. The primary inoculum for FHB is mainly ascospores produced in overwintering perithecia, which form on crop residues in the spring as

temperatures warm up. Ascospores are forcibly discharged from perithecia due to turgor pressure when conditions are favourable. Disease in wheat is initiated when airborne ascospores land on flowering spikelets, germinate, and enter the plant through natural openings such as the base of the lemma and palea or through degenerating anther tissues (Bushnell et al. 2003) and stomata. The pathogen expresses genes for DON biosynthesis almost immediately after infection of wheat florets (Jansen et al. 2005). However, there is a short period during the infection process when the pathogen acts in a biotrophic manner before it changes to a necrotrophic state.

Deoxynivalenol is a virulence factor in wheat causing tissue necrosis (Proctor et al. 1995; Desjardins et al. 1996) and allowing the fungus to spread into the rachis from infected florets (Jansen et al. 2005). Deoxynivalenol is also known to function as a virulence factor in maize (Harris et al. 1999), but in barley, spread of the disease is limited and virulence does not appear to be due to the presence of the toxin (Jansen et al. 2005). Colonization of developing seeds is accompanied by DON accumulation, resulting in shriveled, undersized grain, referred to as Fusarium damaged kernels (FDK) or tombstone kernels.

Anthesis (flowering) is the growth stage that wheat is most vulnerable and susceptible to infection (Bai and Shaner 2004), but infection can occur from anthesis up to the hard dough stage of kernel development (McMullen 2008). During warm temperatures (25˚C to 30˚C) and wet conditions (over 85% relative humidity), blight symptoms develop within two to four days after infection. The symptoms appear as water soaked lesions; bleaching on glumes and usually extends into the rachis and into the stem tissue as the fungus spreads (Bai and Shaner 2004). In wheat, FHB symptoms appear as premature bleaching of one or more spikelets on a spike. This bleaching can continue until the entire spike is whitened. *Fusarium graminearum* sporulates on infected spikelets and glumes during prolonged wet weather, resulting in the production of pink

to salmon- colored spore-bearing structures called sporodochia at the base of the glumes, which are a diagnostic feature of FHB under heavy disease pressure (Schmale et al. 2003; McMullen et al. 2012). Infection of the peduncle immediately below the spike may also occur, causing a brown or purple discoloration.

2.3.5 Morphological factors affecting FHB infection in wheat

As previously stated, mechanisms of resistance to FHB in wheat are classified as morphological (passive) or physiological (active) (Rudd et al. 2001). Fusarium head blight disease avoidance is conditioned by morphological and developmental characteristics such as height, awnedness, anther extrusion/retention (AE/AR) and width of flower opening during anthesis (Buerstmayr et al. 2009). Physiological resistance presumably involves a biochemical pathway that produces compounds that inhibit the pathogen after infection, while morphological resistance allows the plant to escape infection during its most susceptible stage (Wiese 1978). Passive resistance in FHB is associated with plant height (greater height>greater resistance); presence of awns (lack of awns increase resistance); spikelet density (loose ears>greater resistance); anther extrusion (extruded anthers increase resistance) and escape (flowering in the boot).

2.3.5.1 Effect of flowering time and flower opening on FHB in wheat

Genotypes show different degrees of anther extrusion, in addition to cleistogamous florets where anthers are completely enclosed (Graham and Browne 2009; Nair et al. 2010; Kubo et al. 2013). FHB resistance QTL overlapped with QTL for plant height and flowering time in wheat Renan/ Recital (Gervais et al. 2003) and Arina/Forno (Paillard et al. 2004) mapping populations. Narrow flower opening and short floral opening duration were correlated to low FHB incidence in a Patterson/Goldfield population with a major QTL reported on chromosome

2B associated with narrow flower opening and low FHB incidence (Gilsinger et al. 2005). Singh et al. (2007) reported a strong positive correlation between AE and duration of flower opening. Flowering time was associated with FHB severity where early flowering types were more susceptible than later flowering types (Srinivasachary et al. 2009; Buerstmayr et al. 2011). It is generally assumed that early or late maturing lines escape infection by not being at anthesis when optimal conditions are present for infection (Somers et al. 2003), or by slowing down disease spread within the spike when environmental conditions are not optimal for disease development (Lin et al. 2006).

In wheat, cleistogamous (closed flowering) cultivars showed less initial FHB infection (enhanced Type 1 resistance) than chasmogamous (open flowering) cultivars (Kubo and Kawada 2009; Kubo et al. 2013). Fusarium head blight severity was lowest in closed flowering lines, followed by lines with full anther extrusion, while lines with partially exposed anthers were most sensitive to FHB (Kubo et al. 2013). Similar results have been reported in barley (Yoshida et al. 2005). For example, Yoshida et al. (2005) reported that tests using spray inoculation of *F. graminearum* at anthesis in greenhouse environments showed that two-rowed and cleistogamous barley varieties from Japan belonged to the highest resistance group, while six-rowed and chasmogamous barley varieties were mostly susceptible. In a winter wheat population developed from the cross Dream /Lynx, Schmolke et al. (2005) detected four QTL for resistance to disease severity, one of which was associated with plant height and spike compactness, and another QTL was associated with flowering date. The correlation between AE and open flowering is a two edged sword, as observed by Gilsinger et al. (2005), who found that wide flowers and long flowering duration worked as a spore trap and promoted *F. graminearum* infection.

2.3.5.2 Relationship between presence of awns on FHB of wheat

Mesterházy (1995) observed that the genotypes with awns were more susceptible to FHB when tested under natural epidemic condition in the field; but this trait did not influence FHB severity in artificial inoculations. He speculated that the larger surface of awned spikes makes infection by airborne macroconidia more likely and dew caught by the awns provide potentially more humid conditions for successful infection. In contrast, Snijders (1990) reported the linkage between FHB resistance and awnedness in winter wheat infected with *F. culmorum* and suggested that in progenies in which the resistant parent was awned, selection for awned spikes indirectly selected for partial resistance to FHB. Ban and Suenaga (2000) demonstrated that one of the resistance genes in the FHB resistant Chinese wheat cultivar Sumai 3 may be linked in repulsion to the dominant suppressor *B1* gene for awnedness. Gervais et al. (2003) also showed that the FHB resistance QTL located on the long arm of chromosome 5A was linked to the *B1* gene in a population of Renan/Recital.

In Canada, a recombinant inbred lines (RIL) population derived from the cross of two spring wheat genotypes, AC Brio /TC 67 (derived from *T. timopheevii*), showed that the genotypes with awns, like the TC 67 parent, had less FHB disease than lines without awns under both field and greenhouse conditions (Malihipour et al. 2016). Chrpova et al. (2010) reported that the presence of awns reduced both DON content and FHB index in wheat in two RIL populations derived from two crosses, Sumai 3/Swedget and Sumai 3/SG-S 191-01. The presence or absence of awns affected both DON content and FHB index in both populations, but was only significant in the cross Sumai 3/SG-S 191-01, where the effect of selection for fully awned genotypes resulted in reduction of both DON and FHB index in classes without *Fhb1* gene.

2.3.5.3 Relationship between spike density/wheat spike compactness and FHB

In some studies, compactness of wheat spikes was considered to lead to more FHB while in other studies it reduced disease. For example, in the cross Frontana/Remus, Steiner et al. (2004) observed a significant negative, but low correlation between FHB and wheat spike compactness. The QTL for spike compactness were reported on chromosomes 1A and 7A. In a population of G16-92/Hussar, a QTL for ear compactness was detected on chromosome 5A (Schmolke et al. 2008). In contrast, Mesterházy (1995) reported that plants with a dense spike tend to be more susceptible to spike diseases because of micro-climatic conditions. In a RIL population from the cross YZI/NX188, six additive QTL for spike compactness were detected on chromosomes 2D, 4A, 5A, 6B and 7B and each explained between 2.8% and 17.3% of the phenotypic variation. NX188 contributed alleles for compactness except for that on chromosome 4A and they increased spike compactness contributing 39.3% phenotypic variation (Lv et al. 2014). However, only QTL on chromosome 2D co-localized with that of FHB resistance.

2.3.5.4 Relationship between plant height and FHB

Mesterházy (1995) reported a negative correlation between plant height and FHB symptoms. Reduced height in wheat has been associated, or coincides, with increased susceptibility to splash dispersed pathogens like FHB (Draeger et al. 2007; Miedaner and Voss 2008; Srinivasachary et al. 2009). Generally, taller genotypes have lower levels of FHB symptoms (McCartney et al. 2007; Tamburic-Ilincic et al. 2007). This may be due to the shorter plants being closer to the inoculum source because their spikes are closer to the soil and crop residues that harbour the pathogen. When ascospores are propelled from the perithecia they are more likely to land on spikes that are closer to the soil surface and canopy structure alteration

may provide a micro-climate that is more favorable to the pathogen for establishment (Scott et al. 1985).

Research has shown that not all plant height QTL are associated with FHB reaction (Draeger et al. 2007), suggesting that resistance is not an effect of height per se, but rather of linkage or pleiotropy. The *Rht-B1* and *Rht-D1* loci are coincident with FHB loci suggesting that they may have a pleiotropic effect on FHB (Srinivasachary et al. 2009). For example, Srinivasachary et al. (2009) and Liu et al. (2013) demonstrated that lines carrying the *Rht-1Db* semi-dwarfing allele have higher initial FHB infection, but spread within the spike was not associated with the presence or absence of the semi-dwarfing allele. Yan et al. (2011) observed similar results that tall isolines had superior Type 1 resistance compared to dwarf isolines, but there was no clear effect on Type 2 resistance. The dwarf allele *Rht-D1b* also increased FHB symptoms in a double haploid (DH) population in a cross between Avle and Line 685. Avle contained *Rht-D1a* allele while Line 685 had *Rht-D1b* dwarf allele with Sumai-3 and Nobeokabouzu-Komugi FHB resistance sources in their genetic background (Lu et al. 2011). Three winter wheat populations (Apache/Biscay, Romanus/Pirat and History/Rubens with 190, 216 and 103 progenies) were developed after crossing susceptible varieties Biscay, Pirat and Rubens carrying mutant-type allele *Rht-D1b* with the more resistant varieties Apache, Romanus and History containing the *Rht-D1a* wild-type allele. High mean FHB severities ranging from 28% to 49% were observed for the three segregating winter wheat populations with significant genotypic variation for FHB reaction and plant height within all populations (Voss et al. 2008). The *Rht-D1b* allele resulted in 7–18% shorter plants than those with the *Rht-D1a* alleles, depending on the population, but increased FHB severity by 22–53% relative to *Rht-D1a* with a severity of 6 -30%. Selection for moderately FHB resistant genotypes among lines carrying the

Rht-D1b allele was feasible given that significant genotypic variance for FHB resistance remained in all tested *Rht-D1b* sub-populations (Voss et al. 2008). According to Lu et al. (2011), two major resistances QTL, the *Fhb1* gene and 5A QTL, were required to counteract the negative effect of the dwarf *Rht-D1b* allele. The *Rht-D1b* allele was associated with increased yield and decreased lodging, making it desirable for wheat breeders.

2.3.5.5 The relationship between anther extrusion/retention and FHB

Anther extrusion (AE) refers to the process where anthers are pushed out of the floret during flowering, while anther retention (AR) means the anthers are retained in the floret. High AE has been suggested as one of the resistance mechanisms to FHB in wheat. Anther extrusion has high negative correlation with FHB symptoms (the higher the anther extrusion the lower the FHB symptoms) and may be used as an indirect selection target for enhancing FHB resistance (Buerstmayr and Buerstmayr 2015). Graham and Browne (2009) concluded that selection for high AE among European wheat could improve FHB resistance, without negatively impacting agronomic traits. It was suggested that high AE led to lower infection rates, contributing to Type 1 resistance (Skinnes et al. 2010, Buerstmayr and Buerstmayr 2015). Some plant breeders suggested that AE is a stable character (Salvatore 1978; Lu et al. 2013; Buerstmayr and Buerstmayr 2015), while others have concluded that the trait is highly influenced by environmental factors, especially drought (Abdel-Ghani et al. 2005, Stråbø 2014).

Studies show that the more anthers are retained, the greater the FHB susceptibility (Graham and Browne 2009; Skinnes et al. 2008, 2010; Buerstmayr and Buerstmayr 2015). Transgressive segregation for FHB severity and anther retention were reported in several studies (Skinnes et al. 2010; Lu et al. 2013; Buerstmayr and Buerstmayr 2015). For example, transgressive segregates were found in the Arina/NK93604 double haploid (DH) population

(ArNK), suggesting that both parents carry positive and negative alleles, as has been previously reported for FHB and DON for the same population (Semagn et al. 2007). Skinnes et al. (2005) found a high correlation of -0.61 between AE and FHB after inoculation with *Fusarium culmorum*. Graham and Browne (2009) examined the relationship between AE and FHB in 60 European winter wheat and found significant negative correlations(r = -0.25 to -0.26). Skinnes et al. (2010) reported that the mean anther extrusion (AE) showed highly negative correlations both with FHB (*r* = -0.53 to -0.69, P = 0.0001) and DON (*r* = -0.39 to -0.46, P = 0.0001). Anther retention was positively correlated with FHB severity (*r* = 0.63) reflecting the high FHB severity on plants with high anther retention in a study of 171 RILs from an Arina (resistant) /Capo (moderately resistant) cross (Buerstmayr and Buerstmayr 2015). High broad-sense heritability for AE in the ArNK population was reported (H = 0.91) (Skinnes et al. 2010), which is in agreement with Singh et al. (2007) who studied 400 genetically diverse genotypes of spring wheat and found high heritability for anther extrusion. Singh et al. (2007) also found genetic and environmental variation in anther extrusion, anther and stigma length, duration of flowering, and angle of separation of florets, but only duration of flowering was associated with anther extrusion.

2.3.5.5.2 Quantitative trait loci associated with anther extrusion/ retention and FHB

Quantitative trait loci alleles for low AE co-localized with the *Rht-B1b* dwarfing allele and increased FHB infection after spawn and spray inoculation (Lu et al. 2013; He et al. 2016). In the SHA3//CBRD/Naxos RIL population, high FHB severity was associated with low AE and short plant height (Lu et al. 2012). Skinnes et al. (2010) showed that AE was controlled by three major QTL on chromosomes 1AL, 4DL and 6AS, as well as two minor QTL on chromosomes 1BL and 7AL in the Arina/NK93604 population consisting of 93 DH lines. Semagn et al. (2007)

reported a major coincident QTL on 1AL for FHB resistance, DON accumulation and AE. Although all the three QTL for FHB resistance, DON and AE on chromosome 1AL originated from NK93604 alleles, the QTL for AE was 38 cM proximal to that of the FHB and DON, and likely independent. The QTL for AE on chromosome 6AS originated from Arina (Skinnes et al. 2010) and was located at the vicinity of Xbarc3 and Xbarc17, in the same region that Draeger et al. (2007) detected a QTL for resistance to Fusarium damaged kernels and yield in the cross Arina/Riband. The QTL for AE on chromosome 4DL was located at a confidence interval between 46 and 62 cM and explained 13.3% of the phenotypic variance over two years in Arina/NK93604 (Skinnes et al. 2010). Similarly, QTL for FHB related traits in the Arina/Riband population were located in the same region. The exact location of the flanking markers varied among maps, though these markers mapped between 35 and 46 cM (Somers et al. 2004; Song et al. 2005). These two cases point to possible common QTL for AE and FHB severity and not disease incidence because retained anthers act as a source of nutrients to FHB pathogen, thus increasing the FHB infection in the spike.

In the Arina/NK93604 DH population, high AE was correlated with low FHB and a QTL for AE and FHB was detected on chromosome 1B (Semagn et al. 2007). In addition, closely linked QTL for the AE and FHB were identified on chromosome 7A (Skinnes et al. 2010) in the same DH population (Arina/NK93604). The phenotypic distribution suggested that in lines with low AE, anthers trapped between the lemma and palea provided dead tissue readily colonized by Fusarium spp. Also in the Arina/Capo population, Buerstmayr and Buerstmayr (2015) reported QTL for AR on chromosomes 4A, 5A and 6B.

2.4 Management of Fusarium head blight

Cultural practices such as tillage (Schaafsma et al. 2005; Lori et al. 2009), harvesting dates (Xue et al. 2004), nitrogen management (Yang et al. 2010), or fungicide application (Blandino and Reyneri 2009; Haidukowski et al. 2012) have a direct impact on the incidence of FHB and DON concentrations in the harvested grain of spring wheat (Hollingsworth et al. 2008). In addition, choice of more resistant cultivars, crop rotation, residue management, and date of planting may be used to reduce FHB damage.

2.4.1 Cultural practices

Fusarium graminearum survives on the crop debris and burying the debris results in faster decomposition than if the debris is left on the soil surface (Khonga and Sutton 1988; Dill-Macky and Jones 2000). Cultural practices such as conventional tillage may reduce disease, compared to conservation tillage. However, conventional tillage is not likely to be widely practiced because of the energy costs, degradation of soil structure, and erosion. Pereyra et al. (2004) found that *F. graminearum* infested wheat residue that was left on the surface of the soil decomposed slower that buried residue and provided a substrate for the pathogen for a longer period of time. These observations and current knowledge of the aerobiology of *F. graminearum* (Keller et al. 2014) suggest that overwintered wheat, barley or corn residue are a source of both local and regional atmospheric inoculum causing FHB in wheat fields, including those with little within-field inoculum. Fusarium is a necrotroph and it can survive on many types of crop residues. Previously infected wheat or corn may provide the original source of the inoculum, but it can also grow on canola stubble that gets infected from the previous wheat crop residues.

Crop rotation is another cultural practice that could reduce disease. The disease may be more severe in fields with corn and/or wheat residue on the soil surface or where plants are

irrigated due to the splashing effect. Rotations that provide time for degradation of corn, barley, or wheat residues prior to replanting these crops can reduce inoculum levels and subsequent disease. Another cultural method to reduce diseased kernels in harvested grain is to increase the air flow on the combine during harvest to blow out FDKs which are less dense, thus improving the overall quality of the grain harvested (Salgado et al. 2011).

2.4.2 Chemical control

Timely application of fungicides is one of the most important disease management strategies for FHB control. Fungicides should be used with other management strategies such as tillage, crop rotation and resistant cultivars. Fungicides are used at either the flowering stage, or slightly before the flowering stage, to reduce yield loss and mycotoxin contamination (Mesterházy 2003; Ye 2015). The level of resistance of the cultivar, timing and coverage of fungicide application, the rate of application and aggressiveness of the pathogen determine the success of fungicide application (Mesterházy et al. 2003; Amarasinghe et al. 2011; Ye 2015). Many studies have reported inadequate, or inconsistent, control of FHB when fungicides are used on highly susceptible cultivars (Parry et al. 1995; McMullen et al. 1997). The most widely used fungicides are in the demethylation inhibitor (DMI- sterol biosynthesis inhibitors) class (McMullen et al. 2012).They include metconazole (Caramba), propiconazole (Banner Pro), prothioconazole (Proline), tebuconazole (Folicur), and prothioconazole + tebuconazole (Prosaro) (Gilbert and Haber 2013). Fungicides lead to suppression of FHB, but do not eliminate the disease completely (Xue et al. 2009). Fungicides in the quinone outside inhibitor (QoI) class which include strobilurins have been shown to increase DON levels in grain (Ellner 2005; Blandino et al. 2006; Blandino and Reyneri 2009) and are not recommended for FHB and DON control.

2.4.3 Biological control of FHB

The need for alternative disease management strategies to augment host resistance and fungicides has driven a worldwide search for biological control agents to protect flowering spikes against FHB (Luz et al. 2003). Bio-control agents are living organisms that require specific conditions to be effective and are most effective if these conditions are similar to those of the pathogen (Gilbert and Fernando 2004). Bio-controls applied at anthesis are thought to overcome pathogens by aborting, curtailing, or delaying germination of *F. graminearum* spores (Gilbert and Fernando 2004). Effective bio-control agents are able to reduce disease incidence and severity, while minimizing DON concentration. Efficacy in reducing FHB severity or DON accumulation with bacterial and yeast strains has been documented, but consistent field performance over time and across locations has been difficult to achieve (Schisler et al. 2001; Khan et al. 2004). Strains of *Bacillus* spp. and *Pseudomonas* spp. have been the most commonly investigated bacterial agents (Luz et al. 2003; Khan et al. 2004). Yeast strains, including those of *Cryptococcus* species also have proven to be promising biocontrol agents against FHB (Khan et al. 2001). Biological control is an important tool when integrated with host resistance (Luz et al. 2003, Khan et al. 2004). Hue et al. (2009) suggested that strain ACM941 of *Clonostachys rosea* is a promising biocontrol agent against *Gibberella zeae* and may be used as a control measure in an integrated FHB management program. Under simulated disease epidemic conditions during 2005-2007, strain ACM941 reduced the FHB index by 58%, infected spikelets by 46%, FDK by 49%, and DON in kernels by 21%.

2.4.4 Genetic resistance and sources of resistance

Host plant resistance is the most effective, economical and environmentally sound method for controlling FHB and reducing the agricultural and food contamination problems

posed by mycotoxins (Zhang et al. 2008). Resistance to FHB is controlled by a few genes of major effect, plus several genes of more minor effect (Liu et al. 2005). This, combined with the fact that the expression of symptoms is variable and confounded by large environmental effects and genotype x environment interactions (Miedaner et al. 2001), makes selection for improved resistance to FHB difficult and costly. As previously mentioned, multiple types of resistance to FHB described by Schroeder and Christensen (1963) and Mesterházy (1995) further complicate selection for plant resistance. The complexity of resistance is demonstrated in a review by Buerstmayr et al. (2009) who indicated that 100 QTL have been associated with FHB resistance in over 50 diverse wheat sources worldwide, covering all chromosomes except chromosome 7D (Buerstmayr et al. 2009; Liu et al. 2009; Löffler et al. 2009).

Most resistant sources characterized to date are spring wheat genotypes from Asia (Sumai 3, Ning 7840, Ning 8331 etc) and South America (Frontana). Studies have shown that the resistance genes in Sumai 3 have been incorporated into adapted wheat backgrounds and resulted in a few accession releases (Costa et al. 2010; Niwa et al. 2014).The most prominent QTL associated with FHB resistance are *Fhb1* on chromosome 3BS (Anderson et al. 2001; Cuthbert et al. 2006) and *Fhb2* on chromosome 6BS (Anderson et al. 2001; Cuthbert et al. 2007). *Fhb1* was mapped as a single Mendelian gene in high resolution mapping populations segregating for the *Fhb1* gene (Cuthbert et al. 2006). Flanking STS (sequence tagged sites) markers bracketing *Fhb1* within a 1.2 cM interval are now available (Cuthbert et al. 2006; Liu et al. 2006). Recently, the *Fhb1* from a Chinese wheat cultivar Sumai 3 was map-based cloned using mutation analysis, gene silencing and transgenic over-expression, which revealed that a pore-forming toxin-like (*PFT*) gene at *Fhb1* confers FHB resistance. The pore-forming toxin-like gene is assumed to encode a chimeric lectin with two agglutinin domains and an ETX/MTX2 toxin domain. This

study identified a new type of durable plant resistance gene conferring quantitative disease resistance to plants against *Fusarium* species (Rawat et al. 2016). Another candidate gene that controls Type 2 resistance is still being considered by Bai (United States Department of Agriculture-USDA). Deoxyivalenol control in this region appears to be the result of another nearby gene (McCartney, personal communication).

Fhb1 has the largest effect of all loci on Type 2 resistance and accumulation of DON. *Fhb1*is the most extensively used FHB resistance source in breeding programs worldwide (Pumphrey et al. 2007; Buerstmayr et al. 2009). Sumai 3 is the primary source of *Fhb1* and is not well adapted to North American conditions and as a result, there is significant linkage drag when introgressing this gene into a breeding program. For example, McCartney et al. (2007) observed that *Fhb1* was associated with decreased grain protein content, flour yield, and falling number, which are important end-use quality traits for wheat and are the target of selection in most wheat breeding programs.

The Brazilian spring wheat cultivar-Frontana has been used as a source of FHB resistance for both Type 1 and Type 2 resistances. Steiner et al. (2004) mapped the main QTL from the cultivar Frontana on chromosome 3A, which explained 16.2% of phenotypic variation. Srinivasachary et al. (2008) also reported QTL on chromosomes 1B, 3A, 6A, 2B and 7A in a cross with a FHB resistant spring wheat line RL4137 derived from Frontana and the moderately FHB resistant variety Timgalen. The *Fhb4* gene has been mapped to chromosome 4B from the RIL population that was derived from the cross Nanda2419/Wangshuibai (Xue et al. 2010). The 4B QTL may be associated with the major *Rht* genes located on this chromosome (McCartney et al. 2007) because taller genotypes may escape infection under light inoculum loads.

In winter wheat, resistance QTL have been mapped mainly in populations from European descent such as Sincron and F201R from Romania (Ittu et al. 2000; Shen et al. 2003), Renan from France (Gervais et al. 2003), and Dream, Ritmo, and GS16-92 from Germany (Klahr et al. 2007; Schmolke et al. 2008). These European sources mainly contain *Rht-B1* and *Rht-D1* dwarfing genes which are associated with FHB symptoms. Only a few soft red winter (SRW) wheat sources from the US including Ernie, Goldfield, Freedom, IL 94-1653, and VA00W-38 (Gilsinger et al. 2005; Liu et al. 2007; Abate et al. 2008; Bonin and Kolb 2009 ; Liu et al. 2012) have been shown to contain some resistance to FHB. The spring wheat resistance sources are mainly from *Fhb1* which has been identified from Sumai 3 and its derivatives, and 5A QTL from Frontana. No winter wheat sources for *Fhb1* are known, making the winter wheat sources distinct sources of FHB resistance. The major QTL for FHB resistance are located on chromosomes 2DS, 3BL, 4BS, 4DS (Liu et al. 2013) in soft red winter wheat of the US.

Other sources of resistance are available from exotic gene pools such as *Triticum macha* (Mentewab et al. 2000; Buerstmayr et al. 2011), *T. dicoccoides* (Otto et al. 2002; Stack et al. 2002) and *Lophopyrum elongatum* (Shen et al. 2004). In Canada, currently, there are two highly resistant cultivars that are commercially available, namely Emerson (Graf et al. 2013) and AAC Tenacious (Brown et al. 2015) with only a few of the current cultivars being moderately resistant to FHB. Other QTL include *Fhb3* introgressed from *Leymus racemosus* (Qi et al. 2008). In a recent study, FHB resistance QTL on the chromosomes 5AL and 6A were detected from a RIL population from the cross AC Brio (a Canadian bread wheat cultivar moderately susceptible to FHB) and TC 67 (an FHB-resistant wheat line derived from *T. timopheevii*). A novel QTL that originated from *T. timopheevii* which was reported for the first time mapped on chromosome 5AL for FHB resistance in the marker interval of *cfd39-cfa2185*. This QTL explained 19.4% and

20.6% of phenotypic variation for FDK under field conditions and disease severity in the greenhouse (Type 2 resistance), respectively (Malihipour et al. 2016). Limited information is available on FHB resistance derived from wheat relatives. Genetic analysis of FHB resistance derived from these wheat relatives may be important in broadening FHB resistance genetic base.

The genetics of resistance to DON accumulation has been less studied, but QTL were reported on chromosomes 2A and 5A (Ma et al. 2006). In a DH population Wuhan 1/Nyu Bai (McCartney et al. 2007), several QTL were detected for different components of resistance depending on the phenotyping methods applied. Two Type 2 resistance QTL were found on chromosome 2DL (resistant allele from Wuhan 1) and chromosome 3BS (resistant allele from Nyu Bai). For disease severity after spray inoculation, two QTL were detected on chromosomes 3BSc (resistant allele from Nyu Bai) and 4BS (resistant allele from Wuhan 1). Two QTL for DON content after spray inoculation were detected on 3BS and 5AS (both resistant alleles derived from Nyu Bai). In a population of DH181 (a line selected from the cross Sumai 3/ HY368) crossed with AC Foremost, Yang et al. (2005) reported seven QTL for Type 1 resistance, four QTL for Type 2 resistance and six QTL for resistance to kernel infection. QTL on 2DS, 3BS and 6BS were associated with all three traits.

In a marker haplotype study, McCartney et al. (2004) analyzed allele sizes of 41 SSR markers on 79 wheat lines. The same marker haplotype as Sumai 3 for the SSR markers Xgwm493, Xbarc147 and Xgwm533 (spanning *Fhb1*), was found in seven genotypes, including the highly resistant genotypes Ning 7840, Ning 984037, ND2710, and CM-82036. McCartney et al. (2007) reported marginal additivity among the particular FHB QTL studied and reported significant linkage drag, such as negative correlation with plant height, and association of the Sumai 3 5AS resistant allele with reduced grain protein content. In Canada, *Fhb1* and *Fhb5* were

combined in the cultivar Cardale (Fox et al. 2013). In a cross Wangshuibai /Nanda 2419, Lin et al. (2004, 2006) found four QTL for FHB on chromosomes 2B, 3B, 4B and 5A. The 4B QTL were fine-mapped later by Xue et al. (2010) and is flanked by the markers *Xhbg226* and *Xgwm149*. Other resistance QTL have been reported on chromosomes 2B (Gervais et al. 2003), 3A (Steiner et al. 2004), 4AL (Paillard et al. 2004), 5AL (Gervais et al. 2003; Singh et al. 2007, and 6DL (Paillard et al. 2004).

2.5 Molecular mapping in wheat

2.5.1 Mapping populations

Different types of populations can be used to perform genetic mapping with the four primary mapping population types being F_2, backcross (BC_1F_1 and BC_2F_1), DH lines, and RILs (Young 1996); (Mohan et al. 1997; Gupta et al. 1999). Studies have shown that using more lines is always better than using few lines (Beavis, 1998). A large number of lines are needed for meaningful QTL estimation when the QTL have moderate to small individual effects contributing to trait expression (Vales et al. 2005).

Backcross and F_2 populations are easy to develop, but are inadequate for replicated studies, since they are not immortalized lines, and cannot be easily reproduced to maintain a homozygous genetic background for indefinite evaluation. Double haploid lines and RILs are homozygous at all, or the majority, of gene loci (Bun et al. 1988). Self pollination of these lines generates offspring that are genetically identical, or nearly identical, to the parent. Use of dominant and co-dominant markers of the DH lines and RILs provide the same amount of genetic information in these populations (Poehlman 2013). Double haploid and RIL lines can be tested for an unlimited number of traits in an unlimited number of environments (Baenziger et al. 2009). Accurate assessment of the genetic component of variance can be made for quantitative

traits because a genotype is represented by a line instead of a single individual (Bun et a1. 1988). Double haploid lines development is quicker, but they are labor intensive to produce (Poehlman 2013). The ease of DHs use is restricted by the technical difficulty of producing a sufficiently large population in a desired genetic background (Seymour et al. 2012). The development of RILs requires multiple cycles of self pollination. Recombinant inbred lines have more recombination events than DH lines because of the additional meioses in RIL development (Burr et al. 1988). The RILs produce genetic maps of higher resolution than a similar sized DH population.

Introgression lines (ILs) (Eshed and Zamir 1995), are also immortal populations obtained through repeated backcrossing and extensive genotyping. They are called near-isogenic lines (NILs) (Monforte and Tanksley 2000). The NIL mapping strategy involves creating a set of lines in which each NIL carries only a small region of the donor parent genome. The NILs offer more accurate QTL effect estimates than RILs when multiple QTLs are segregating in a population since RIL mapping studies are often confounded by many segregating QTL (Szalma et al. 2007). When QTL with large effects are present, the power of NILs to detect QTL is comparable to RIL populations (Keurentjes et al. 2007). The NILs are very good for fine mapping studies of regions that have previously been marked as important QTL. The NILs have been suggested as a resource to theoretically improve QTL detection and estimation because of the importance of background effects.

2.5.2 Phenotyping

To detect the level of genetically determined resistance of each line of the mapping population as accurate, phenotyping is required. One of the major problems in testing for Fusarium resistance is reproducibility of test results (Groth et al. 1999; Dill-Macky 2003).

Different types of resistance may require different methods of phenotyping. Resistance to FHB is a complex trait and different FHB resistance measurements are used. Type 1 and Type 2 resistance may be independently controlled. For mapping purposes, the main FHB traits phenotyped include; incidence, severity, Fusarium head blight index (incidence x severity/100), FDK, DON and yield. FHB symptoms may be evaluated visually on the spikes, or by visual scoring of percentage of FDK in harvested samples, or measurement of yield or yield components relative to non-inoculated controls, or DON content. Greenhouse based QTL mapping studies have been conducted (Buerstmayr et al. 2009; Liu et al. 2009). Evaluation of FHB resistance expressed under field conditions is more comprehensive and provides information on the different resistance types and overall level of FHB resistance compared to greenhouse assessments. Macroconidia spray inoculations in mist-irrigated nurseries have been used to invoke the disease in many studies (Buerstmayr et al. 2009; Liu et al. 2012).

Uniform inoculated nurseries provide better information for mapping than naturally infected nurseries. Uniform inoculum application during anthesis in the field or greenhouse to provoke Fusarium infections leads to more consistent phenotypic results, but does not prevent the potential of having GxE. Reaction to FHB is a quantitative trait that is modulated by genetic factors of the host (resistance factors in the plant) and of the pathogen (aggressiveness of the fungus) and environmental influences on disease establishment and development. There may be confounding effects because the different mechanisms of resistance are phenotypically related. The environment affects the expression of the trait for each genotype, while GxE measures how a group of genotypes responds to different environments. Significant GxE interactions (Campbell and Lipps 1998; Fuentes et al. 2005) can affect QTL estimates (Ma et al. 2006).

A major challenge in QTL mapping is to separate the effect of tightly linked, or pleiotropic, genes involved in morphological or developmental traits that influence FHB response from effects of true FHB resistance genes. Including resistant and susceptible checks with a broad range of flowering dates helps to separate and interpret effects due to flowering date, but positive correlations between plant height and disease resistance are more difficult to separate, especially under field conditions. A good example is when dwarfing genes which may mask the effects of other QTL involved with resistance (Cuthbert et al. 2008).

2.5.3 Mapping and marker assisted selection

2.5.3.1 Genetic markers

Deoxyribonuclei acid based markers linked to traits of importance are very useful for traits such as FHB that are difficult and expensive to phenotype. Markers that are linked to resistance genes can be used effectively for selection of combinations of genes for resistance (Anderson 2007). Marker assisted selection (MAS) uses the presence or absence of a marker linked to traits of interest for selection which increases efficiency, effectiveness, reliability, and reduces costs compared to conventional plant breeding (Collard et al. 2005). Kolb et al. (2001) identified steps that are typically followed to develop molecular markers based on QTL for MAS: developing the mapping population, phenotyping, developing a linkage map, conducting QTL analysis and then generating user-friendly makers. There have been many types of molecular markers used for mapping and QTL detection; Amplified fragment length polymorphism (AFLP), restriction fragment length polymorphism (RFLP), and random-amplified polymorphic DNA (RAPDs), but until recently, simple sequence repeats (SSRs) were dominant in mapping studies because they are highly polymorphic, exhibit co-dominant inheritance, are abundant and reproducible (Landjeva et al. 2007). Simple sequence repeats are

not expensive to develop (can mine from Chinese Spring sequence) but are expensive to genotype relative to single nucleotide polymorphism SNPs. Single nucleotide polymophims markers are markers of choice and SSR are still used to supplement SNP maps.

2.5.3.1.1 Single nucleotide polymorphism

Single nucleotide polymorphisms (SNPs) are a type of polymorphism involving variation of a single base pair in any part of genome. Genetic markers based on a SNP are the markers of choice in QTL identification and are converted to more high-throughput platforms like KASP markers (Cabral et al. 2014) because of their abundance in the genome. Single nucleotide polymorphism marker data is widely used to detect marker-trait associations in QTL mapping trials and genome-wide association studies (GWAS) (Zhao et al. 2011; Jia et al. 2013). Next-generation sequencing technologies have enabled SNPs discovery by whole genome (Berkman et al. 2012; Xu et al. 2011), transcriptome (Cavanagh et al. 2013; Oliver et al. 2013) or reduced-representation sequencing in diverse populations of individuals (Elshire et al. 2011; Poland et al. 2012; Saintenac et al. 2013). The SNPs may be present in exons, introns, and promoters (Khlestkina and Salina 2006). Functional effects of SNPs are either anonymous, where polymorphism results in unknown effects, or suggestive of some functional effect (Khlestkina and Salina 2006). Generally, only two bases exist at a particular position in individuals from across between two genotypes (only two allele options for a specific position). Assays to test SNPs are designed to differentiate two bases (Brookes 1999) and SNPs can be automated and analyzed simultaneously. True SNPs may be adapted for use as molecular markers after validation in more than one population.

Single nucleotide polymorphism detection in polyploid wheat is complicated because of three homeoelogous A, B and D genomes (Ganal et al. 2011). To detect regions of the wheat

genome subject to selection during improvement, Cavanagh et al. (2013) developed a high-throughput Illumina Infinium bead chip assay with 9,000 SNPs that adequately covered the three wheat genomes. A worldwide sample of 2,994 hexaploid wheat accession, landraces, and modern cultivars were genotyped with about 86% to100% of the SNPs being polymorphic. A consensus map consisting of six mapping populations was used for mapping of 7,504 polymorphic markers (Cavanagh et al. 2013). More recently, Wang et al. (2014) developed a genotyping array including 90,000 gene-associated SNPs and used it to characterize genetic variation in allohexaploid and allotetraploid wheat populations. The array consists of common genome-wide distributed SNPs that are represented in populations of diverse geographical origin. A total of 46,977 SNPs from the wheat 90K array (Illumina platform) were genetically mapped using a combination of eight mapping populations (Wang et al. 2014). The developed array is a great tool for QTL mapping and gene cloning in wheat.

2.5.4 Genetic map construction

To construct a linkage map, the genotype of each line in the mapping population relative to the parental genotypes must be determined. Rarely, more than 300 lines are used in QTL mapping in plants due to practical limitations, although over 300 lines are desirable for quantitative traits controlled by multiple loci (Melchinger et al. 2004; Schön et al. 2004). In the past, many studies used about 100 lines for mapping, but currently the number of lines used varies from 100-250 lines. The number and choice of markers should allow full coverage (no gaps >20 cM) of the genome and should include suspected QTL regions based on previous work. At least three independent biological experiments (locations or years/site years) are recommended to estimate the repeatability of trait evaluation and determine the stability of QTL estimates across environments.

The wheat genome is complex and has been fully sequenced, and a high quality version (RefSeq v 1.0) is available through the International Wheat Genome Sequencing Consortium (IWGSC) at http://www.wheatgenome.org/News/Latest-news/RefSeq-v1.0-URGI. However, consensus maps are still important tools for genome analysis and understanding the genetic basis of phenotypic variation. These populations have been broadly used for genetic analyses of rice (http://www.gramene.org/species/oryza), maize (http://maizegdb.org/ map.php), arabidopsis ((Koornneef and Meinke 2010), and wheat (Wang et al. 2014). Detailed genetic analysis of complex traits can be done using reference populations. The polyploid nature of the wheat genome and abundance of repetitive DNA complicate the analysis of genetic variation and the development of high-density genetic maps. Several studies were done on the analysis of complexity-reduced fraction of the wheat genome (Trebbi et al. 2011) or cDNA (Lai et al. 2012; Trick et al. 2012). The SNPs discovered in these datasets were then successfully used to develop genotyping assays based on GoldenGate (Akhunov et al. 2009; Chao et al. 2010), BeadExpress (Trebbi et al. 2011), KASPar (Allen et al. 2011) and Infinium platforms (Cavanagh et al. 2013). The shift from SNP-based genotyping to direct sequencing of populations (Hamilton and Robin Buell 2012) is due to increased throughput sequencing technologies which allow genetic variation analysis, thus reducing the effect of ascertainment bias caused by the SNP discovery process. The bias can be solved by using multiple reference genomes (Gan et al. 2011).

2.5.4.1 Methods of QTL detection and logarithm of odds score (LOD)

Phenotypic and genotypic data of the population to be mapped are used in QTL analysis using various types of software such as Qgene (Joehanes and Nelson 2008) which calculate QTL statistics such as LOD score, r^2 (phenotypic variation explained by a QTL) and additive effect. The LOD thresholds of >3 are generally used to construct linkage maps, but may be lowered to

detect weakly linked loci. Different LOD scores may be evaluated before a final one is selected for mapping. With a LOD score exceeding a threshold of 3, it is inferred that a QTL is located near that marker. This is based on the observation that the genotypes of loci that are closer together are more likely to be inherited together. The conventional threshold of LOD ≥ 3 may not necessarily reflect the true significance threshold (Szabó-Hevér et al. 2014). Permutation analyses (Rudolph 1995) with 1000 runs may address problems associated with multiple testing problems and determining significance thresholds because of the correlation among the statistics due to linkage. A QTL is statistically significant, when the peak exceeds a specified significance level.

The main QTL detecting methods are single-marker analysis, simple interval mapping, composite interval mapping (CIM) (Liu 1998; Tanksley 1993) and multiple interval mapping (MIM) (Kao et al. 1999). Linear regression is most commonly used because the coefficient of determination (R^2) from the marker explains the phenotypic variation arising from the QTL linked to the marker. Composite interval mapping (Zeng 1994) is the most commonly used QTL analysis method with populations derived from biparental crosses (Zhang et al. 2014). The CIM (Otto et al. 2002; Löffler et al. 2009) includes in the model background cofactors which are markers in the genetic map that are selected to reduce residual genetic variation arising from QTL not linked to the QTL being tested. It allows more precise QTL location estimation by giving narrower peaks and more accuracy compared SIM, when linked QTL are involved.

According to Kao et al. (1999), MIM improves upon CIM by fitting multiple putative QTL simultaneously directly into the model for mapping QTL. Currently MIM is commonly used in QTL mapping and it uses maximum likelihood for estimating genetic parameters. A stepwise selection procedure with the use of the likelihood ratio test statistic as a criterion is

proposed to identify QTL in MIM. Multiple interval mapping (Kao et al. 1999), is known to be more accurate than CIM.

2.5.6 Marker assisted selection (MAS)

According to Bernardo (2008) many QTL have been mapped, but there has been limited use of these QTL in MAS. The difficulty in using QTL for MAS is that many identified QTL are specific to the population and environment in which they were identified, thus making it hard to apply them to a wide range of breeding material. Marker-assisted selection using known QTL such as 3BS and 6BS is an effective strategy in the development of FHB resistant wheat cultivars (Wilde et al. 2007). Yield, quality and FHB resistance need to be incorporated into elite winter wheat in Canada, and this is a challenge for breeders since FHB resistance sources are from non-adapted sources. Von der Ohe et al. (2010) used MAS to introgress the 5A QTL for FHB resistance in European winter wheat without quality and yield penalties. Meidaner et al. (2009) concluded that MAS used in conjunction with traditional phenotypic selection may be the most efficient means of increasing resistance. Similarly, Miedaner and Korzun (2012) suggested that use of QTL associated with FHB resistance and MAS for breeding FHB resistant lines cannot be a complete substitute for conventional phenotypic selection.

Validation of QTL is important in genetics and MAS, but Fusarium resistance QTL are not always reproducible in different populations. For example, studies with Arina showed a high number of QTL in three different populations, but only the QTL for the *Rht-D1* gene co-localizing with a Fusarium QTL was shown to be common (Draeger et al. 2007; Paillard et al. 2004). This was due to the complexity of the genetic background for the small and intermediately strong QTL which exhibit many different unknown regulatory mechanisms.

CHAPTER 3

INHERITANCE OF FUSARIUM HEAD BLIGHT RESISTANCE AND MOLECULAR MAPPING OF QUANTITATIVE TRAIT LOCI CONTROLLING FUSARIUM HEAD BLIGHT IN DH99W19H*16 /CDC BUTEO DOUBLE HAPLOID WINTER WHEAT POPULATION

3.1 Abstract

Fusarium head blight (FHB), caused by *Fusarium graminearum*, is an important disease of wheat (*Triticum aestivum* L.) worldwide. The hard red winter wheat cultivar, CDC Buteo, is moderately resistant to FHB and is well adapted to production in the Canadians prairies; however, the genetics of its resistance has not been studied. The objectives of this study were to identify FHB resistance QTL in a double haploid (DH) population (n=228) from the cross DH99W19H*16 /CDC Buteo. CDC Buteo is moderately resistant while DH99W19H*16 (19H*16) is susceptible to FHB. The DH population was phenotyped in six inoculated field environments. Six DH lines had < 10% FHB index when combined data analyses were done for all cropping years. Transgressive segregants were observed for all traits measured, indicating that resistance was contributed by both parents.

High density mapping of single nucleotide polymorphism (SNP) markers from an Illumina 90K Infinium, and analysis for *Rht-B1* and *Rht-D1* SNPs identified 614 unique markers covering a distance of 1768.24 cM. Multiple QTL were detected on 13 chromosomes with no QTL on chromosomes 1A, 1D, 2A, 2D, 3A, 5A, 6A and 7B. Major QTL for all FHB resistance traits measured were detected on chromosomes 4B and 4D which mapped to the positions for dwarfing genes *Rht-B1* and *Rht-D1*, respectively. CDC Buteo has *Rht-B1b/Rht-D1a* while 19H*16 has *Rht-B1a/Rht-D1b* allele combinations. Consistent QTL from 19H*16 alleles for

increased FHB resistance were identified on chromosomes 4B and 6B while from CDC Buteo alleles they were identified on chromosomes 2B, 4A, 4D and 7A. This is the first report of the mapping of CDC Buteo-derived QTL that influence FHB resistance. The level of resistance in Canada's elite cultivars is limited, making CDC Buteo a valuable source of FHB resistance.

3.2 Introduction

Fusarium head blight (FHB) is a disease of small grain cereals. It is one of the most important diseases of wheat in Eastern Canada, Saskatchewan, Manitoba and the United States (Gilbert and Tekauz 2000; Goswami and Kistler 2004; Goswami and Kistler 2005). Amongst the Fusaria, *Fusarium graminearum* Schwabe (teleomorph *Gibberela zeae* (Schwein and Petch) is the primary causal pathogen of FHB in the United States, Canada, and many other countries (Bai and Shaner 2004; Parry et al. 1995; McMullen et al. 1997). The primary etiological agents of FHB belong to the *F. graminearum* species complex (Fg complex), which consists of at least 16 phylogenetically distinct species (O'Donnell et al. 2000; O'Donnell et al. 2004; Starkey et al. 2007; O'Donnell et al. 2008; Yli-Mattila et al. 2009; Sarver et al. 2011).

Fusarium graminearum represents over 97% of Fusarium isolates found on wheat in Manitoba (Gilbert et al. 2010). The fungus reduces yield and end-use grain quality and contaminates the grain with mycotoxins such as deoxynivalenol (DON), reducing its use for food or feed (Gilbert and Fernando 2004; Pestka and Smolinski 2005). Maximum allowed levels for the most prevalent Fusarium mycotoxins in cereals and cereal products (Van Egmond 2004) and animal feeds have been established in many countries to protect consumers and livestock from mycotoxicosis. According to the joint FAO/WHO expert committee on food additives, the provisional maximum tolerable daily intake (PMTDI) for DON was converted to a group (class of food contaminant/toxicological evaluations) PTMDI of 1 $\mu g/kg^{-1}$ body weight for DON and its acetylated derivatives 3-acetyl deoxynivalenol (3-ADON) and 15-acetyl deoxynivalenol (15-ADON) (FAO/WHO, 2010).

In 2009, the losses to FHB in the previous 30 years were estimated to be one billion dollars in Canada (Clear and Nowicki, 2009). Deoxynivalenol is one of the most important

mycotoxins produced by *Fusarium* species; however, not all species produce DON. Cultural practices or timely applications of fungicides may suppress disease severity, thereby decreasing inoculum levels and losses from FHB (Dill-Macky 2008; McMullen 2008; Gilbert and Haber 2013). Development of host genetic resistance is key to achieving meaningful control (Anderson 2007; Bonin and Kolb 2009). The well-studied sources of resistance are in backgrounds with poor agronomic and quality traits (Comeau et al. 2011) such as Sumai 3 and its derivatives. Other resistance sources have been identified in Asian spring wheat, Brazilian spring wheat and European winter wheat (Snijders 1990).The resistance may be due to combined action of multiple genes interacting in complex ways, making their use in breeding more challenging (Miedaner and Korzun 2012). Globally, there is continuous evaluation of FHB resistance sources involving elite cultivars (Graf et al. 2013), landraces and wheat wild relatives such as *Triticum macha* (Buerstmayr et al. 2011). Lack of vertical resistance in FHB-host interactions precludes the prospect of immunity. The limited numbers of strong resistance sources are heavily utilized through various breeding programs, a phenomenon that may ultimately lead to breakdown of resistance due to selection pressure on *F. graminearum* (Shaner and Buechley 2001; McCartney et al. 2004).

Large numbers of QTL conferring resistance to FHB have been mapped in diverse genetic sources, though very few have been integrated in wheat breeding (Liu et al. 2009; Xue et al. 2010). Buerstmayr et al. (2009) noted more than 100 QTLs for FHB resistance in wheat have been published. A meta-QTL analysis identified 43 QTL clusters and 19 important QTL (Liu et al. 2009; Löffler et al. 2009). Among the types of FHB resistance described by Mesterházy (1995), FHB reductions in both initial infection and disease spread in the spike are important in breeding for resistance to FHB. Reduced FDK and low DON accumulation are important for the

end-use of the grain as food or animal feed. Many QTL regions have been detected in more than one mapping population, thus greatly increasing the chances that the QTL are a real effect.

Molecular markers linked to QTL associated with varying aspects of resistance have been identified, mainly from the FHB-resistant Chinese cultivar Sumai 3, which has been used in many breeding programs and is the most widely studied. The most prominent Type 2 resistance QTL from Sumai 3 is located on chromosome 3BS (Waldron et al. 1999; Ban and Suenaga 2000; Anderson et al. 2001). The major QTL on 3BS and 6BS in Sumai 3 were designated *Fhb1* (Cuthbert et al. 2006) and *Fhb2* (Cuthbert et al. 2007), respectively. Other Sumai 3 FHB resistance QTL have been located on chromosomes 2AL, 4BL (Waldron et al. 1999), 5A (Buerstmayr et al. 2002), 3AL, 6AS, 6B (Anderson et al. 2001), and 7D (Sneller et al. 2001). Huapei 57-2 (Bourdoncle and Ohm 2003), and Ning 894037 (Shen et al. 2003), also carry an FHB resistance QTL in a similar region on chromosome 3BS. Other Asian resistances sources that differ from Sumai 3 showing presence of QTL on chromosomes 3BS, 5AS and 6BS, similar to those of Sumai 3 have been reported. They include Wangshuibai (Jia et al. 2005; Lin et al. 2006; Yu et al. 2008), Wuhan-1 (Somers et al. 2003), Nyubai (Somers et al. 2003; McCartney et al. 2004), and W14 (Chen et al. 2006).Wuhan-1, is also associated with a QTL on chromosome 2DL that differs from those in other Asian sources (Somers et al. 2003). Liu et al. (2007) identified two major QTL on 3BS and 5AS and two minor QTL on 2B and 4BL associated with Type 2 resistance in the cultivar, Ernie. The QTL on 3BS, which is proximal to the centromere, differs from the distal 3BS QTL in Sumai 3 as does the 5AS QTL. Liu et al. (2013) reported three additional QTL on 4BS, 4DS, and 5AL associated with field disease incidence and severity in Ernie. Quantitative trait loci on 4BS and 4DS co-localised with height alleles *Rht-B1* and *Rht-D1* while the QTL on 2DS was near the photoperiod insensitivity allele *Ppd-D1a*. Abate et al.

(2008) reported three QTL on chromosomes 3BSc, 4BL, and 5AS that were associated with lower DON and four QTL on 2B, 3BSc, 4BL and 5AS associated with reduced FDK in Ernie. All were coincidental with those identified by Liu et al. (2007).

The Brazilian spring wheat Frontana is also frequently used as a source of FHB resistance for both Type 1 and Type 2 resistances. Steiner et al. (2004) mapped the main QTL from the cultivar Frontana on chromosome 3A, which explained 16.2% of phenotypic variation. Srinivasachary et al. (2006) reported QTL on 1B, 3A, 6A, 2B and 7A in a cross between Frontana and the spring wheat line RL4137.

Among the QTL mapped in European wheat, most are in a winter wheat background. QTL for incidence, severity, DON and/or FDK have been mapped in Arina (Paillard et al. 2004); (Draeger et al. 2007), Fundulea 201R, (Shen et al. 2003), Renan (Gervais et al. 2003), Remus (Steiner et al. 2004), Dream, (Schmolke et al. 2005), and NK93604 (Semagn et al. 2007).Three potentially unique QTL on 2ASc, 2DS and 3DS all coming from the Truman alleles were identified from 169 RILs derived from the Truman /MO 94-317 cross (Islam et al. 2016). The QTL analyses clearly show that some QTL determine resistance to FHB, FDK or DON, or their combination (Mesterházy 1995; Agnes et al. 2014; Szabó-Hevér et al. 2014). Most of the QTL analyses utilised visual symptoms (incidence and severity) on the spike (Buerstmayr et al. 2009) with few studies measuring FDK and DON in the first 10-15 years when FHB epidemics were studied (Mesterházy et al. 1999). Pyramiding known FHB resistance QTL from exotic sources was verified in elite wheat lines in Europe (Miedaner et al. 2008). Von der Ohe et al. (2010a) recommended introgression of the 5A QTL for FHB resistance in European winter wheat without quality and yield penalties.

CDC Buteo is a hard red winter wheat cultivar with good yield potential, intermediate plant height, moderate stem and leaf rust resistance, good winter hardiness and excellent grain quality (Fowler 2010). Repeated field testing of FHB reaction in CDC Buteo has shown that it produces a moderately resistant reaction to FHB (Brûlé-Babel, personal communication; Seed Manitoba, 2017). The overall objectives of this project were to: evaluate FHB reactions in a double haploid (DH) population involving CDC Buteo and DH99W19H*16, identify QTL associated with FHB reactions in the population, and determine whether CDC Buteo could be successfully used as a source of resistance to FHB in winter wheat in Canada.

3.3 Materials and methods

3.3.1 Population development

Molecular marker analysis of CDC Buteo with known FHB QTL associated with chromosomes 2D, 3A, 3B, 5A and 6B indicated that it does not possess the most common resistant haplotypes (Brûlé-Babel, personal communication). The positive controls used in haplotyping included Sumai 3, Wuhan, Nyubai and Frontana. The SSR markers used were : 2D (gpw5141, gpw8003, cfd73, cfd233, gwm608, gwm539), 3A (wmc664, gwm5, gpw3036, gwm30, wmc651, wmc627, gpw2118, gwm494, cfa2193), 3B (gwm533, sts80, sts142, sts66, gwm493), 5A (gwm293, wmc705, barc186, gwm304, gwm156) and 6B (wmc104, barc136, barc1169, wmc398, gwm133, gwm644, wmc397). Therefore, it is possible that CDC Buteo possesses a unique form of FHB resistance. The 249 DH population was developed from the cross CDC Buteo/DH99W19H*16 at the University of Manitoba, Plant Science Department using wheat-maize pollination and the embryo rescue technique (Laurie and Bennett 1988). CDC Buteo was considered to be moderately resistant, while DH99W19H*16 was susceptible to FHB

but high yielding. The population designation in this study was 9HBT and the susceptible parent was abbreviated as 19H*16.

The susceptible parent, 19H*16, was a selected DH line from the cross UM1174/CDC Clair. UM 1174 was a breeding line at the University of Manitoba from a GN567/Norstar cross. GN567 is a winter wheat accession obtained from France with the designation VT2222. The UM 1174/CDC Clair cross was made in Winnipeg in 1998. F_1 seed from the UM 1174/CDC Clair cross was used to produce the double haploid DH99W19H*16 in 1999. CDC Buteo (Fowler, 2010) was selected from the progeny of a cross S86-808/Abilene where S86-808 = Norstar*2/Vona (Welsh et al. 1978; Grant, 1980; Roberts, 1989) and the final cross made in 1988.

3.3.2 Experimental design

The 9HBT population was evaluated for reaction to FHB in inoculated field nurseries at the Ian Morrison Research Station, Carman and the Point Field Research Laboratory of the University of Manitoba Fort Garry Campus, Manitoba, in 2011, 2012, and 2013. The 249 DH lines and parents were planted in the fall of 2010, 2011 and 2012 in separate trials in a randomized complete block design with three replicates in each site. Each plot was a single 1 meter row plot. The sowing density was approximately 70 seeds per row. In addition, checks FHB 148 and DH00W32C*17 (resistant), Freedom and 431*18 (intermediate) and Hanover and Caledonia (susceptible) were placed at the end of each block to monitor disease development throughout the field.

3.4 Phenotypic screening for Fusarium head blight field resistance

Days to heading and 50% anthesis were recorded for each DH line. The inoculum was produced by first culturing the isolates in spezieller nahrstoffar agar (SNA) media plates

(Nirenberg 1981) for one week. The SNA cultures were then transferred to aerated liquid carboxyl methyl cellulose (CMC) media (Tuite 1969) for another week to produce macroconidia under UV light. The media were strained and decanted into sterile glass bottles using sterile cheese cloth. A haemocytometer was used to determine the macroconidia concentration. Equal concentrations of each isolate were combined and adjusted to 5×10^4 macroconidia spores/ml just before inoculation. The surfactant Tween 20 was added to the inoculum prior to spraying at a volume of 2 ml per one liter of inoculum. Each plot was spray inoculated with a mixture of four *F. graminearum* isolates. An equal mixture of four virulent isolates of *F. graminearum* Schwabe, originally provided by Dr. Gilbert's lab at the Cereal Research Centre, Agriculture and Agri-Food Canada was used. Two of the isolates were of the 3-ADON chemotype (M7-07-1 and M9-07-1), and two (M1-07-2 and M3-07-2) were of the 15-ADON chemotype. The isolates were previously collected from infected wheat in Manitoba, to represent a sample of isolates prevalent in Manitoba. The spikes of the entire row were spray inoculated at 50 % anthesis with 50 ml/row of inoculum consisting of 50,000 macroconidia spores/ml using a CO_2 backpack sprayer calibrated at 30 psi. Each row was inoculated a second time three to four days after the first inoculation. After each inoculation, the plots were mist irrigated for 10 minutes every hour from 6:00 pm to 6:00 am (12 hours) to maintain high humidity in the plot area. Eighteen to twenty-one days after the initial inoculation, or when symptoms were well developed (Cuthbert et al. 2007); plots were visually assessed for disease incidence and severity. Fifty spikes were randomly harvested in each plot of one replicate at each location and stored at -20°C to later verify the visual rating in the field in 2011. During 2012 and 2013 field season, the ratings were done by two people per site and then the results averaged before statistical analysis.

The FHB incidence was estimated as a percentage of spikes in the plot that were infected and severity as the percentage of spikelets that were infected within the infected spikes. This was done visually for each plot and numerically for the 50 spike samples that were collected from the plots. FHB index was calculated as FHB index = (disease incidence (%) x disease severity (%))/100. This constituted the data for field resistance where the effects of disease incidence and disease spread were combined. At maturity, each plot was hand harvested. In 2011, the spikes were threshed using a belt thresher and a blower that was set at a low fan speed to remove the chaff and avoid loss of small and light kernels. In 2012 and 2013, the plots were hand cut and threshed using a stationary Wintersteiger Elite combine. The air flow of the combine was reduced from the normal air flow by 60% to retain as many FDK as possible.

For the 2011 FHB nursery, the subsamples from the two replicates per genotype (the third replicate was hand harvested during rating for confirmation of visual ratings of incidence and severity by counting infected spikes and spikelets) were used for FDK testing. In 2012 and 2013 all the three replicates were tested individually for FDK while for DON analyses, composites from the three replicates were used. Fusarium damaged kernels were distinguished from healthy kernels by their shriveled shape, light weight and pink coloration. Fusarium damaged kernels were assessed by visually separating diseased kernels from a random sample of 200 seeds and expressing the FDK as a percentage of total number of seeds.

Deoxynivalenol measurements were made on composite samples developed by pooling 20g /replicate/genotype for each site. A representative 50g sample was obtained from the pooled composite. Deoxynivalenol analysis was conducted at Biovision-Seed and Grain Testing Laboratory (Winnipeg, Manitoba). The 50g samples were ground on a hammer mill for about two minutes to pass through a 20 mesh sieve, the particle size of a fine instant coffee (less than

0.85mm). A 10g sub- sample was vigorously shaken in 100 ml of de-ionized water for three minutes. The material was left to set for two to three minutes to enable the sediment to settle, leaving the supernatant for analysis. The extract was filtered by pouring at least 5 ml through a Neogen filter syringe and the filtrate was collected as a sample. Deoxynivalenol analysis was done using competitive enzyme linked immunosorbent assay (ELISA) (Veratox DON 5/5-Quantitative test) with a DON quantification limit of 0.5 mg kg¯1. Dilutions were used when DON content exceeded the limits of the test to ensure accurate DON quantification.

Height data were collected from a separate field trial in Winnipeg in a 2010 rust nursery. Each plot was a single three-meter row plot. Plant height was measured in centimetres, and three measurements per plot were taken and then averaged to give the final reading.

Data for total precipitation and average monthly temperatures for May-August in 2011 - 2013 cropping seasons at Carman were retrieved from Environment Canada Weather station Archives. The weather station was located near the experimental sites. Data for the Winnipeg site was retrieved from the Forks location (Table 3.1).

3.5 Phenotypic screening for Type 2 resistance in greenhouse

A random sample of 81 DH lines from 9HBT population was evaluated for Type 2 resistance using dual-floret inoculation. The greenhouse experiments were arranged as a completely random design with three replicates for parents, DH lines and checks planted in pots and evaluated according to the procedures described in Cuthbert et al. (2007). The same isolates as those used in the field experiment were used for point inoculation in greenhouse. Five spikes per genotype were inoculated at 50% anthesis using 10 µl macroconidia suspension (50,000 spores/ml) placed between the lemma and palea. The inoculation points on each spike were the spikelet positioned 2/3 of the way from the base of the spike and the spikelet immediately above

that point (Cuthbert et al. 2007). To maintain high relative humidity, the spike was covered with a glassine bag for 48 hours after inoculation. The ratings were done 7, 14 and 21 days post inoculation by counting the number of infected spikelets directly below the inoculated spikelet, excluding the spikelets that were inoculated. The percentage of infected spikelets was calculated as (number of infected spikelets below inoculated spikelets / total number of spikelets below the inoculation point x100).

3.6 Data analysis

The disease incidence, severity, FHB index and FDK data from all field trials were analyzed using the PROC GLM of SAS (SAS v9.3, SAS Institute Inc., Cary, NC, USA, 2009) with all effects in the model considered random. Each environment was analyzed separately. Further analyses were done by combining all sites for each year, combining all years for each site, and combining all sites and years. The environments were combined as site-years rather than by site and by year. For the analyses of variance for each site, the model was incidence, severity, FHB index, FDK = Genotype replicate with all variables being random. For the analyses of variance for combined data, the model statement was incidence, severity, FHB index, FDK = siteyear replicate(siteyear) genotype siteyear*genotype with all factors being random. The least square mean values for each DH line evaluated from each dataset were used for QTL analyses. The error terms used in the Proc GLM for the factors included; (a) siteyear; MS(rep(siteyear) + MS(siteyear*genotype) - MS(residual Error); (b) genotype-MS(siteyear*genotype), (c) rep(siteyear) and siteyear*genotype, the error terms used was MS(Error). Proc Univariate of SAS was used to test for normality of residual distribution. Homogeneity test using Levene test at P = 0.05 was conducted to determine if the data sets could be combined across site years rather than by site and by year. The decision to combine data over

the years and sites was made by examining the results of the Proc Univariate‘s plot of residual by predicted values for disease incidence, severity, FHB index, and FDK. Despite finding significant differences between the site-years for most of the variables, the residual error deviations were homogenous along the range of the predicted vs residual. The error deviations had similar variance across the range of data, and the error deviations were normally distributed suggesting that combining of the data for analyses was appropriate (data not shown). To determine the associations between traits, correlation coefficients among FHB variables and other traits were calculated for each environment and combined site years using SAS Proc Corr (9.3). For the greenhouse experiment on Type 2 resistance the severity data were analyzed using the Proc GLM of SAS (SAS v9.3, SAS). Deoxynivalenol analysis for the pooled field data for four site-years was done using Proc Mixed of SAS 9.3 using the model statement; DON= siteyear genotype. The genotype was considered fixed factor while siteyear was random.

3.7 Construction of linkage maps for 9HBT population

3.7.1 DNA extraction

Three seeds per DH line were seeded in the greenhouse with one plant per pot. Supplemental incandescent light was set for 16h daylight and the temperature ranged from 18 to 24 °C. Two to three pieces of young leaves (~5cm in length) from one plant per DH line were collected and immersed in liquid nitrogen. The leaf tissue for each plant was lyopholized in a separate perforated envelope. The leaf samples were freeze dried for 48 hours and stored at -20 °C until deoxyribonucleic acid (DNA) extraction. DNA was extracted from a single plant of each DH line from two-week-old seedlings of 249 9HBT DH lines using Qiagen DNeasy 96 plant kit (Qiagen, Mississauga, Ontario). QuantiT PicoGreen dsDNA quantification reagent was used to

quantify double stranded DNA samples and working DNA samples were adjusted to a concentration of 50 ng/ul.

3.7.2 Genotyping using an Illumina 90K Infinium iSelect Custom Beadchip genotyping assay

The 9HBT population was genotyped using the 90K SNP wheat beadchip of the Infinium platform from Illumina (Wang et al. 2014) to identify SNP variation and independently order SNPs along the wheat chromosomes in A, B and D genomes.

3.7.3 Data analyses

The assays were performed according to the manufacturer's protocols (Illumina). The SNP allele clustering and genotype calling for this population was performed with Genome Studio (GS) and the algorithm in GS was used to identify assays that produced three distinct clusters corresponding to the AA, AB and BB genotypes expected for bi-allelic SNPs for polyploid wheat. The markers were called into scores; AA= 0, AB=1 and BB=2 (AB genotype is as a result of wheat genome comprising of three genomes whereas the algorithm in GS used is for identification of diploid wheat not polyploid wheat). The 0, 1 and 2 were converted into either A, B or - based on sorting of markers by both parents. All markers with similar parental genotype were converted into A for the female (19H*16) and B for male (CDC Buteo) after sorting by both parents. A missing value (-) was recorded for those markers which could not be scored reliably for a particular line. Markers with more than 10% missing data were culled from the list of markers to be used in mapping. Manual curation was performed for assays that could not be discriminated by the default algorithm and the accuracy for SNP clustering was validated visually.

Minimum Spanning Tree Map (MSTMap), a software tool capable of efficiently constructing genetic linkage maps, was used to build the initial 9HBT genetic linkage map by first constructing a framework linkage group (Wu et al. 2008). This resulted in 78 linkage groups for the 9HBT DH population. All redundant markers were removed before using MapDisto and only one marker per linkage bin was used. The resulting markers from MSTMap were used for mapping in MapDisto, version 1.7.7 (Lorieux 2012). Linkage between loci and the map distances were calculated using the Kosambi mapping function (Kosambi 1943). To test and verify Mendelian segregation of all markers in MapDisto, the segregation X^2 was used to obtain chi squared values to remove highly skewed markers ($p<0.01$). Markers showing severe segregation distortion were removed from mapping populations before map construction. Different LOD scores and recombination values were tried to generate linkage groups. The LOD score of 4 and a maximum recombination frequency (RF) of 0.20 were used to identify linkage groups in this population. The LOD = 4 and RF = 0.2 are used as an initial starting point to identify linkage groups and then relax these criteria to LOD of 3 and RF =0.3 (McCartney, personal communication). The fitting criteria used in loci ordering were 'AutoMap', 'Order sequence', and 'Compare all order' functions. The Branch and Bound II ordering methods were used in combination with the sum of adjacent recombination fractions (SARF) as criteria for ordering and ripple. The markers were sorted based on the same position with the most informative marker from each bin (with least missing data) being used for QTL analyses. Marker data were checked and error candidates in markers were replaced by missing data (-). Linkage groups were assigned to chromosomes based on knowledge of previously mapped markers (Wang et al. 2014).

The DH lines' marker data and phenotypic data (from all the field trials) were used to perform QTL analyses using QGene software (version 4.3.10) using multiple interval mapping (MIM) (Joehanes and Nelson 2008). The QTL scan walking speed was 1cM. There was no significant difference between means for DH lines used for Type 2 resistance (greenhouse trial) and the phenotypic variation was small making it unsuitable for QTL analysis. However, QTL analyses were done separately for each environment (Carman, Winnipeg for 2011, 2012 and 2013). Further QTL analyses were done by combining all years for each site, combining all sites for each year, and combining all sites and years. The LOD score for claiming a significant QTL was determined based on a permutation test with 1000 runs for each QTL. The QTL position, additive effect and the percentage of phenotypic variation explained (R^2) were estimated and the linkage maps were drawn using MapChart v. 2.2 (Voorrips 2002).

3.7.3.1 Fixing the 9HBT population for the 4B and 4D alleles

The number of DH lines was reduced from 249 to 228 because 21 DH lines had many double recombinants or they had more than 10% missing marker data. After QTL analysis for the 9HBT population using 228 DH lines, two QTL with very high LOD scores were detected on chromosomes 4B and 4D, which could have masked other QTL. They mapped on positions known for the *Rht-B1* and *Rht-D1* genes for semi-dwarfism. In order to determine whether additional QTL could be detected, the population was subdivided into four subpopulations fixed for the parental alleles at the 4B and 4D *Rht* loci using the dwarfing-gene markers, wMAS000001 and wMAS000002.

The dwarfing genes *Rht-B1b* and *Rht-D1b,* derived from the Japanese variety Norin 10, are gain-of-function mutant alleles of the *reduced height-1* genes (*Rht-B1* and *Rht-D1*) encoding wheat DELLA proteins, which act to repress GA-responsive growth. The mutant alleles *Rht-B1b*

and *Rht-D1b* are thought to confer dwarfism by producing more active forms of these growth repressors. Genes *Rht-B1* and *Rht-D1* are GA-insensitives genes that are classified according to their sensitivity to externally applied gibberellins (GA). The alleles conferring the dwarf phenotype are *Rht-B1b* and *Rht-D1b* and the wild-type alleles are *Rht-B1a* and *Rht-D1a* (Würschum et al. 2015).

Two groups based upon *Rht-B1* (4B) were created by classifying the population using the *Rht-B1* marker (wMAS000001) for this gene (consisting of 131 DH lines (CDC Buteo allele (*Rht-B1b1*) and 97 DH lines (19H*16 allele - tall allele-*Rht-B1a*)) and QTL analysis was done for each subpopulation. Two other groups based upon 4D alleles (consisting of 116 DH lines - 19H*16 allele-*Rht-D1b*) and 112 DH lines - CDC Buteo allele *Rht-D1a*) were created by classifying the population using the *Rht-D1* marker (wMAS000002) and QTL analysis was done for each. The adjustment allowed other important QTL to be detected because they may have been masked by the huge effect of these two dwarfing genes. Permutation tests were done for all sub-populations (data not shown).

3.7.3.2 Effect of the *Rht* genes on Fusarium head blight

The number of DH lines was reduced from 249 to 228 because 21 DH lines were either heterozygous or they had more than 10% missing marker data. The 228 DHs were grouped into genotypic groups based on their *Rht* allele combinations in homozygous conditions (*Rht-B1b/Rht-D1a, Rht-B1a/Rht-D1a, Rht-B1b/Rht-D1b, and Rht-B1a/Rht-D1b)* to see if there was a difference in their trait performance. The genotypic data from the two dwarfing gene markers wMAS000001 and wMAS000002 were used for classification. The analysis of variance of each *Rht* allele combination was calculated using Proc Mixed of SAS (SAS v9.3, SAS) and mean difference of the groups compared using least significant difference (LSD, $p \leq 0.05$). Model: Inc

Sev FHBI = siteyear genotype rep(siteyear) siteyear*genotype. The factors which were considered random included siteyear, genotype rep(siteyear), and siteyear*genotype. The means difference of the groups were calculated using the statement "LSMeans genotype/PDIFF".

3.8 Results

3.8.1 Weather conditions in Carman and Winnipeg during the growing period

Fusarium head blight development is affected by environmental conditions (rainfall and temperature). In both sites, the temperatures were shown to be higher in 2012 than 2011 and 2013 and the precipitation was highest in 2013 and very low in 2011 (Table 3.1) for the period from anthesis, inoculation, disease development and rating (June-July). The temperatures were generally higher in Winnipeg than in Carman during the three seasons. However, incidence, severity, FHB index and FDK were reported to be generally higher in Carman than in Winnipeg (appendices 2.1 and 2.8). The highest FHB infection in both sites was observed in 2013 for all FHB traits and lowest in 2012 (appendix 2.1). This could be explained by dry conditions experienced during disease development in June and July in 2012 even though misting irrigation was provided during disease inoculation in both sites.

Table 3.1 Cropping season mean temperatures and total precipitation for 2011-2013 at the Ian Morrison Research Station, Carman and Winnipeg (Forks), Manitoba

		Mean air temperatures (°C)					Precipitation (mm)				
	Year	May	June	July	August	Mean	May	June	July	August	Total
	2011	10.4	16.7	20.3	19.3	16.7	72	59.2	37.6	12.2	181
	2012	12.1	17.7	21.9	19	17.7	60.5	86.2	27.8	47.2	221.7
Carman[1]	2013	10.4	17.7	18.6	18.7	16.4	111	50.6	49	59.4	270
		Mean air temperatures (°C)					Precipitation (mm)				
	Year	May	June	July	August	Mean	May	June	July	August	Total
	2011	12	18.3	23	21.9	18.8	49.4	41.4	17.8	16	124.6
	2012	13.1	18.9	24.1	20.6	19.2	94	69.2	20.4	42.8	226.4
Winnipeg[2]	2013	12.2	18.7	20.7	20.7	18.2	71.1	62.6	66.6	41.8	242.1

Source: National Climate Data and Information Archive, Environment Canada [1], Winnipeg (Forks) [2]

3.8.2 Field phenotypic data and deoxynivalenol results

The population size was reduced to 228 DH lines since genotypic data for 21 DH lines was removed due to many double recombinants (heterogenous) and missing marker data of more than 10%. Analysis of variance (ANOVA) revealed significant differences between genotype, siteyear, replicate(siteyear), siteyear*genotype interaction effects (Table 3.2). The significant siteyear effect for all evaluated FHB traits explained a large proportion of the total variation, emphasizing the importance of environment in disease development. The frequency distributions displayed significant broad and continuous variations for all measured disease parameters (Fig 3.1). The distributions for the different FHB parameters exceeded the range of the parents (Table 3.3 and Fig. 3.1). Table 3.3 shows that the population mean for FHB incidence was 54.2% which was higher than that of CDC Buteo (44.7 %), but almost similar to mid parent value of 56.8%. The population mean for FHB severity was 52% which was similar to CDC Buteo and different from the mid-parent value of 57.9%. For FHB index the population mean was 30.5% with a mid-parent value of 33.7% which was higher than that of CDC Buteo (23.4%). Similar results were reported for FDK where CDC Buteo had a lower mean of 14.5% compared to population mean and mid parent mean of 18.3 and 18.5%, respectively. Transgressive segregants were observed for all FHB traits measured having higher or lower FHB reaction than both parents (Fig. 3.1), but the proportion of lines on either side of the parents was not consistent between years, locations and traits measured (appendices 2.2-2.4,2.6 and 2.7). There were more transgressive segregants with disease levels greater than the susceptible parent than segregants with lower disease levels than the moderately resistant parent. CDC Buteo showed a moderately resistant reaction to FHB compared to 19H*16 which was susceptible (Table 3.3 and Fig. 3.1). Similar results were reported when data were analysed for individual site-years, combined for each site across three

years or combined across years (appendices 2.1, 2.5 and 2.8). The phenotypic variation observed in this DH population was suitable for QTL analysis. According to Seed Manitoba (2012), CDC Buteo was 76.2 cm cm tall and 19H*16 was 68.6 cm tall. Six DH lines had a FHB index of less than 10% with 56 DH lines having an FHB index of 10- 20%. Some of these DH lines could be used as a source of resistance for development of elite cultivars or could be developed directly into cultivars for the Prairies.

Table 3.2 Analysis of variance for incidence, severity, Fusarium head blight index (FHB index) and Fusarium damaged kernel (FDK) for pooled data for Carman and Winnipeg 2011, 2012 and 2013 (six site years) FHB nurseries

Source of variation	DF	Incidence (%)		Severity (%)		FHB index (%)		FDK (%)		
		MS	P>F	MS	P>F	MS	P>F	DF	MS	P>F
Siteyear	5	119745.3	<.0001	108814.7	<.0001	45595.7	0.0002	5	135686.0	<.0001
Rep(Siteyear)	12	3668.4	<.0001	3005.4	<.0001	3691.6	<.0001	10	1280.6	<.0001
Genotype	227	3955.0	<.0001	2220.1	<.0001	3367.0	<.0001	227	1065.8	<.0001
Siteyear*Genotype	1,133	361.7	<.0001	315.3	<.0001	284.4	<.0001	1135	95.9	<.0001
Error	2,711	201.5		144.2		140.4		2,260	33.9	

Table 3.3 Mean and range for incidence, severity, Fusarium head blight index (FHB index), and Fusarium damaged kernel (FDK) for pooled data for Carman and Winnipeg for 2011, 2012 and 2013 (six site years) FHB nurseries

Genotypes	Parents		DH population		
Trait	Buteo	19H*16	Mean	Min	Max
Incidence	44.8	68.8	54.2	16.9	85.2
Severity	51.3	64.5	52.0	26.5	78.1
FHB index	23.4	44.0	30.5	6.4	67.2
FDK	14.5	22.5	18.3	2.8	43.6

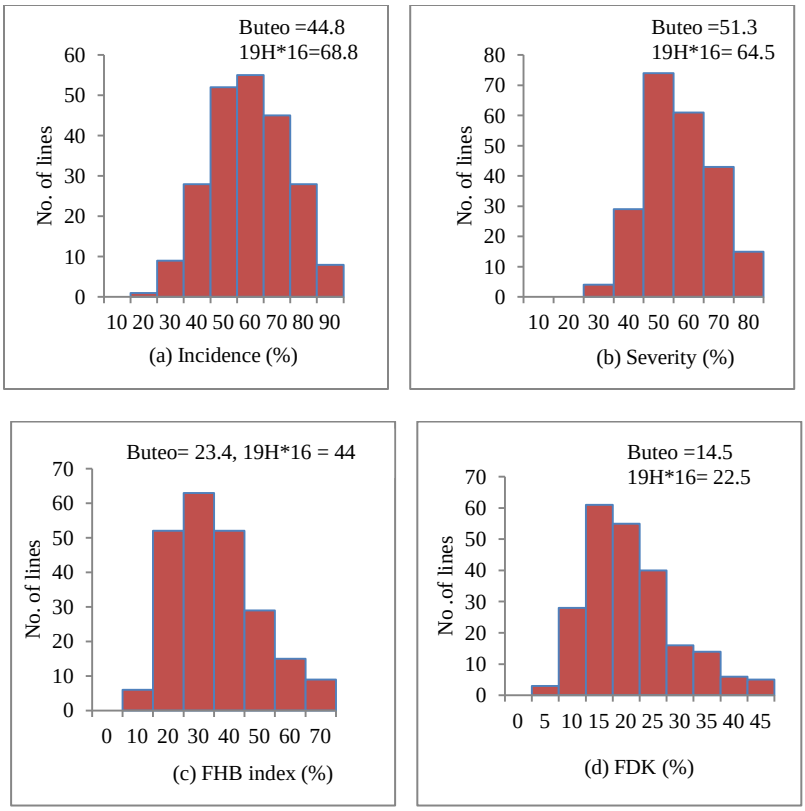


Figure 3.1. Frequency distributions of 228 9HBT lines derived from the cross 19H*16 /CDC Buteo for field incidence (a), severity (b), Fusarium head blight index (FHB index) (c) and Fusarium damaged kernel (FDK) (d). The pooled data are for six site years (Carman and Winnipeg, MB across 2011, 2012 and 2013). The heights of the bars represent the number of lines in the population in each phenotypic class

3.8.3 Deoxynivalenol results for Carman and Winnipeg FHB nurseries in 2012 and 2013

Due to the high numbers of samples and the high cost associated with DON tests, DON analysis was done on one pooled sample from the three replicates per genotype per location for the 2012 and 2013 cropping seasons (Fig. 3.2). The DON accumulation levels were higher in Carman than Winnipeg in both years the DON tests were done. However, more DON accumulation was reported in 2013 than 2012 FHB nurseries in both sites. In the Winnipeg 2012 nursery, CDC Buteo and 19H*16 had DON concentrations of 1.4 ppm (parts per million) and 1.5 ppm respectively, while in Carman 2012, the DON concentration was 2.8 ppm for CDC Buteo and 10 ppm for 19H*16. In Winnipeg 2013 and Carman 2013, CDC Buteo had DON values of

17.5 ppm and 42.5 ppm, respectively, while for 19H*16 the values were 18 and 65 ppm, respectively.

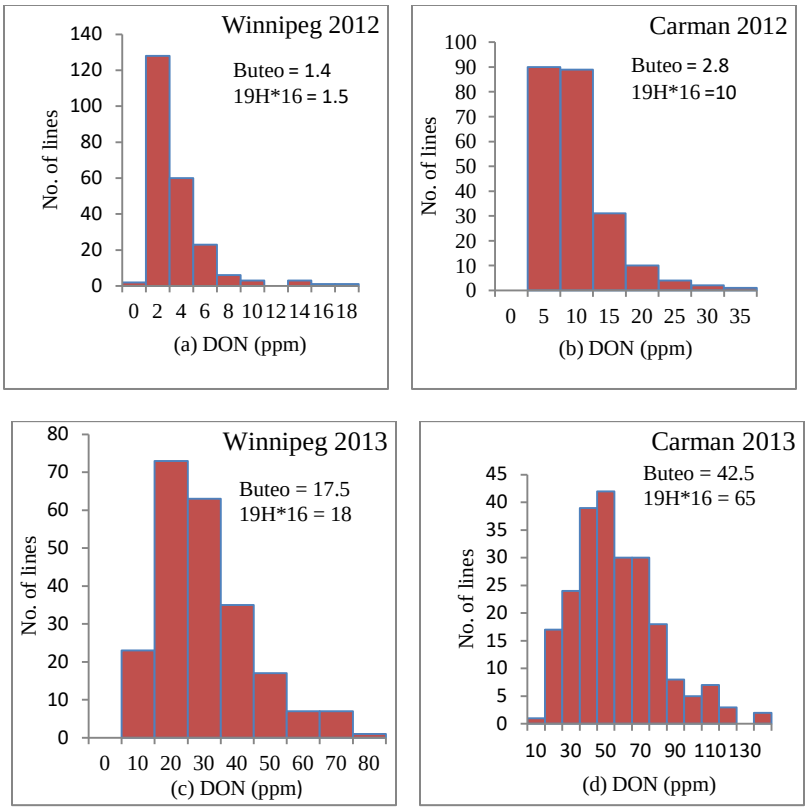


Figure 3.2 Frequency distributions for deoxynivalenol (DON) concentrations in parts per million (ppm) in Winnipeg 2012 (a), Carman 2012 (b), Winnipeg 2013 (c) and Carman 2013 (d) for 228 9HBT lines derived from the cross of 19H*16 /CDC Buteo. The data were collected from Winnipeg and Carman FHB nurseries in 2012 and 2013 from pooled samples consisting of three replicates per genotype at each siteyear. The heights of bars represent the number of lines in the population in each phenotypic class

Table 3.4 Analysis of variance for deoxynivalenol (DON) concentration (ppm) for combined data from Carman and Winnipeg for 2012 and 2013 (four site years) FHB nurseries

Source of variation	DF	Mean Square	P > F
Siteyear	3	119000.6	<.0001
Genotype	227	492.3	<.0001
Error	681	127.5	

The ANOVA of the pooled data indicated that the site years and DH lines were significantly different (Table 3.4). The frequency distribution of means across all site years

displayed significant broad and continuous variation (Fig. 3.3) with transgressive segregants. The significant site year effect explained a large proportion of the variation in DON accumulation in each site year which could be attributed to differences across the environments (Table 3.1). The significant difference in genotypes was due to different genetic composition of each DH. Figure 3.2 supports this as there are 10-fold differences in DON concentration between 2012 and 2013. The genotypes accumulated different levels of DON ranging from 3.2-57.4 ppm with a mean of 22.6 ppm (Fig. 3.3). The moderately resistant parent, CDC Buteo had a lower average DON concentration of 16.1ppm compared to susceptible parent, 19H*16 with 23.6 ppm.

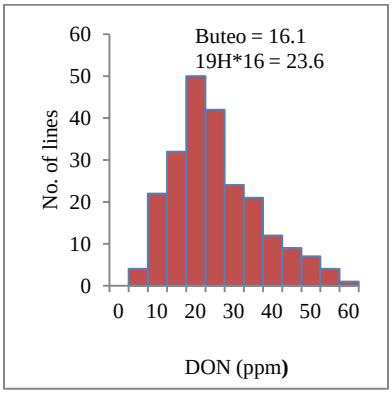


Figure 3.3 Frequency distributions for mean deoxynivalenol (DON) concentrations in parts per million for pooled data for Winnipeg 2012, Carman 2012, Winnipeg 2013 and Carman 2013 for 228 9HBT lines derived from the cross of 19H*16/CDC Buteo. The data were collected from Winnipeg and Carman FHB nurseries in 2012 and 2013 from composite samples consisting of three replicates per genotype in each site year. The heights of bars represent the number of lines in the population in each phenotypic class

3.8.4 Relationship between FHB traits and height

Correlation coefficients for all FHB traits measured were all positive and highly significant ($P<0.0001$), ranging from $r = 0.52$ to $r = 0.97$ (Tables 3.5-3.6 and appendices 2.9-2.11). Plant height was negatively correlated with all FHB traits. The correlations between incidence, severity, FHB index and FDK for the mean data for all field trials were very high, but lower between FHB traits and height (Table 3.5). This could be attributed to the fact that height

data were not taken in the same field trials as the FHB data and/or there is not as strong a correlation between height and the FHB traits than between the FHB traits. However, the highest relationship between height and FHB traits evaluated was between incidence and height (r = 0.71) and lowest between height and severity (r = 0.60). The highest relationship was between FHB index and FDK (r = 0.92).

When correlations were determined for the site years in which DON was measured, the correlation between DON and FDK was the highest (r = 0.95) with the lowest one being between severity and height (r = 0.52) (Table 3.6). Similar results were reported for DON where the association between FHB traits and height were the lowest and height was negatively correlated to DON. Correlations between severity and DON (r = 0.77) were lower compared to those of DON and FDK (r = 0.95) and incidence and DON (r = 0.85). However, higher correlations were observed between incidence and FDK (r=0.89), and incidence and DON (r = 0.85) (Table 3.6).

Table 3.5 Correlation coefficients (r) between incidence, severity, Fusarium head index (FHB index) and Fusarium damaged kernel (FDK), calculated using mean field data measurements from two field locations (Winnipeg and Carman, MB) over three years (2011, 2012 and 2013) and one season height data (2010)

Trait	Severity	FHB index	FDK	Height
Incidence	0.91	0.96	0.90	-0.71
Severity		0.97	0.88	-0.60
FHB index			0.92	-0.67
FDK				-0.67

*All correlations are significant at P=0.0001
*The r value is the degree of relationship between the two traits
*Height N= 76 genotypes, one environment data
*FHB traits N=228

Table 3.6 Correlation coefficients (r) between deoxynivalenol (DON) and field data measurements for the means across the four site years (Winnipeg and Carman for 2012 and 2013 samples for which DON analysis was done) for incidence, severity, Fusarium head blight (FHB index) and Fusarium damaged kernel (FDK)

Trait	Severity	FHB index	FDK	DON	Height
Incidence	0.84	0.98	0.89	0.85	-0.68
Severity		0.91	0.77	0.77	-0.52
FHB index			0.90	0.86	-0.66
FDK				0.95	-0.67
DON					-0.64

All correlations are significant at P=0.0001
*DON accumulation data were collected from Winnipeg and Carman nurseries for 2012 and 2013 from a pooled sample consisting of three replicates each (Winnipeg 2012, Carman 2012, and Winnipeg 2013 Carman 2013)
*Height N=76 genotypes, one environment data
*FHB traits N=228

3.9 Mapping results for 9HBT population

3.9.1 Genotyping and construction of the linkage map for 9HBT population

A total of 7,375 polymorphic markers were generated from the 9HBT SNP data. The number of markers within this map was reduced to 1,215 markers (binning) from MTSMap (Minimum Spanning Tree of a graph) (Wu et al. 2008), which were used for mapping in MapDisto (version 1.7.7), resulting in 51 linkage groups. Fifteen markers were not linked with any other markers. Where there were multiple markers in a bin, the markers from MapDisto were sorted based on position, with the most informative marker from each bin (with least missing data) being used for QTL analyses. Out of these 1,215 markers, after using one marker per bin, only 614 markers consisting of 45 linkage groups were used for linkage map construction and subsequently QTL analyses in Qgene. The 19H*16 /CDC Buteo genetic map spanned a total of 1,768.2 cM covering less than half the wheat genome.

Table 3.7 Distribution of polymorphic SNP markers across the three genomes (A, B and D) and the seven chromosome groups for the genetic map of 228 DH 9HBT wheat lines

Chromosome number	**A genome**	**B genome**	**D genome**
1	389	460	44
2	235	411	101
3	120	421	149
4	380	138	40
5	295	556	20
6	410	278	53
7	577	243	107
Total	2406	2507	514
Percent	44.3	46.2	9.5

*****Total number of SNP markers used in mapping in Mapdisto

The 78 linkage groups (from MSTMap) were resolved into different chromosomes by comparing the 9HBT map with the Wang et al. (2014) consensus map. Out of 7375 markers, only 6123 were used in mapping in Mapdisto with only 5427 markers being assigned to chromosomes (696 markers could not be assigned to any chromosomes). Genetic distance and marker coverage varied between the A, B and D genomes (Table 3.7). There was a good coverage of the A genome with 2406 markers. The B genome map contained 2507 markers, while the D genome map was poorly covered with only 514 markers. The lowest numbers of markers were reported in this genome with chromosomes 1D, 4D, 5D and 6D having less than 55 markers each. The A genome had a better distribution of markers except in chromosome 3A. Loci were more or less uniformly distributed along the chromosomes except for chromosomes 3A, 4D, 5B and 7D, which had distances between marker loci as large as 18.4, 12, 16.8 and 15 cM, respectively. The average marker interval was 2.87 cM when unique markers were used for linkage mapping.

3.9.2.1 QTL analyses for 9HBT population (228 DH lines)

Quantitative trait loci analyses were conducted for individual site years and pooled data for six site years. Other combinations of sites and years were analysed for QTL, but did not reveal additional significant QTL that were not previously identified through the individual site-year and six site-year analyses (data not shown). Multiple QTL were detected for the 19H*16/ CDC Buteo population on 13 chromosomes out of 21 (Table 3.8 and appendix 3.2) for 228 DH lines. No QTL were detected on chromosomes 1A, 1D, 2A, 2D, 3A, 5A, 6A and 7B**.** Based on data collected during the three years at two locations, major QTL were detected on chromosomes 4D and 4B in Winnipeg and Carman for all FHB traits measured (Table 3.8 and appendix 3.2). A QTL that explained more than 10% of the variance (R^2) in at least one environment was classified as a major QTL and those explaining less than 10% as minor QTL. The 4B and 4D QTL were responsible for most of the phenotypic variation in incidence, severity, FHB index, FDK and DON (Table 3.8). The FHB QTL hereby referred to as 4B and 4D, mapped to the expected locations of *Rht-B1* and *Rht-D1*, respectively. The dwarfing genes were confirmed by genotyping the 9HBT population using two SNP markers for dwarfing genes *Rht-B1* (marker wMAS000001) and *Rht-D1* (marker wMAS000002) (from http://www.cerealsdb.uk.net/cerealgenomics/CerealsDB/kasp_download.php?URL=)). CDC Buteo was confirmed to have *Rht-B1b/Rht-D1a* alleles while 19H*16 has *Rht-B1a/Rht-D1b* alleles.

The 19H*16 alleles decreased FHB symptoms for QTL in chromosomes 3D, 4B, 6B and 6D and increased FHB disease for QTL in chromosomes 1B, 2B 3B, 4A, 4D, 5B, 5D, 7A and 7D. The 19H*16 allele (*Rht-B1a*) led to decreased FHB disease; including major disease incidence, severity, FHB index, FDK and DON QTL on 4B than the dwarf type (*Rht-B1b).* A

plant height QTL was detected on chromosome 4D in which 19H*16 allele (*Rht-D1b*) lines were on average shorter in height by 11.7cm than the wild-type (Table 3.8). The 19H*16 allele (*Rht-D1b*) also increased FHB disease on chromosome 4D for all traits evaluated relative to the wild type. On chromosome 4B, the lines with the 19H*16 allele, *Rht-B1a* were taller on average by 6.5cm than the dwarf type. Plant height varied widely with the shortest DH line measuring 50cm and the tallest 125cm (height data for one season).

The 4D QTL explained between 20.7-49.2% of phenotypic variation in incidence, 7.4-35% in severity, 22.2-43.4% in FHB index, 32.9-54.2% in FDK, 26.4-49.0% in DON and 51.8% for plant height in the environments where these traits were measured (Table 3.8). The 19H*16 allele *Rht-D1b* on chromosome 4D increased the FHB symptoms and the lines on average were shorter in plant height by 11.7cm than the wild type. Chromosome 4D was poorly covered by markers and the QTL interval was 12.1cM.

The major QTL on chromosome 4B which mapped to the *Rht-B1* locus was detected for most FHB traits except for severity in the Winnipeg 2012 and Carman 2012 FHB nurseries (Table 3.8). The chromosome 4B QTL explained phenotypic variation ranging from 16.2-38.2% for incidence, 7.7-21.6% for severity, 8.4-29.8% for FHB index, 17.5-42.4% for FDK, 12-40.4% for DON and 25.9% for plant height. The 4D QTL explained a larger proportion of phenotypic variation than 4B QTL in all FHB traits evaluated. The 19H*16 alleles (*Rht-B1a*) on chromosome 4B reduced incidence, severity, FHB index, FDK and DON, but increased height.

Other major QTL observed included those on chromosome 6B at position (0-2 cM) for all traits measured except for incidence, which had 6B QTL (5.2% phenotypic variation) at position 38 (LOD score 3.05) in Winnipeg 2013. The 19H*16 alleles from 6B decreased the FHB disease. The 6B QTL was reported in all environments for FDK and DON, five sites for severity and

three sites for FHB index. However, the 6B QTL was shown to be very effective in reducing percent FDK and DON accumulation. The 6B QTL explained 6.1-11% of the variation for severity, 5.7-7.8% for FHB index, 5.6-14.6% for FDK and 6.9-24.1% phenotypic variation for DON.

Other QTL were detected on chromosome 4A in which 19H*16 alleles increased FHB disease and explained 6-13% of the variation for incidence, 6-7.9% for severity, 5.7-11.3 % for FHB index and 7.4 % for FDK. The 4A QTL was identified for incidence in six environments, severity in four environments, and only one environment for FDK and DON. The 19H*16 allele at the 4A loci led to increased disease. The 19H*16 alleles from the 2B and 3B QTL played a major role in increasing FHB disease, especially the 2B QTL which was associated with increased % FDK in three environments.

Other minor QTL for DON were observed on chromosomes 3B, 4A, 5D and 7A, each explaining 7.7%, 9.2%, 6.8%, and 7.6% of the phenotypic variation, respectively, with the 19H*16 alleles increasing DON concentration. For DON concentration, the major 4B and 6B alleles from 19H*16 decreased DON concentration in this population.

Table 3.8 Fusarium head blight (FHB) resistance quantitative trait loci (QTL) detected using multiple interval mapping (MIM) for deoxynivalenol (DON), incidence, Fusarium damaged kernels (FDK), Fusarium head blight index, (FHB index), height and severity, detected for 19H*16/CDC Buteo 228 DH lines for individual environments and pooled environments (Carman and/or Winnipeg) for 2011, 2012 and 2013 (6siteyrs). For DON, the analysis was done for four site years (Carman, 2012 and 2013 and Winnipeg, 2012 and 2013), and for height only one season data (2010) was used

Deoxynivalenol (four site years)

Trial dataset	Chr. [a]	Marker at the QTL peak [b]	Pos (cM) [c]	Add [d]	% PVE [e]	LOD [f]	LOD α=0.05 [g]
Winnipeg2013	3B	-	2	3.26	7.7	3.97	3.51
Winnipeg2013	4A	Excalibur c23921 738	18	3.1	9.2	4.76	3.54
Carman2012	4B	Kukri_c26905 392	4	-1.76	12	6.31	3.80
4siteyrs	4B	RAC875_c15872_141	5	-5.67	40.4	25.59	3.62
Winnipeg2012	4B	Kukri_c26905 392	4	-1.15	21.3	11.86	3.87
Carman2013	4B	Kukri_c26905 392	4	-12.28	35.3	21.56	3.52
Winnipeg2013	4B	Kukri_c26905 392	4	-7.39	33.7	20.36	3.51
4siteyrs	4D	wMAS000002	11	6.99	49	33.37	3.62
Carman2012	4D	wMAS000002	13	2.84	26.4	15.17	3.80
Winnipeg2012	4D	wMAS000002	13	1.52	32	19.13	3.87
Carman2013	4D	wMAS000002	11	14.49	41.2	26.30	3.52
Winnipeg2013	4D	wMAS000002	10	9.63	43.4	28.18	3.51
Winnipeg2013	5D	RAC875_c39430_181	2.4	3.45	6.8	3.46	3.51
4siteyrs	6B	CAP8_c3629_486	0	-3.81	24.1	13.63	3.62
Carman2012	6B	CAP8_c3629_486	2	-1.76	10.7	5.63	3.80
Winnipeg2012	6B	CAP8_c3629_486	0	-5.59	6.9	3.55	3.87
Carman2013	6B	CAP8_c3629_486	2	-9.07	20.9	11.58	3.52
Winnipeg2013	6B	CAP8_c3629_486	2	-4.73	15.4	8.30	3.51
Carman2013	7A	IAAV6131	54	4.64	7.6	3.92	3.52

FDK

Trial dataset	Chr. [a]	Marker at the QTL peak [b]	Pos (cM) [c]	Add [d]	% PV [e]	LOD [f]	LOD α=0.05[g]
Carman2012	1B	Excalibur_c64866_259	0	0.94	6.2	3.17	3.81
6siteyrs	2B	Bobwhite c54073 99	1	1.78	8	4.11	3.30
Carman2012	2B	Bobwhite c54073 99	1	1.23	6.5	3.31	3.81
Carman2013	2B	Bobwhite c54073 99	1	5.08	11.8	6.22	3.73
Winnipeg2013	3B	BS00095515_51	28	1.76	6.5	3.35	3.43
Carman2012	4A	IAAV4351	27	1.37	7.4	3.79	3.81
6siteyrs	4B	Kukri_c26905 392	4	-4.46	42.4	27.30	3.30
Carman2011	4B	Kukri_c26905 392	4	-3.86	34.1	20.65	3.50
Winnipeg2011	4B	Kukri_c26905 392	4	-5.3	34.2	20.70	3.43
Carman2012	4B	Kukri_c26905 392	4	-1.77	17.5	9.53	3.81
Winnipeg2012	4B	Kukri_c26905 392	4	-2.02	30.4	17.91	3.90
Carman2013	4B	Kukri_c26905 392	4	-8.83	38.7	24.20	3.73
Winnipeg2013	4B	Kukri_c26905 392	4	-4.99	34.2	20.71	3.43
6siteyrs	4D	wMAS000002	11	5.74	54.2	38.63	3.30
Carman2011	4D	wMAS000002	12	4.39	38.9	24.39	3.50
Winnipeg2011	4D	wMAS000002	12	6.7	43.7	28.41	3.43
Carman2012	4D	wMAS000002	13	2.67	32.9	19.73	3.81
Winnipeg2012	4D	wMAS000002	13	2.38	37.6	23.36	3.90
Carman2013	4D	wMAS000002	15	9.95	43.6	28.37	3.73
Winnipeg2013	4D	wMAS000002	10	7.03	49.3	33.63	3.43
Winnipeg2013	5D	RAC875_c39430_181	2.4	2.28	6.3	3.24	3.43
6siteyrs	6B	CAP8_c3629_486	0	-2.11	14.6	7.79	3.30
Carman2011	6B	CAP8_c3629_486	0	-1.88	11.3	5.95	3.50
Winnipeg2011	6B	CAP8_c3629_486	0	-2.52	10.9	5.70	3.43
Carman2012	6B	CAP8_c3629_486	2	-1.28	9.0	4.67	3.81
Winnipeg2012	6B	CAP8_c3629_486	0	-0.73	5.6	2.84*	3.90

Trial dataset	Chr. [a]	Marker at the QTL peak [b]	Pos (cM) [c]	Add [d]	% PV [e]	LOD [f]	LOD α=0.05 [g]
Carman2013	6B	CAP8_c3629_486	2	-3.68	9.3	4.83	3.73
Winnipeg2013	6B	CAP8_c3629_486	2	-3	13.9	7.43	3.43
Carman2012	7A		54	0.97	6.1	3.14	3.81

FHB index

Trial dataset	Chr. [a]	Marker at the QTL peak [b]	Pos (cM) [c]	Add [d]	% PVE [e]	LOD [f]	LOD α=0.05 [g]
Carman2013	2B	Bobwhite c54073 99	1	3.61	7.1	3.66	3.72
Winnipeg2012	3B	wsnp_JD_c828_1226159	2	3.02	7.2	3.70	3.42
Carman2011	3D	wsnp_JD_c828_1226159	36	-4.08	5.8	2.97	3.71
6siteyrs	4A	Excalibur c23921 738	19	2.9	8.1	4.19	3.43
Winnipeg2012	4A	IAAV4351	28	2.76	7.3	3.78	3.42
Carman2013	4A	-	4	2.93	5.7	2.90	3.72
Winnipeg2013	4A	Excalibur c23921 738	19	3.76	11.3	5.94	3.38
6siteyrs	4B	Kukri_c26905 392	4	-6.38	29.8	17.54	3.43
Carman2011	4B	Kukri_c26905 392	4	-7.99	18.3	10.02	3.71
Winnipeg2011	4B	Kukri_c26905 392	4	-10.37	29.2	17.10	3.14
Carman2012	4B	Kukri_c26905 392	4	-2.32	8.4	4.33	3.23
Winnipeg2012	4B	Kukri_c26905 392	4	-4.39	16.5	8.94	3.42
Carman2013	4B	Kukri_c26905 392	4	-5.26	19.6	10.77	3.72
Winnipeg2013	4B	Kukri_c26905 392	4	-6.62	28.1	16.36	3.38
6siteyrs	4D	wMAS000002	12	8.04	39	24.5	3.43
Carman2011	4D	wMAS000002	12	9.63	23.7	13.41	3.71
Winnipeg2011	4D	wMAS000002	12	13.14	38	23.68	3.14
Carman2012	4D	wMAS000002	13	4.36	25.1	14.32	3.23
Winnipeg2012	4D	wMAS000002	11	5.49	22.2	12.45	3.42
Winnipeg2013	4D	wMAS000002	10	9.66	43.4	28.15	3.38
Carman2013	4D	wMAS000002	16	6.04	23.4	13.22	3.72
6siteyrs	6B	CAP8_c3629_486	0	-2.69	7.8	4.01	3.43

Carman2011	6B	CAP8_c3629_486	0	-4.45	6.7	3.43	3.71
Winnipeg2011	6B	CAP8_c3629_486	0	-3.79	5.7	2.89*	3.14
Carman2012	6B	CAP8_c3629_486	0	-1.77	5.7	2.90*	3.23
Winnipeg2013	6B	CAP8_c3629_486	0	-2.88	7.6	3.91	3.38
Carman2012	6D	BS00022523_51	7	-1.89	6.3	3.21	3.23
Carman2012	7A	wsnp JD c 38071 27729378	53	2.03	6.5	3.35	3.23

Height

Trial dataset	Chr. [a]	Marker at the QTL peak [b]	Pos (cM) [c]	Add [d]	% PVE [e]	LOD [f]	LOD α=0.05 [g]
2010	4B	Kukri_c26905 392	4	6.49	25.9	4.94	4.36
2010	4D	wMAS000002	8	-11.67	51.8	12.04	4.36

Incidence

Trial dataset	Chr. [a]	Marker at the QTL peak [b]	Pos (cM) [c]	Add [d]	% PVE [e]	LOD [f]	LOD α=0.05 [g]
Carman2013	2B	IAAV8795	25	2.88	6	3.06	3.62
Winnipeg2012	3B	wsnp_CAP7_c5097_2266314	22	4.07	7.6	3.90	3.57
6siteyrs	4A	Excalibur c23921 738	19	3.68	13.3	7.04	3.52
Winnipeg2011	4A	Excalibur c23921 738	19	5.36	8.9	4.61	3.18
Carman2012	4A	Excalibur c23921 738	18	2.82	6.0	3.05	3.61
Winnipeg2012	4A	IAAV4351	28	3.66	7.7	3.96	3.57
Carman2013	4A	RAC875_c19534_68	16	2.73	7.8	4.00	3.62
Winnipeg2013	4A	Excalibur c23921 738	19	3.56	10.1	5.25	3.52
6siteyrs	4B	Kukri_c26905 392	4	-7.45	38.2	23.81	3.52
Carman2011	4B	Kukri_c26905 392	5	-7.54	21.2	11.78	2.83
Winnipeg2011	4B	Kukri_c26905 392	4	-13.22	36.9	22.79	3.8
Carman2012	4B	Kukri_c26905 392	4	-4.95	16.2	8.73	3.16
Winnipeg2012	4B	Kukri_c26905 392	4	-6.33	19.7	10.86	3.57
Carman2013	4B	Kukri_c26905 392	4	-5.6	25.8	14.78	3.62

Trial dataset	Chr. [a]	Marker at the QTL peak [b]	Pos (cM) [c]	Add [d]	% PVE [e]	LOD [f]	LOD α=0.05 [g]
Winnipeg2013	4B	Kukri_c26905 392	0	-7.04	29.5	17.31	3.52
6siteyrs	4D	wMAS000002	12	9.32	47.6	32.00	3.52
Carman2011	4D	wMAS000002	15	7.62	20.7	11.45	2.83
Winnipeg2011	4D	wMAS000002	11	17.75	49.2	33.58	3.18
Carman2012	4D	wMAS000002	13	7.31	29.5	17.28	3.16
Winnipeg2012	4D	wMAS000002	11	7.83	25.9	14.85	3.57
Carman2013	4D	wMAS000002	16	5.78	26.1	15.01	3.62
Winnipeg2013	4D	wMAS000002	11	9.95	44.4	29.05	3.52
Winnipeg2012	5D	RAC875_c39430_181	3.4	3.96	6.4	3.29	3.57
Winnipeg2013	6B	wsnp_Ex_c4815_8597139	38	-3	6.0	3.05	3.52

Severity

Trial dataset	Chr. [a]	Marker at the QTL peak [b]	Pos (cM) [c]	Add [d]	% PVE [e]	LOD [f]	LOD α=0.05 [g]
Carman2011	2B	RAC875_c19042_443	31	3.93	6	3.05	3.57
Carman2013	2B	IAAV8795	25	3.4	7.3	3.78	3.25
Winnipeg2012	3B	wsnp_JD_c828_1226159	2	3.48	0.093	4.81	3.14
Carman2011	3D	Excalibur_c22852_401	36	-4.01	6.1	3.11	3.57
6siteyrs	4A	Excalibur c23921 738	19	2.37	7.9	4.08	3.66
Winnipeg2011	4A	Excalibur c23921 738	19	4.47	7.5	3.85	3.35
Winnipeg2012	4A	Excalibur c23921 738	19	2.09	5.7	2.93	3.14
Winnipeg2012	4A	IAAV2596	28	2.03	5.5	2.78*	3.14
Carman2013	4A	Excalibur_c24664_1308	14	3.05	6	3.08	3.25
Winnipeg2013	4A	Excalibur c23921 738	19	2.53	7.9	4.09	3.61
Winnipeg2013	4A	IAAV2596	28	2.37	7	3.57	3.61
6siteyrs	4B	Kukri_c26905 392	4	-4.44	21.6	12.05	3.66
Carman2011	4B	Kukri_c26905 392	4	-6.77	14.9	7.97	3.57
Winnipeg2011	4B	Kukri_c26905 392	4	-9.68	27.4	15.83	3.35
Carman2013	4B	Kukri_c26905 392	4	-2.82	7.7	3.95	3.25

Trial dataset	Chr. [a]	Marker at the QTL peak [b]	Pos (cM) [c]	Add [d]	% PVE [e]	LOD [f]	LOD α=0.05 [g]
Winnipeg2013	4B	Kukri_c26905 392	4	-4.16	17.4	9.49	3.61
6siteyrs	4D	wMAS000002	11	6.41	35	21.36	3.66
Carman2011	4D	wMAS000002	12	8.27	19.9	10.98	3.57
Winnipeg2011	4D	wMAS000002	12	11.95	35	21.32	3.35
Carman2012	4D	wMAS000002	11	2.45	10.3	5.39	3.68
Winnipeg2012	4D	wMAS000002	7	2.56	7.4	3.81	3.14
Carman2013	4D	wMAS000002	16	3.91	13.2	7.03	3.25
Winnipeg2013	4D	wMAS000002	15	6.23	31.3	18.58	3.61
6siteyrs	5B	BS00009789_51	8	2.03	5.9	3.01	3.66
Winnipeg2013	5B	BS00009789_51	9	2.34	6.9	3.51	3.61
6 site years	6B	CAP8_c3629_486	0	-2.93	11	5.78	3.66
Carman2011	6B	CAP8_c3629_486	0	-4.74	8.1	4.18	3.57
Winnipeg2011	6B	CAP8_c3629_486	0	-4.37	7.9	4.08	3.35
Carman2012	6B	CAP8_c3629_486	0	-1.71	6.1	3.10	3.79
Winnipeg2012	6B	CAP8_c3629_486	2	-2.42	6.1	3.11	3.14
Winnipeg2013	6B	CAP8_c3629_486	0	-2.84	9.3	4.82	3.61
Carman2012	6D	BS00022523_51	7	-2.47	11	5.79	3.79
6 site years	7A	wsnp JD c 38071 27729378	54	2.47	8.3	4.28	3.66
Carman2012	7A	IACX2471	75	2.29	9.1	4.73	3.79
Winnipeg2013	7D	RAC875 c68368 99	6	3.2	6.8	3.49	3.61

[a] Chr., Chromosome
[b] Marker at the peak of the QTL
[c] Pos, Position on linkage group in cM
[d] Add, Additive effect (Additive effect of allele substitution of the trait in question. A negative sign indicates that 19H*16 alleles decreased the trait and vice versa)
[e] %PVE, Phenotypic variation explained (R^2; %)
[f] LOD, Peak LOD score
g LOD α=0.05, Significant LOD score threshold at 5%
* Detected QTL with a LOD score <3.00

3.9.3 Results for QTL analyses for four sub-populations, fixed for the 4B or 4D alleles

The 9HBT population was classified into four sub-populations containing the different combinations of the semi-dwarfing alleles for the dwarfing genes *Rht-B1*and *Rht-D1*. The analysis of QTL of these subdivided-populations did not detect new QTL (data not shown).

3.9.4 The *Rht* allele combinations and their effect on Fusarium head blight traits

The subdivision of the 9HBT population according to *Rht* (Reduced height) genes resulted in four sub-populations comprising of combinations (*Rht-B1b/Rht-D1a* and *Rht-B1a/Rht-D1b*), double mutants (*Rht-B1b/Rht-D1b*), and double wild-types (*Rht-B1a/Rht-D1a*). The ranking of these genotypic groups was consistent for all FHB symptoms for all traits measured with *Rht-B1a/Rht-D1a< Rht-B1b/Rht-D1a < Rht-B1a/Rht-D1b <Rht-B1b/Rht-D1b* (Figure 3.4 and Table 3.13). The double wild-type genotypes (*Rht-B1a/Rht-D1a)* had the lowest disease incidence, severity, FHB index and FDK and DON while the double mutant genotypes *(Rht-B1b/Rht-D1b)* had the highest FHB infection for all traits measured. The mutant allele *Rht-D1b* had higher FHB disease than *Rht-B1b* for all the traits measured, although in years where the disease was not high due to unfavorable weather conditions, there was no significant difference between the two classes, e.g. in Winnipeg 2012 severity and FHB index (Figure 3.4 and Table 3.13).

For height, the double mutant genotypes were shown to be the shortest with a mean of 62.9 cm compared to the double wild genotypes (101.8 cm). The allele combination *Rht-B1a/Rht-D1b* was shorter than its counterpart *Rht-B1b/Rht-D1a* by 7.6 cm (Table 3.9) and had higher FHB infection. The total phenotypic variation explained by these two dwarfing genes was 87.4%, 62.4%, 73 %, 96.6%, 78.7% and 77.7% for incidence, severity, FHB index, FDK, DON

and height respectively. The largest phenotypic variation was explained by the *Rht-D1* gene (Table 3.8).

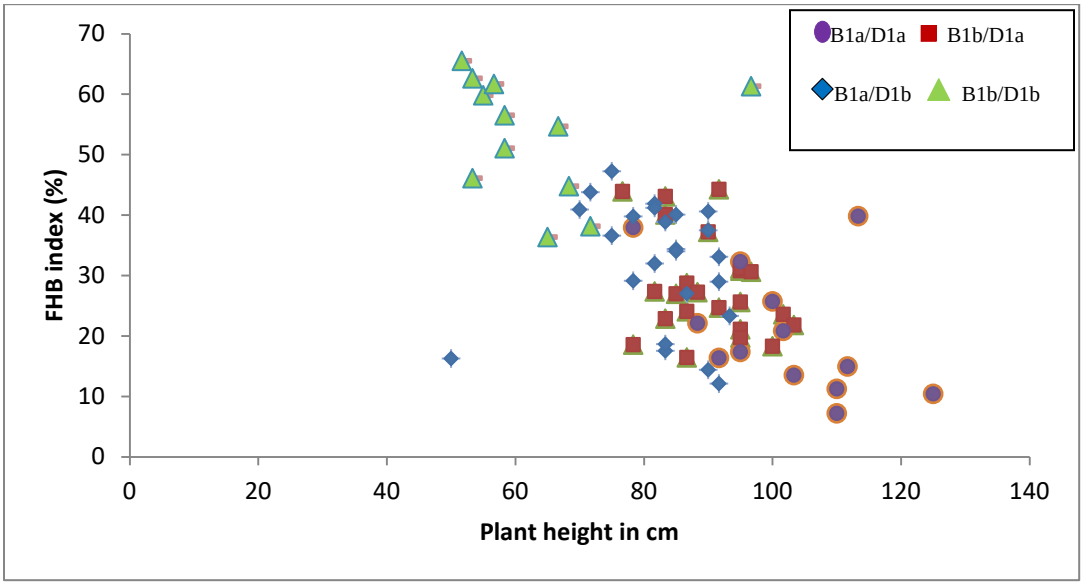


Figure 3.4 A scatter plot for pooled data for Fusarium head blight index (FHB index) versus plant height (cm) for 76 double haploid (DH) lines. The allele combinations for various *Rht* genes of individual genotypes is shown by different symbols

Table 3.9 Means of reduced height (*Rht*) groups indicating which means are statistically different based on least significance difference (LSD) for Fusarium head blight (FHB) incidence, severity, Fusarium head blight index (FHB index), Fusarium damaged kernels (FDK), deoxynivalenol (DON) and height in the 9HBT population across sites, Carman (CRM) and Winnipeg (WNP) and years (2011, 2012 and 2013) and for the pooled data for Carman and Winnipeg for 2011, 2012 and 2013 FHB nurseries. For height, only one of season data (2010) was used. Ten genotypes could not be classified for both loci due to the failure of the PCR reaction and they have been removed from these tables

Trait/*Rht* Genotype	N	Pooled data	CRM 2011	WNP 2011	CRM 2012	WNP 2012	CRM 2013	WNP 2013
				FHB incidence				
B1a/D1a	47	38.4 a	58.3 a	24.1 a	36.2 a	27 a	47 a	38 a
B1b/D1a	62	52.6 b	77.11 b	55.4 b	39.3 a	33.3b	57.2 b	53 b
B1a/D1b	72	56.8 c	79.3 b	62.7 c	46.3 b	36.4 b	58.5 b	57.5 c
B1b/D1b	37	74.4 d	90.7 c	88.2 d	65.1 c	57.3 c	71.6 c	73.3 d
				FHB Severity				
B1a/D1a	47	42.6 a	32.6 a	15.9 a	43.1 a	50.1 a	58.6 a	55.5 a
B1b/D1a	62	50.4 b	41 b	30.5 b	44.6 a	56 b	64.3 b	65.9 b
B1a/D1b	72	53.7 c	44.5 b	35.5 c	49.8 b	56.2 b	66.7 b	69.1 c
B1b/D1b	37	64.9 d	63.4 c	63.2 d	54.8 c	59.7 c	71.4 c	77 d
				FHB index				
B1a/D1a	47	18.8 a	22 a	8 a	16.8 a	14.4 a	29 a	22.5 a
B1b/D1a	62	27.5 b	33.5 b	19.5 b	18.9 a	19.5 b	38.3 b	35.6 b
B1a/D1b	72	31.7 c	37.3 b	25.2 c	24.5 b	21.7 b	40.7 b	40.5 c
B1b/D1b	37	50 d	58.8 c	58.8 d	37.2 c	35.4 c	52.6 c	57 d
				FDK				
B1a/D1a	47	10.6 a	11.3 a	6.3 a	3.7 a	1 a	28.4 a	12.8 a
B1b/D1a	62	16.3 b	16.8 b	13.6 b	5.2 b	1.8 b	40.5 b	19.9 b
B1a/D1b	72	18.2 c	17.6 b	15.8 b	7.1 c	2.8 c	43.5 c	22.3 c
B1b/D1b	37	31.7 d	28.6 c	31.2 c	13.5 d	11.1 d	68.5 d	37.5 d

Trait/*Rht* Genotype	N	Pooled data	CRM 2011	WNP 2011	CRM 2012	WNP 2012	CRM 2013	WNP 2013
				DON				
B1a/D1a	47	12.6 a			4.2 a	1.1 a	31.2 a	14 a
B1b/D1a	62	20.9 b			5.9 a	1.8 a	51.1 b	24.9 b
B1a/D1b	72	22.8 b			8.3 b	2.6 b	53.5 b	27 b
B1b/D1b	37	39.3 c			14.2 c	6.9 c	87.4 c	48.7 d

*Numbers followed by a different letter in a column are significantly different based on LSD ($p \leq 0.05$)

		Plant Height (cm) 2010
B1a/D1a	13	101.8 a
B1b/D1a	25	89.8 b
B1a/D1b	23	82.2 c
B1b/D1b	11	62.9 d

*Numbers followed by a different letter in a column are significantly different based on LSD ($p \leq 0.05$)

3.10 Discussion

3.10.1 Inheritance of FHB in 9HBT Double haploid population

The frequency distributions displayed significant continuous variations for means across all experiments. CDC Buteo was shown to be moderately resistant on average over all environments with 19H*16 being susceptible. This agrees with other reports that CDC Buteo is moderately resistant to FHB (Seed Manitoba, 2017). Transgressive segregants were observed for all traits, but the proportion of lines on either side of the parents was not consistent between years, locations, and traits measured. This indicated that both parents contributed some resistance to FHB. The phenotypic data suggested that there was significantly large genetic variation in FHB resistance among lines ranging from resistant to highly susceptible, making the DH population suitable for QTL analysis. FHB disease was associated with the semi-dwarfing loci where taller genotypes (*Rht-B1a/Rht-D1a*) had lower disease symptoms compared to their shorter counterparts (*Rht-B1b/Rht-D1b*) (Table 3.9). The DH lines with 19H*16 allele (*Rht-B1a*) on chromosome 4B, were on average, taller by 6.5cm than the dwarf types while the 19H*16, (*Rht-D1b)* allele on 4D led to shorter lines on average, by 11.7cm than the wild type. The corresponding 19H*16 alleles on chromosome 4B were associated with decreased FHB symptoms, while the alleles from this parent on 4D increased FHB symptoms. McCartney et al. (2007) reported similar results that the Wuhan-1 4B resistance allele was the most effective resistance allele, but was associated with a 9.3 cm increase in plant height. Abilene, one of the parents for CDC Buteo contains *Rht-B1b* and *Rht-D1a* (Guedira et al. 2010). From the genotyping data for the two *Rht* genes, CDC Buteo was confirmed to have the *Rht-B1b*/*Rht-D1a* genotype, while the 19H*16 allele combination was *Rht-B1a*/*Rht-D1b*. Others studies have shown the relationship between the dwarfing genes and FHB. For example, in the three mapping

populations tested by (Buerstmayr et al. 2012) *Rht-B1b* was associated with increased FHB susceptibility, with phenotypic effects ranging from 3-18%. The negative effects of the two dwarfing genes on field FHB resistance have also been reported in Chinese and US wheat materials (Liu et al. 2013; Lv et al. 2014).

Reduction in mycotoxins, especially DON, is important because DON adversely affects humans and livestock health (Aakre et al. 2005). Deoxynivalenol was proven to inhibit protein synthesis in eukaryotic cells and acts as a virulence factor during fungal pathogenesis, therefore resistance to DON is considered an important component of resistance against FHB (Arunachalam and Doohan 2013). In Winnipeg 2012, there was no significant difference between CDC Buteo and 19H*16 in terms of DON accumulation, while in Carman in both years there was a significant difference with the highest DON accumulation reported in 2013 in Carman for both parents. This could be attributed to high temperatures and more rainfall in Carman in 2013 that created a conducive environment for FHB development. Champeil et al. (2004) suggested that FHB is widely spread, in areas with inductive climatic conditions (hot/warm temperatures and high/medium high humidity).

Correlation coefficients for FHB traits between environments were all positive and highly significant ($P<0.0001$) and they ranged from $r=0.39$ to $r=0.97$ for all traits in all FHB nurseries. Correlations for severity and DON were moderately low compared to those of DON and FDK, and incidence and FDK. However, better correlations were observed between incidence and FDK than incidence and DON. The correlations for overall means in this study are quite high ($r=0.77$ for severity and 0.85 for incidence) which suggests that disease incidence and severity in 9HBTpopulation are good predictors of DON. This also would suggest that the total amount of disease is the primary determinant of DON in this population, rather than the presence of a

detoxification mechanism that could show high disease but low DON. However, the correlations between DON and severity are lower than those for incidence in most site years tested (Appendix 2.9).

Liu et al. (2013) in a study of crosses between Becker/Massey (B/M) and Ernie/MO 94-317 (E/MO) that were evaluated for FHB resistance reported similar results that correlations of DON concentration with incidence and FDK were significant in most environments, while DON was not significantly associated with severity in most of the tests. This suggests that DON concentration in most environments is determined to a larger extent by FHB incidence than severity (Liu et al. 2013). The best relationship was between DON and FDK, and FHB index and FDK. This means that FDK may be a good measure of DON in this population.

A strong relationship was identified between increased plant height and improved FHB resistance in 9HBT population. The 4B and 4D QTL mapped on the exact positions of dwarfing genes *Rht-B1* and *Rht-D1* respectively. All FHB traits were negatively correlated with plant height, with the highest associations noted between plant height and incidence (r= -0.71) and with FDK and FHB index (r = -0.67, each (Table 3.5)) meaning that the taller DH lines were more resistant than their dwarf counterparts. Buerstmayr et al. (2011) reported a negative correlation between FHB severity and plant height, spike length and glaucousness of the spikes in a RIL population from a cross between *T. macha* (Georgian spelt wheat) and Furore (an Austrian winter wheat).

Understanding the relationship between DON and FHB severity can be useful in developing DON predictive models which can be used in management of FHB and DON due to the costs associated with DON tests. Paul et al. (2005) analyzed 163 studies and found more than 65% of all correlation coefficients between FHB severity and DON to be >0.50 and showed that

the strongest relationship was between FDK and DON, followed by index and DON. Similar results were reported for 9HBT population where the highest correlation for DON was between FDK and DON at r=0.95 (Table 3.6). Deoxynivalenol is known to play a role in the development of FHB and reports of positive relationships between fungal biomass and DON content of grain (Edwards et al. 2001) suggest that DON accumulation may be related to the level of FHB damage. Mesterházy et al. (2003) demonstrated the effect of genotype on DON accumulation in winter wheat where susceptible genotypes had low to moderate accumulation, compared to little or no accumulation of the mycotoxin in the most resistant genotypes.

The 9HBT population had six lines with FHB index of less than 10% and 56 lines having an index 10- 20%. Of these, about 14 of these moderately resistant lines out of 76 lines (for which height measurements were available), would be of an acceptable height ranging from 50-93.3 cm and a FHB index range of 12.1-24.7% based on distribution figure for height and FHB index (Figure 3.4). Some of these DH lines could be used as a source of resistance for development of elite cultivars or be developed into cultivars for the Prairies.

3.10.2 Discussion for QTL mapping for 9HBT 228 DH lines

The majority of mapped markers were located in the A (44.3%) and B (46.2%) genomes. About 9.5% of the markers mapped to the D genome. The D genome is known to be more conserved (Janda et al. 2004; Linkiewicz et al. 2004) and the D genome maps are particularly lacking full marker coverage (Buerstmayr 2009). Despite some chromosomes having very few markers, especially in the D genome, several QTL were identified across the three genomes. Numerous sources of FHB resistance from many countries in Asia, North America, South America, and Europe have been genetically mapped. Many of these studies include sources from Sumai 3 and its derivatives or other varieties of Chinese origin with a limited number of studies

on known resistance sources from elsewhere (Buerstmayr et al. 2009). In the current study, DH lines from 19H*16 /CDC Buteo were challenged for FHB resistance in three field experiments at two locations using four *F. graminearum* isolates consisting of two chemotypes. Transgressive segregants of FHB resistance were observed indicating that both parents contributed unique alleles which reduced the FHB disease. In total 15 QTL were detected in this population. 19H*16 alleles reduced FHB disease on chromosomes, 4B, 6B and 3B. For the rest of QTL the 19H*16 alleles increased FHB symptoms especially with 4D and 4A QTL having the largest effect in increasing FHB susceptibility followed by QTL 7A. The QTL on chromosome 4A was reported for all traits, but was more common for incidence than severity, FDK and DON accumulation. This means that for all the QTL in which 19H*16 increased FHB symptoms, the corresponding alleles in CDC Buteo reduced FHB symptoms and vice versa.

The 4B and 4D FHB resistance QTL were the most effective in reducing FHB infection in 9HBT population with the QTL in chromosome 4D explaining the largest proportion of phenotypic variation in all traits. The results of this study suggested that the CDC Buteo alleles for incidence, severity, FHB index, FDK and DON at chromosomes 2B, 4A, 4D, 5A, 7A and 19H*16 alleles for severity, FHB index, FDK and DON at 4B and 6B may be suitable for reducing FHB symptoms in Western Canada. Three recombinants that were not very tall (88.3-95 cm), but had better FHB symptoms were identified, with allele combinations *Rht-B1a/Rht-D1a* and an FHB index ranging from 7.24 -13.1%. Other recombinants included two from *Rht-B1b/Rht-D1a* with an FHB index of 16.5 and 18.6% and plant height of 86.7 and 78.3 cm, respectively, and three recombinants from *Rht-B1a/Rht-D1b* with an FHB index of 12.2-18.7% and plant height of 83.3-91.7cm (data not shown). The plant height data for all genotypes was not available and the total number of shorter lines with better FHB resistance could not be

determined. However, this region was also strongly associated with plant height in this population. *Rht-B1* is a major dwarfing allele and its location in the 4BS FHB resistance QTL region (McCartney et al. 2007) explains the association between height and FHB reaction. In a study from a cross Wuhan-1/Nyuubai, QTL for plant height was detected near the 4B FHB resistance QTL and Somers et al. (2003), suggested that the association between plant height and FHB reaction detected in their study, was due to linkage and not pleiotropy. Efforts should be made to break this deleterious linkage. Results from our study could not clearly suggest if the variation in FHB symptoms may be due to pleiotropic effects of the *Rht* genes or to nearby genes that are in very high linkage disequilibrium. The most frequently used height reducing genes with strong effect on plant height shortening are *Rht-B1b* and *Rht-D1b*, derived from the Japanese cultivar Norin 10 which were the basis of Borlaug's Green revolution in wheat breeding. In this study, CDC Buteo was found to contain allele combination *Rht-B1b/Rht-D1a* while 19H*16 contained *Rht-B1a/Rht-D1b*. This suggests that both parents contributed alleles which reduced FHB symptoms. Gibberellic acid tests have been used in determining dwarfing gene presence, but development of molecular markers technique facilitated their direct detection at the DNA level (Ellis et al. 2002). The two parents carried contrasting alleles on 4B and 4D such that the tall 19H*16 alleles on 4B and the tall alleles from CDC Buteo on 4D were associated with decreased FHB symptoms.

In winter wheat, FHB resistance QTL are known to occur especially on chromosomes 1B, 2B, 3A, 4AL, 5AL, and 6DL (Gervais et al. 2003; Shen et al. 2003; Paillard et al. 2004). Similarly, in a RIL mapping of Kenyon/86ISMN 2137, FHB resistance QTL were identified on chromosomes 4A, 4D, and 5B and they mapped to known locations of FHB resistance (McCartney et al. 2016). 19H*16 alleles reduced FHB on 4B QTL and also a major QTL on 6B

pos 2 and less environmentally stable QTL on 2B for FDK, 3D, and 6D. When an allele from 19H*16 increases FHB, the corresponding allele from CDC Buteo decreases FHB. Therefore, CDC Buteo alleles decreased FHB disease on chromosomes 1B, 2B, 3B, 4A, 4D, 5B, 5D, 7A and 7D. The inconsistent identification of resistance QTL across sites may be due to the effects of other uncontrollable environmental factors, such as temperature and humidity at each experimental location, or environment and genotypes interaction. These inconsistencies in FHB QTL resistance expression in different environments was also observed in other studies in wheat (Buerstmayr et al. 2009; McCartney et al. 2016). Bonin and Kolb (2009) suggested that environment may affect the expression of important FHB resistance QTL and the effectiveness of minor resistance QTL varies by environment. The QTL identified in multiple environments (at least four site years) may have a more consistent and significant effect on FHB control, and may be useful in marker-assisted breeding programs especially the ones with at least one semi-dwarf allele.

The 6B QTL did not contribute any alleles for FHB incidence for the 228 DH population except for an inconsistently identified QTL on pos 38 cM both for incidence and severity (one environment each). The 19H*16 allele at the 6B QTL was associated with decreased severity, FHB index, reduced FDK and reduced DON accumulation in all locations and years tested for FHB infection. It was found to be effective in reducing FDK (Type 3) and DON accumulation (Type 5) (Mesterházy 1995) and may be useful in increasing resistance to the existing Canadian elite cultivars. It was identified in three out of four environments where DON tests were done including the combined data for DON four site years and all environments where FDK tests were carried out. Cuthbert et al. (2007) qualitatively mapped the resistance gene from the cross BW278/AC Foremost as a Mendelian factor and named it *Fhb2*. However, it is not clear if this

QTL on 6B pos 2 cM from CDC Buteo is similar to that from the cross BW278/AC Foremost *(Fhb2)*. Comparative mapping using Mapchart to compare Cuthbert et al. (2007) and 9HBT map indicates that this could be same QTL as that on chromosome 6BS pos 13, flanked by microsatellite markers GWM133 and GWM644.

Other major QTL were reported in chromosomes 4A and 7A and the 19H*16 alleles increased FHB infection indicating that the Buteo alleles would be the desired alleles to select for reduced FHB infection.

The trichothecene mycotoxin DON may be produced by *F. graminearum* to facilitate spread, from the point of infection, into adjacent spikelets (Bai et al. 2002; Wegulo et al. 2015). Interestingly, in the current study, QTL analysis for DON tolerance identified five major QTL which co-localized with the other FHB QTL. Exploration of FHB resistance in 4B and 6B in 19H*16 and 2B, 4A, 4D and 7A in CDC Buteo which reduced FHB symptoms may provide breeders with alternative adapted genes for the improvement of FHB resistance in wheat.

3.10.3 Conclusion

Six DH lines from the cross 19H*16/CDC Buteo had < 10% FHB index with 56 DH lines having an index of 10-20%. For all traits investigated, FHB resistant QTL were identified on 13 chromosomes with consistent QTL on 2B, 4A, 4B, 4D, 6B and 7A. The 4B and 4D mapped on positions for the *Rht-B1* and *Rht-D1* dwarfing genes, respectively. The 4B and 4D FHB resistance QTL were the most effective in reducing FHB symptoms in the 9HBT population with the QTL in chromosome 4D explaining the largest proportion of phenotypic variation in all traits. CDC Buteo was found to contain allele combination *RhtB1b/Rht-D1a* while 19H*16 contained *Rht-B1a/Rht-D1b*. Exploration of FHB resistance in 4B and 6B in 19H*16 and 2B, 4A, 4D and 7A in CDC Buteo which reduced FHB symptoms may provide breeders with

alternative adapted genes for reducing FHB symptoms in Western Canada. Marker assisted selection may be used for selection of semi-dwarf lines which can be converted to new cultivars or used to introgress FHB resistance to existing elite cultivars. Overall the 19H*16 alleles for the 4B and 6B QTL and the CDC Buteo alleles for the 4A, 4D and 7A QTL were the most important QTL in this population, contributing to reduced FHB symptoms in all the FHB traits measured.

CHAPTER 4

INHERITANCE OF FUSARIUM HEAD BLIGHT RESISTANCE AND MOLECULAR MAPPING OF QUANTITATIVE TRAIT LOCI CONTROLLING FUSARIUM HEAD BLIGHT IN DH99W22A*13/ CDC BUTEO DOUBLE HAPLOID WINTER WHEAT POPULATION

4.1 Abstract

Fusarium head blight (FHB) is a destructive wheat disease of global importance. Resistance breeding depends heavily on the *Fhb1* gene from Sumai 3. In Canada, numerous wheat genetic studies of FHB resistance have been published with only limited information available on FHB resistance derived from elite cultivars. CDC Buteo is a winter wheat cultivar developed in Canada that has been reported to be moderately resistant to FHB. The objective of this study were to evaluate the inheritance of FHB resistance and map FHB resistant QTL in a double haploid (DH) population (n= 218) from the cross DH99W22A*13/CDC Buteo. The population was evaluated for FHB resistance in six FHB nurseries. The genetic variation ranged from moderately resistant to susceptible with transgressive segregants observed for all traits, an indication that both parents contributed FHB resistance. About 93 out of 218 lines had less than 20% FHB index for pooled data for all environments. Both parents showed similar FHB index (FHBI) rates, although CDC Buteo had lower disease incidence and deoxynivalenol (DON), but higher FDK (Fusarium damaged kernels) levels than DH99W22A*13.

Single nucleotide polymorphism (SNP) genotyping using the 90K Infinium assay (Illumina) resulted into 66 linkage groups with 6049 polymorphic markers. Nineteen FHB resistance QTL were identified. Alleles from DH99W22A*13 reduced FHB symptoms at eight of the loci while CDC Buteo alleles reduced FHB symptoms at 11 QTL loci. Major QTL where

DH99W22A*13 alleles reduced FHB were identified on chromosomes 3B and 6B for incidence, FHBI, FDK and DON and increased disease on chromosomes 2B, 2D, 4A, 5B and 6A in most of the environments.

4.2 Introduction

Fusarium head blight (FHB), caused predominantly by *Fusarium graminearum* Schwabe (teleomorph *Gibberella zeae* (Schwein. Petch)) is a wheat disease that has generated considerable economic and health concerns worldwide due to yield and quality losses, and mycotoxin accumulation in infected grain (Bai and Shaner 2004). Breeding for FHB resistance is a common goal for wheat breeders. Molecular markers can be used to complement phenotyping and classical breeding to select for FHB major resistance QTL (Buerstmayr et al. 2009; Kang et al. 2011; Agostinelli et al. 2012). To provide a high level of FHB resistance in wheat, marker assisted selection (MAS) may be used to pyramid resistant alleles into an agronomically desirable background. Currently, breeders rely on phenotypic selection, MAS, or a combination of the two in development of FHB-resistant wheat cultivars. Agostinelli et al. (2012) proposed that an initial round of phenotypic selection at moderate selection intensity enriches the population with major QTL resistance alleles while maintaining variation at minor FHB resistance loci and for other traits. Genotyping can then be used to extract lines homozygous for resistance alleles at the major QTL.

The QTL analyses have identified loci for resistance to FHB index, FDK or DON, or their combination (Mesterházy 1995; Agnes et al. 2014; Szabó-Hevér et al. 2014). The components of FHB resistance have been postulated (Mesterházy et al. 2005). Type 1 resistance has been defined as resistance to initial infection and is thought to be primarily passive in nature. In contrast, Type 2 resistance reduces fungal spread from infected florets to other florets along the rachis and is considered to be an active form of resistance. Type 1 and Type 2 resistance were first described by Schroeder and Christensen (1963), and are widely accepted and extensively studied. Mesterházy (1995) described Type 3 resistance as reduction in kernel

infection; Type 4 as tolerance where yield and quality are maintained despite disease pressure; and Type 5 resistance as the ability of the host to degrade mycotoxins.

Host resistance to FHB is a quantitative trait, with more than 100 QTL for resistance identified throughout the wheat genome (Buerstmayr et al. 2009; Liu et al. 2009; Löffler et al. 2009). Type 2 resistance is measured through single or dual floret inoculation. As a result, the majority of QTL identified confer Type 2 resistance (Buerstmayr et al. 2009). Type 1 resistance is more difficult to assess because it is difficult to separate from Type 2 resistance in inoculated nurseries (Mesterházy et al. 2008).

Asian spring wheat varieties and landraces like Sumai 3, Nobeoka Bozu and Wangshuibai are the most widely investigated resistance sources. The 3BS QTL derived from Sumai-3, *Qfhs.ndsu-3BS* (Anderson et al. 2001), which was mapped as a single Mendelian gene termed *Fhb1* (Cuthbert et al. 2006), is the most important. Recently, the *Fhb1* from a Chinese wheat cultivar Sumai 3 was map-based cloned, which revealed that a pore-forming toxin-like (*PFT*) gene at *Fhb1* confers FHB resistance (Rawat et al. 2016). The pore-forming toxin-like gene is assumed to encode a chimeric lectin with two agglutinin domains and an ETX/MTX2 toxin domain. This study identified a new type of durable plant resistance gene conferring quantitative disease resistance to plants against *Fusarium* species. Mesterházy et al. (2008) described the non-specific nature of the 3BS and 5AS FHB QTL (*Fhb1, Qfhs.ifa*-5*A*) (Anderson et al. 2001; Buerstmayr et al. 2009) suggesting that the large-effect QTL confer protection against both Type 1 and Type 2 resistances when spray inoculation is used. Mesterházy (1995) reported that full resistance or immunity has not been found with many studies concentrating and reporting on Type 2 resistance which is thought to be incomplete. Other studies have shown that the progress based on studying the 3BS (*Fhb*1) QTL was much slower than expected. This is due

to the fact that Type 1 resistance determined by the 5A QTL is almost as strong as Type 2 *Fhb*1 QTL on 3BS from CM82036 (from Sumai 3) and their common effect secure the high resistance in Sumai 3 (Mesterházy et al. 2008). The *Fhb2* and *Fhb5* genes do not contain resistance as strong as Sumai 3. Transgressive segregation is where you get some phenotypes on the two extremes of a distribution that fall beyond their parental phenotypes. Liu and Wang (1991) analyzed transgressive segregation for FHB in wheat crosses and observed that a few lines were more resistant than the resistant parent. The FHB-resistant spring wheat cultivar Sumai 3 was developed in China by crossing two moderately susceptible parents, Funo and Taiwanxiaomai. This phenomenon could explain that FHB resistance was accomplished by transgressive segregation using an appropriate combination of alleles at different QTL (Suzuki et al. 2012; Schweiger et al. 2013).This suggests that, acting alone, these QTL determine only an intermediate-level of resistance. Other studies reported Type 1 resistance QTL identified on chromosomes 5A (Buerstmayr et al. 2002; Steiner et al. 2004), 4B (Liu et al. 2007) and 4A (Steed et al. 2005).

Validation of QTL is crucial in plant breeding, but Fusarium resistance QTL are not always reproducible in different populations. For example, studies involving Arina as a parent showed a high number of QTL in three different populations, but only the QTL for the *Rht-D1* gene co-localizing with a Fusarium QTL was common to all populations (Paillard et al. 2004; Draeger et al. 2007). This is due to the complexity of the genetic background for the small and intermediately strong QTL which exhibit many different unknown regulatory mechanisms. Szabo-Hever et al. (2012) demonstrated that the validation of molecular markers is necessary for breeders to confirm the presence of a locus responsible for an investigated trait. In FHB resistance QTL analysis, differentiation between QTL responsible for resistance and those of

other morphological traits is paramount to avoid the transfer of an undesired morphological trait with FHB resistance (e.g. pleiotropy or linkage drag) into elite cultivars (Kosová et al. 2009). However, phenotyping is a key issue in QTL analysis, where reduction of the ‘‘background noise’’ of morphology and other traits operating on disease development is very important. Many QTL have been mapped with limited use in MAS (Bernardo 2008) for development of FHB resistant cultivars. Marker-assisted selection may be used to enhance FHB resistance through selection of favorable alleles of significant QTL, while considering morphological traits like plant height. Many breeders face challenges in using QTL for MAS because many identified QTL are specific to the population and environment in which they were identified, making it difficult to apply them to a wide range of breeding material.

Development of FHB resistant cultivars plays a key role in integrated Fusarium control and the prevention of DON contamination. Large genetic variation for FHB resistance is available in the wheat gene pool including the wild relatives, but often the best regionally adapted and highly productive cultivars are susceptible to FHB. To establish the resistance in CDC Buteo, a large DH population consisting of 229 lines was developed by crossing DH99W22A*13/ CDC Buteo (hereby referred to as 2ABT population). The objectives of the study were:

(a) To study the inheritance of FHB in a double haploid population developed with CDC Buteo as the resistant parent

(b) To quantify the phenotypic variation for FHB resistance and map QTL associated with FHB resistance in a double haploid population using CDC Buteo as the resistant parent.

4.3 Materials and methods

4.3.1 Phenotyping Fusarium head blight field resistance

4.3.1.1 Mapping population development

A large double haploid (DH) wheat mapping population of the cross DH99W22A*13/ CDC Buteo (2ABT) consisting of 229 DH lines was developed at the University of Manitoba, Plant Science Department using the wheat x maize hybridization and embryo rescue technique (Laurie and Bennett 1988). CDC Buteo was considered to be moderately resistant, while DH99W22A*13 (abbreviated 22A*13) had an intermediate reaction to FHB. Genotype 22A*13 is a selected DH line from the cross CDC Kestrel/UM1174. CDC Kestrel was selected from the progeny of a cross Norstar*2/Vona made in 1979 (Fowler 1997) and UM1174 was a breeding line at the University of Manitoba from the cross GN567/Norstar. GN 567 is a winter wheat accession obtained from France with the designation VT2222. CDC Buteo was selected from the progeny of a cross S86-808/Abilene where S86-808 = Norstar*2/Vona (Welsh et al. 1978; Grant, 1980; Roberts, 1989). The DH population from DH99W22A*13/ CDC Buteo is identified in this study as 2ABT.

4.3.1.2 Phenotypic screening for Fusarium Head Blight Field Resistance

The FHB trials were carried out at the Ian N. Morrison Research Station, Carman and the Point Field Research Laboratory of the University of Manitoba Fort Garry Campus, Manitoba, in 2011, 2012, and 2013. Data for total precipitation and average monthly temperatures for May-August in 2011 -2013 cropping seasons at Carman were retrieved from Environment Canada Weather station Archives (Table 4.1). For Winnipeg, the weather data were obtained from the Forks Weather Station.

Parents, checks and 229 DH lines were screened for FHB in replicated field trials conducted in Carman and Winnipeg, Manitoba from 2011 to 2013. Single one-meter row plots were planted in a randomized complete block design with three replications for all tests. Days to heading and anthesis were recorded for each plot. The plots were inoculated and mist-irrigated as described in Chapter 3 section 3.4 for the 9HBT population. Four aggressive *F. graminearum* isolates from Manitoba consisting of two 3ADON and two 5ADON chemotypes were used for spray inoculations (1×10^5 macroconidia spores per ml) as described in section 3.4. In field experiments, data for FHB variables including disease incidence, disease severity, and Fusarium head blight index (FHB index) were collected following methods of (Cuthbert et al. 2007). The Fusarium damaged kernels (FDK) and deoxynivalenol (DON) accumulation were also determined. The DON concentrations were measured using competitive Enzyme Linked Immunosorbent Assay (ELISA) as described in section 3.4. Heading date, flowering time (date of 50 % anthesis), and FHB traits data were taken in all six field environments. Fusarium damaged kernels counts were done for all field seasons, but DON was measured only on 2012 and 2013 samples from Carman and Winnipeg using methods previously described for the 9HBT population (section 3.4).

Height data were collected from a separate field trial in Winnipeg in a 2010 rust nursery. Each plot was a single three-meter row plot. Plant height was measured in centimetres, and three measurements per plot were taken and then averaged to give the final reading.

4.3.1.3 Data analysis

The disease incidence, severity, FHB index and FDK data from all field trials were analyzed using the Proc GLM of SAS (SAS v9.3, SAS Institute Inc., Cary, NC, USA, 2009) with all effects in the model considered random. Each environment was analyzed separately. Further

analyses were done by combining all sites for each year, combining all years for each site, and combining all sites and years. The environments were combined as site-years rather than by site and by year. For the analyses of variance for each site, the model was incidence, severity, FHB index, FDK = Genotype replicate with all variables being random. For the analyses of variance for combined data, the model statement was incidence, severity, FHB index, FDK = siteyear replicate (siteyear) genotype siteyear*genotype with all factors being random. The error terms used for (a) siteyear was: MS (rep(siteyear) + MS(siteyear*genotype) - MS(residual error); (b) genotype: MS(siteyear*genotype) and (c) rep(siteyear) and siteyear*genotype: the error term used was: MS(residual error). The least square mean values for each DH line evaluated from each dataset were used for QTL analyses. Proc Univariate of SAS was used to test for normality of residual distribution. Homogeneity test using Levene test at P = 0.05 was conducted to determine if the data sets could be combined across site years rather than by site and by year. The decision to combine data over the years and sites was made by examining the results of the Proc univariate‘s plot of residual by predicted values for disease incidence, severity, FHB index, and FDK. Despite finding significant differences between the site years for most of the variables, the residual error deviations were homogenous along the range of predicted FHB trait value and were sufficiently normal to warrant combining the data for analyses (data not shown). The error deviations had similar variance and were normally distributed. To determine the associations between traits, correlation coefficients among FHB variables and other traits were calculated for each environment and combined site years using SAS Proc Corr (9.3). Deoxynivalenol analysis for the pooled data for four site years was done using Proc Mixed of SAS 9.3 using the model statement; DON= siteyear genotype.

4.3.2 Construction of linkage maps for 2ABT population

4.3.2.1 DNA extraction

Three seeds per DH line were seeded in the greenhouse with one plant per pot. Supplemental light was set for 16h daylight and 8h night. In the greenhouse, the temperature settings were 18-22 ^{o}C daytime and 14-18 ^{o}C at night time. Two weeks after transplanting, two to three pieces of young leaves (~5cm in length per piece) from one plant per DH line were collected and immersed in liquid nitrogen. The leaf tissue for each plant was lypholized in separate perforated envelopes for 48 hours and stored at -20 °C until DNA extraction was done. The DNA was extracted from a single plant of each DH line using Qiagen DNeasy 96 plant kit (Qiagen, Mississauga, Ontario). QuantiT PicoGreen dsDNA quantification reagent was used to quantify double stranded DNA samples and working DNA samples were adjusted to a concentration of 50 ng/ul.

4.3.3 Genotyping using the 90K wheat SNP iSelect genotyping assay

The 90K SNP wheat beadchip using Infinium platform from Illumina (Wang et al. 2014) was used to genotype the population. The assays were performed according to the manufacturer's protocols (Illumina) as described in 9HBT population (section 3.7.3).The genotyping assay identified SNP variation in this population.

4.3.4 Data analyses of SNPs

Single nucleotide polymorphism allele clustering and genotype calling for this population were performed with standard diploid Genome Studio (GS) software (Illumina) and the algorithm implemented in GS was used to identify assays that produced three distinct clusters corresponding to the AA, AB and BB genotypes expected for biallelic SNPs as described in 9HBT population (3.7.3). The AB genotypes cluster was as a result of diploid version of Genome

Studio being used in identification of assays from wheat which consists of three genomes (A, B and D).

The linkage groups were constructed using the MSTMap software (Wu et al. 2008) resulting in 66 linkage groups initially from 6,049 polymorphic markers. All redundant markers were removed before using Mapdisto and kept only one marker per bin (the most informative marker with the least missing data). Linkage between loci and the map distances were calculated using the Kosambi mapping function (Kosambi 1943).The resulting markers were used for mapping in MapDisto version 1.7.7 (Lorieux 2012) using Branch and Bound II method of ordering loci. Markers showing severe segregation distortion were removed from mapping populations before map construction. To test and verify Mendelian segregation of all markers, the segregation X^2 was used to obtain chi squared values which measure the deviation from 1:1segregation. Marker quality was estimated by calculating a percentage chi square value. This study used only SNP markers with chi square value with probabilities associated to F-test of ≥ 0.05 (ns), <0.05 and ≥ 0.01 (*) and <0.01 and ≥ 0.001 (**). Testing for Mendelian segregation of all sequences was done using chi test to remove highly skewed markers. Linkage groups were identified with a minimum LOD score of 4 and a maximum recombination fraction between markers of 0.3. To identify genotyping errors, the functions in MapDisto were used to identify double recombinants. Linkage groups were combined into chromosomes based on knowledge of previously mapped markers and then assigned to chromosomes (Wang et al. 2014).

4.3.5 Quantitative trait loci analyses

QGene software version 4.3.10 and multiple interval mapping (MIM) were used to perform QTL mapping on the DH population marker data and phenotypic data (from all the field trials) (Joehanes and Nelson 2008). The QTL scan walking speed was 1cM. The QTL analyses

were done separately for each environment (Carman, Winnipeg for 2011, 2012 and 2013). Further QTL analyses were done by combining all years for each site, combining all sites for each year, and combining all sites and years. However, only QTL for individual environments and pooled data for all environments and years are shown. The LOD score for claiming a significant QTL was determined based on a permutation test with 1000 runs for each QTL. The options included selecting cofactors with a maximum number of five cofactors using the method of stepwise cofactor selection at F to add (0.05) and F to drop (0.05). The QTL position, additive effect, and the percentage of phenotypic variation explained (R^2) were estimated, and the linkage maps were drawn using MapChart v. 2.2 (Voorrips 2002).

4.4 Results

4.4.1 Weather conditions in Carman and Winnipeg during the growth season

The cropping season mean temperatures and total precipitation for 2011-2013 in Carman and Manitoba are discussed in Chapter 3-section 3.8.1 (Table 3.1).

4.4.2 Field phenotypic and deoxynivalenol results

4.4.2.1 Inheritance of Fusarium head blight

This study evaluated multiple components of FHB resistance in wheat, including Type 1 and Type 2 resistance, resistance to kernel damage (Type 3) and resistance to DON accumulation (Type 4). The number of DH lines was reduced from 229 to 218 lines after genotyping data showed that 11 lines had SNP data of poor quality or had marker data with more than 10% missing values. The incidence, FHB index, FDK and DON were reported to be higher in Carman than Winnipeg, while severity was higher in Winnipeg (appendices 4.7 and 4.14). The highest FHB infection was reported in 2013 for combined data for Carman and Winnipeg in 2013 for all FHB traits and lowest in 2012 (appendix 4.11) although the temperatures were highest in 2012.

Additionally, severity for pooled data for Carman 2011, 2012 and 2013 cropping years was lower than that of Winnipeg for three site years (appendix 4.14). It was observed that within the three cropping years, the infection was highest in 2013 and lowest in 2012. Nevertheless, the different environmental conditions amongst years and sites caused differences in FHB traits means between the parental and the DH lines. Environmental conditions play an important role in determining the severity of FHB infection. However, the overall ranking of double haploid lines showing lower FHB symptoms and those that had higher FHB symptoms were consistent and remained in the same phenotypic classes, while the moderately susceptible lines shifted to various phenotypic classes, based on the environmental conditions in different site years.

Analysis of variance was done for all traits across the six inoculated field trials. The genotype effects for analyzed data for individual and combined data sets were all highly significant (Table 4. 1 and 4.3, and appendices 4.1-4.6). A greater percent of lines fell in the intermediate phenotypic class with most of lines being moderately resistant to FHB disease. The distribution of FHB traits (incidence, severity, FHB index and FDK) means were normally distributed with transgressive segregants observed for all traits measured (Figures. 4.1- 4.3). This suggested that both parental lines may carry resistance alleles that could combine to produce genotypes with increased levels of resistance.

The means of the parental lines, the DH lines and the population ranges for FHB traits across the pooled data for all environments are shown in Table 4.2. The population mean for all the FHB traits were lower than those of CDC Buteo except for FDK which was higher. Similar results were observed for mid-parent incidence, severity, FHB index, FDK and DON which were close to those for CDC Buteo at 52.3%, 46.4%, and 25.2% 10.7% and 22.4% respectively but higher than the population mean value for all traits. This is because the parents are fairly similar

in their reaction to FHB (Table 4.2 and Figure 4.3). About 93 out of 218 DH lines had 10- 20% FHB index for pooled data for all site years.

The small differences for FHB traits between the parental genotypes accounted for moderate genetic variation in the mapping population. The parents showed *Fusarium* infection symptoms which were very close, with the parents falling in the same phenotypic classes for severity and FHB index except in very few instances as shown in Fig 4.1-4.2 and appendices 4.8-4.11 especially DON. Overall, CDC Buteo had lower incidence and DON accumulation, but higher severity, FHB index and FDK than 22A*13. The DH lines showed the lowest FDK count in 2012 in both locations varying from 0.3-8.8 % with a mean of 2.4% with the highest count reported in 2013 varying from 7.3-35% with a mean of 18.1% (appendix 4.11). Varying results were obtained across sites for FDK in parental genotypes where 22A*13 was found to have lower FDK levels than CDC Buteo except in Winnipeg 2011,Carman 2012 and Winnipeg 2012 (appendix 4.7). In 2011, there was possible seed contamination for CDC Buteo, explaining the pattern observed for these results where the 22A*13 was more resistant than CDC Buteo compared to 2012 and 2013 for both locations. CDC Buteo was observed to have a lower incidence compared to 22A*13 (Fig. 4.1) except for combined data for the two sites in 2011 FHB nurseries (appendices 4.8) due to possible seed contamination for CDC Buteo in 2011. This confirms that the resistance from CDC Buteo may be due to lower disease incidence as observed earlier for 9HBT population.

Table 4.1 Analysis of variance for disease incidence, severity, Fusarium head blight index (FHB index) and Fusarium damaged kernel (FDK) for 2ABT DH lines for pooled data for Carman and Winnipeg 2011, 2012 and 2013 FHB nurseries (six site years)

Source of variation	Incidence (%)			Severity (%)		FHB index (%)		FDK (%)		
	DF	MS	Pr > F	MS	Pr > F	MS	Pr > F	DF	MS	Pr > F
Siteyear	5	11408	<.0001	10937	<.0001	29749	0.017	5	33166	<.0001
Rep(siteyear)	12	7015.1	<.0001	6369.9	<.0001	6764.9	<.0001	10	625.6	<.0001
Genotype	216	1213.5	<.0001	929.9	<.0001	816.6	<.0001	217	87.7	<.0001
Siteyear*Genotype	108	313.3	<.0001	215.3	<.0001	154.5	<.0001	108	29.3	<.0001
Error	258	233.8		155.0		102.9		215	22.1	

*FDK degrees of freedom are different from other traits measured because only two rows per genotype were harvested in 2011. The other row was used for hand count for incidence and severity

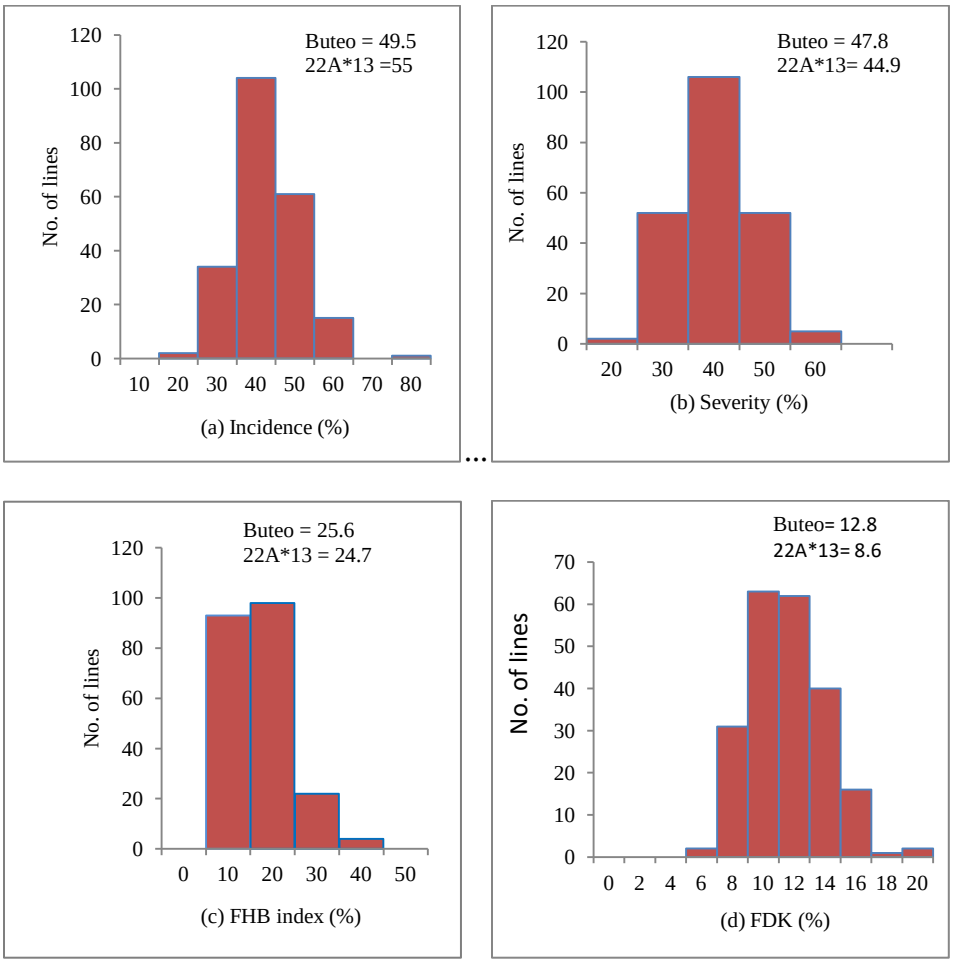


Figure 4. 1 Phenotypic distribution based on FHB infection of 218 DH derived from the cross 22A*13/CDC Buteo. Percent infection was measured from replicated field disease nurseries in 2011, 2012 and 2013. Traits included disease incidence (a), disease severity (b), Fusarium head

blight index (FHBI = DS x DI) (c), and Fusarium damaged kernels (FDK) (d). Mean values for the parents are include in the figure

Table 4.2 Mean and range for incidence, severity, Fusarium head blight index (FHB index) Fusarium damaged kernels (FDK) and deoxynivalenol (DON) for pooled data for Carman and Winnipeg for 2011, 2012 and 2013 FHB nurseries (six site years)

Trait	Parents		DH Population		
	Buteo	22A*13	Mean	Min	Max
Incidence	49.5	55	47.7	28.5	83.6
Severity	47.8	44.9	45.2	27.3	64.3
FHB index	25.6	24.7	22.2	10.1	45.8
FDK	12.8	8.63	10.5	5.6	19.7
DON[1]	21.7	23	19.9	9.7	41.9

[1]DON data was obtained from composite samples from three replicates across four site years (Carman and Winnipeg, 2011 and 2012)

4.4.3 Deoxynivalenol results for Carman and Winnipeg FHB nurseries in 2012 and 2013

Deoxynivalenol concentrations were analyzed from composite samples from three replicates in each individual year and pooled data for four years. The DON distributions for the population differed considerably between the different sites and years (Figure 4.2). The DON ranged from 0.1 -7.4 ppm in Winnipeg 2012 compared to 1.8 - 21 ppm in Carman in the same year. During the 2013 season, the DON ranged from 13 - 57.5 ppm in Winnipeg compared to 14.0- 85 ppm in Carman. There was higher DON accumulation in Carman than Winnipeg for both years DON was tested and there was more DON accumulation in 2013 than in 2012 in both sites. The low DON levels in 2012 may be due to different environments and/or lower precipitation in July in Winnipeg 2012 than Carman 2012 leading to unfavourable conditions for disease development although total precipitation was higher in Winnipeg than Carman for that season.

The analysis of variance for pooled data for DON showed that the site years and the DH lines were significantly different with the site years explaining the largest proportion of variation in DON accumulation (Table 4.3). The frequency distribution had broad and continuous

variation (Fig. 4.3) with trangresssive segregants. The DH lines varied in DON levels ranging from 9.7-41.9 ppm (Fig. 4.3). CDC Buteo had a lower mean DON accumulation compared to 22A*13 for the pooled data for four site years (Fig. 4.3). In general, there was more variation for DON at Carman than at Winnipeg for both 2012 and 2013. CDC Buteo had lower DON than 22A*13 in three individual environments except for Carman 2013 (Figure 4.2). Results for DON and FDK were contrasting amongst the parents with CDC Buteo generally having higher FDK count than 22A*13, but lower DON. However, these differences are relatively small.

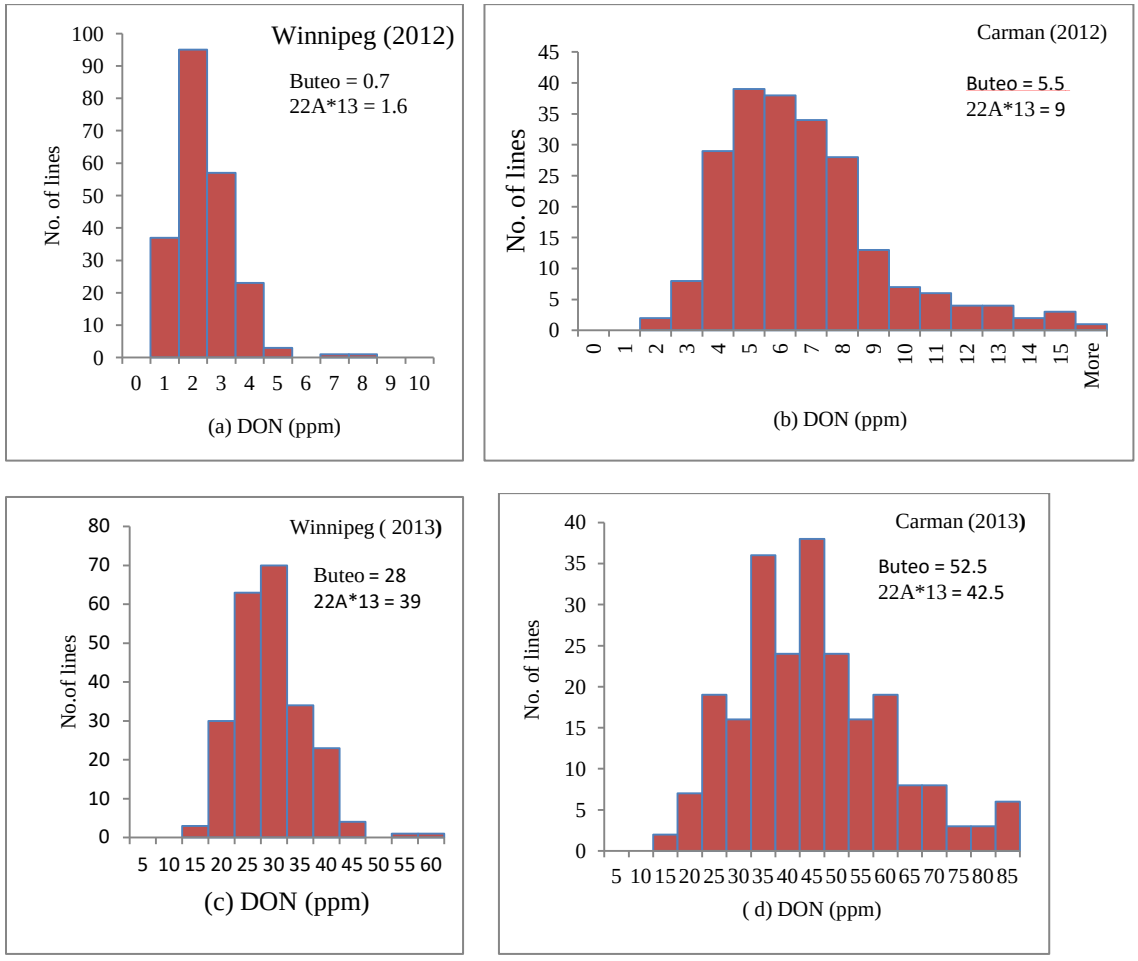


Figure 4.2 Phenotypic distribution based on DON accumulation of 218 DH lines derived from the cross 22 A*13/CDC Buteo. Deoxynivalenol (DON parts per million) was measured from composites samples from three replicates for trials conducted in 2012 and 2013 for both Carman and Winnipeg

Table 4.3 Analysis of variance for deoxynivalenol (DON) concentration (parts per million) for pooled data for Carman and Winnipeg for 2012 and 2013 FHB nurseries (four site years) for 218 DH lines derived from the cross 22 A*13/Buteo.

Source of variation	DF	Mean Square	P > F
Siteyear	3	83762.5	<.0001
Genotype	216	114.7	<.0001
Error	651	59.2	

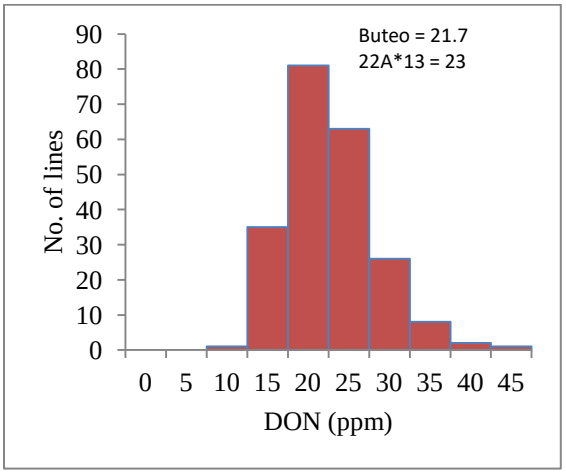


Figure 4.3 Frequency distribution for deoxynivalenol (DON) accumulation in parts per million for the pooled data for Winnipeg 2012, Carman 2012, Winnipeg 2013 and Carman 2013 for 218 DH lines derived from across of 22A*13/ CDC Buteo. The data were collected from Winnipeg and Carman FHB nurseries in 2012 and 2013 from pooled samples consisting of three replicates per genotype. The heights of bars represent the number of lines in the population in each phenotypic class

4.5.4 Correlations between FHB traits

The correlations for all FHB traits evaluated were all positive and highly significant (P=0.0001) ranging from r=0.62 to 0.91(Table 4.4). The highest relationship was between FHB index and severity (r =0.91) and the lowest was between incidence and FDK (r=0.62). The FHB index is a product of incidence x severity/100, thus there was high correlations between these three traits.

Table 4.4 Correlations coefficients between Fusarium head blight (FHB) disease incidence, severity, Fusarium head blight index (FHB index) and Fusarium damaged kernels (FDK) for pooled data for 2ABT population for Winnipeg and Carman FHB nurseries for three years (2011, 2012 and 2013, six site years)

Trait	Severity	FHB index	FDK
Incidence	0.65	0.88	0.62
Severity		0.91	0.63
FHB index			0.69

*All significant at p=0.0001
*The r value is the degree of relationship between the two traits
*N=218

4.5.5 Correlation coefficients between FHB traits measured and DON accumulation in 2012 and 2013

Correlations conducted between data collected in 2012 and 2013 revealed that there were significant correlations between all FHB traits, but no FHB trait was significantly correlated with plant height (Table 4.5). The correlations among FHB traits varied from r=0.55-0.91 with the best relationship being between incidence and FHB index (r=0.91). The lowest association was between severity and DON. The correlations for DON were higher between incidence and DON than between DON and severity, with the highest correlation for DON being between DON and FDK (r=0.73).

Table 4.5 Correlation coefficients between disease incidence, severity, Fusarium head blight index (FHB index), Fusarium damaged kernels (FDK) and deoxynivalenol (DON)for the cross 22A*13/CDC Buteo, after pooling data for Winnipeg and Carman FHB nurseries for 2012 and 2013 (four site years)

Trait	Severity	FHB index	FDK	DON	Height
Incidence	0.58***	0.91***	0.56***	0.60***	-0.04 ^{ns}
Severity		0.84***	0.57***	0.55***	-0.12^{ns}
FHB index			0.65***	0.66***	-0.09^{ns}
FDK				0.73***	-0.09^{ns}
DON					0.007^{ns}

*N (incidence, severity, FHB index, FDK and DON) = 218
*N (Height = one season data) = 155
*FDK were done on pooled samples across the replicates
*** Significant at P<0.001
*ns not significant

4.6 Quantitative trait loci mapping results

4.6.1 Genetic mapping results for 2ABT population

The total number of polymorphic markers detected was 6,049 with about 5069 assigned to chromosomes based on Wang et al. (2014) map (698 markers could not be assigned to any of the chromosomes). Of these assays, 9 markers were unlinked with any other markers. The markers were assigned to linkage bins based on their positions on chromosomes and the most informative marker (with least missing data) from each bin was used for QTL analyses. The 834 unique polymorphic marker loci for this population were assembled into 37 linkage groups which were used in QTL analyses in Qgene. The genetic map spanned a total of 1668.2 cM with an average marker interval of 2 cM with all chromosomes represented from the three genomes A, B and D. The majority of mapped markers were located in the A (45.3%) and B (44.6%) genomes with 10.1 % on the D genome (Table 4.6). The D genome had very few markers (511). The lowest number of markers was reported in D genome with chromosomes 2D, 4D, 5D, 6D and 7D having 22 - 59 markers. The A genome had a better distribution of markers except in chromosomes 1A and 5A with 133 and 69 markers respectively. Loci were more or less uniformly distributed along the chromosomes except for the following chromosomes: 2B (17 cM), 3A (16, 22 and 31 cM), 3B (19, 62 and 93 cM), 4A (26 cM), 6A (24 and 46 cM) and 6D (19 cM).

Table 4.6 Distribution of loci across the three genomes (A, B, and D) and the seven chromosome groups for the genetic map of 2ABT DH population consisting of 218 DH lines

Chromosome group	A	B	D
1	133	210	167
2	360	411	32
3	537	344	165
4	343	283	31
5	69	543	22
6	393	295	35
7	461	176	59
Total	2296	2262	511
Percent	45.3	44.6	10.1

**Total markers that were used for mapping in MapDisto was 5767 but 698 of them could not be assigned to any chromosome markers

4.6.2 The QTL analysis for 2ABT population for Carman and Winnipeg for individual and pooled data for FHB nurseries for 2011, 2012 and 2013 (individual environments and six site years)

The QTL analyses were conducted for individual site years and the means of pooled data for six site years (Table 4.7 and Appendix 4.18). Other QTL analyses were conducted by taking means across years or locations. The results from means across years or locations did not reveal additional QTL (data not shown). In total, QTL were detected on all chromosomes except 2A and 7D for all FHB traits measured (incidence, severity, FHB index, FDK and DON). The poor coverage of D genome could have affected the number of QTL detected in these chromosomes. The 22A*13 alleles reduced FHB disease on seven QTL on chromosomes 1B, 3A, 3B, 3D, 6B, 6D and 7A, but increased FHB symptoms on 12 QTL for the FHB traits investigated. The 6B QTL at pos 17-29 cM was the most common QTL reported for all of the evaluated FHB traits in most of the environments and in the pooled data for some environments (Table 4.7 and appendices 4.18). One QTL was identified on 6B at 1 cM for FDK (not significant), but all other QTL were between 16-29 cM. The 6B QTL was identified in three environments for incidence,

one environment for severity, three environments for FHB index, four environments for FDK and three out four environments for DON. It was also expressed in pooled data for incidence, FHB index FDK and DON. Two other 6B QTL at pos 52 cM were inconsistently reported for incidence and FHB index in one environment each.

Other QTL from 22A*13 alleles which reduced the FHB symptoms included those on chromosomes 1B, 3A, 3B, and 3D, 6D and one QTL on 7A for incidence. The 3B major QTL on pos 23 cM was identified in one, three, four, three and two environments for incidence, severity, and FHB index, FDK and DON, respectively. The 3B (pos 23 cM) QTL was also reported for all FHB traits for pooled data. However, for incidence a second 3B QTL was identified on position 12 cM in two environments. The 1B QTL was reported only in one environment each for FDK and severity. The 3D, 6D and 7A QTL were reported in one environment each for FDK, FHB index and incidence, respectively, and 3A in two environments for severity.

The 22A*13 alleles increased the FHB symptoms at some loci with the most important QTL detected on chromosome 2B at 67 cM, which was identified for all measured FHB traits except FDK and severity. This QTL was observed in five, three and two environments for incidence, FHB index and DON, respectively, and for pooled data for incidence and FHB index. The 2B QTL played a major role in increasing incidence and thus FHB index as it was identified in almost all environments where FHB incidence was measured. This is interesting since there is no QTL for plant height reported at this locus for the 2ABT population.

A QTL on chromosome 5B was identified in at least one environment for all FHB traits measured except severity. This QTL was commonly identified for incidence (three environments) and FDK (two environments) and the pooled data for the two traits, DON and FHB index. Two major QTL for incidence, severity and FHB index were reported on

chromosome 2D at 3 cM and 10 cM. The alleles from 22A*13 on both 2D QTL (10 cM and 3 cM) increased FHB infection with phenotypic variation ranging from 7.3-22% and additive effects of 2.1-6.1. The 2D QTL at 3 cM was identified for incidence, severity, FHB index in one, two and one environments, respectively. One QTL each were reported on 2D at 10 cM for severity and FHB index. There is a high possibility that these two QTL are the same.

Other QTL for FHB traits were identified on chromosome 4A for incidence, severity and FHB index in two, three, two environments, respectively, and for pooled data for incidence and FHB index. Additionally, the 3A QTL was reported in three positions, 13, 216 and 249 cM for DON, FHB index and incidence, respectively, and they were inconsistently reported in different environments. 22A*13 allele increased FHB symptoms on 3A at 216 cM for DON and incidence, (one QTL each). The other 3A QTL at13 cM and 249 cM were inconsistently identified and the 22A*13 allele reduced FHB disease. The 6A QTL was identified for incidence and for FHB index between 103 and128 cM and the 22A*13 alleles increased both traits. Other minor QTL were inconsistently reported on chromosomes 1A, 1D, 4B, and 4D.

The height QTL were reported on chromosomes 1A, 4D, 5D and 7A, explaining phenotypic variation of 6.4-8.4%. For the minor QTL for height detected on 5D, the 22A*13 alleles reduced the height by 1.02cm, while that on 7A increased the height by 1.01cm. The height measurements were taken only once in 2010 on a different set of trials.

Table 4.7 Quantitative trait loci for the cross 22A*13/CDC Buteo detected using multiple interval mapping (MIM) for deoxynivalenol (DON), Fusarium damaged kernels (FDK), Fusarium head blight (FHB index), height, incidence, and severity for individual environments and pooled data for six site years (Carman and/or Winnipeg for 2011, 2012 and 2013). The height data were from one season only.

DON (Four site years)

Trial data set	Chr. [a]	Marker at the QTL peak [b]	Pos (cM) [c]	Add [d]	PVE [e]	LOD [f]	LOD α=0.05 [g]
4Siteyrs	1A	BS00088035_51	63	-1.16	6.2	3.06	3.85
Winnipeg2013	1A	BS00065676_51	50	-1.68	6.3	3.07	3.44
Carman 2012	2B	Kukri_c25281_99	67	0.69	7.5	3.71	4.41
Winnipeg 2012	2B	wsnp_Ra_c3955_7262354	77	0.32	10.4	5.22	4.44
Carman 2012	3A	BS00064039_51	216	0.8	9.9	4.94	4.41
Carman2013	3A	Kukri_c51666_401	13	-5.25	10.9	5.48	4.14
4Siteyrs	3B	BS00071108_51	23	-1.71	11.9	6.01	3.85
Carman 2012	3B	BS00071108_51	22	-0.86	10.6	5.33	4.41
Winnipeg 2012	3B	BS00071108_51	22	-0.27	7.4	3.63	4.44
4Siteyrs	5B	wsnp_Ex_c5915_10378807	13	1.51	6.3	3.07	3.85
4Siteyrs	6B	wsnp_CAP12_c1388_706924	24	-2.25	15.3	7.85	3.85
Carman 2012	6B	Tdurum_contig42655_703	16	-0.68	6.0	2.95*	4.41
Carman2013	6B	Tdurum_contig10149_284	26	-5.52	14.1	7.19	4.14
Winnipeg2013	6B	RAC875_c20634_100	29	-1.9	7.2	3.56	3.44

FDK

Trial data set	Chr. [a]	Marker at the QTL peak [b]	Pos (cM) c	Add [d]	PVE [e]	LOD [f]	LOD α=0.05 [g]
Carman 2013	1A	BS00067652_51	61	-1.39	6.5	3.18	3.90
Winnipeg2012	1B	Excalibur_c11666_996	64	0.68	6.9	3.39	3.46
Carman 2013	1D	D_contig14507_369	13	2.23	6.5	3.19	3.90
6Siteyrs	3B	Excalibur_rep_c103408_632	23	-0.86	15.6	8.00	3.46

Trial data set	Chr. [a]	Marker at the QTL peak [b]	Pos (cM) c	Add [d]	PVE [e]	LOD [f]	LOD α=0.05 [g]
Carman 2012	3B	Excalibur_rep_c103408_632	22	-0.42	7.3	3.59	3.89
Winnipeg2012	3B	Excalibur_rep_c103408_632	22	-0.24	6.1	2.96*	3.46
Carman 2013	3B	Excalibur_rep_c103408_632	23	-2.22	15.1	7.74	3.90
Winnipeg 2011	3D	Tdurum_contig82605_187	27	-1.22	12.4	6.29	3.97
Winnipeg2012	4B	RAC875_c12495_1391	55	0.26	7.4	3.62	3.46
6Siteyrs	5B	wsnp_Ex_c5915_10378807	13	0.69	7.2	3.52	3.46
Carman 2012	5B	wsnp_Ex_c5915_10378807	13	0.51	7.4	3.64	3.89
Winnipeg2012	5B	RAC875_c103396_446	19	0.25	6.7	3.27	3.46
6Siteyrs	6A	Tdurum_contig4885_1870	46	-0.73	6.4	3.11	3.46
6Siteyrs	6B	Tdurum_contig10149_284	24	-1.15	20.4	10.79	3.46
Carman 2011	6B	Tdurum_contig10149_284	27	-1.88	22.2	11.87	3.69
Winnipeg 2011	6B	Tdurum_contig42655_703	17	-1.16	6.1	2.99*	3.97
Carman 2012	6B	CAP11_c1087_327	1	-0.45	6.7	3.29	3.89
Carman 2013	6B	Tdurum_contig10149_284	27	-1.91	11.3	5.67	3.90
Winnipeg2013	6B	Tdurum_contig10149_284	23	-1.72	9.4	4.68	3.71

FHB index

Trial data set	Chr. [a]	Marker at the QTL peak [b]	Pos (cM) c	Add [d]	PVE [e]	LOD [f]	LOD α=0.05 [g]
Carman 2013	1D	D_contig14507_369	12	2.03	7.5	3.68	3.78
6Siteyrs	2B	Kukri_c25281_99	67	2.01	10.9	5.47	3.72
Winnipeg 2011	2B	Kukri_c25281_99	67	4.15	14.9	7.61	3.94
Winnipeg2012	2B	Kukri_c25281_99	67	2.2	9.4	4.65	3.76
Winnipeg2013	2B	Kukri_c25281_99	67	2.42	8.7	4.33	3.70
6Siteyrs	2D	D_GB5Y7FA01EHPZX_186	2	2.09	11.1	5.56	3.72
Winnipeg2013	2D	Excalibur_c55975_231	10	2.19	7.3	3.59	3.70
Carman 2011	2D	D_GB5Y7FA01EHPZX_186	3	5.74	20.5	10.84	3.74
6Siteyrs	3B	Excalibur_rep_c103408_632	23	-2.24	13.1	6.64	3.72

Trial data set	Chr. [a]	Marker at the QTL peak [b]	Pos (cM) c	Add [d]	PVE [e]	LOD [f]	LOD α=0.05 [g]
Carman 2011	3B	Excalibur_rep_c103408_632	23	-2.8	6.5	3.17	3.74
Winnipeg 2011	3B	Excalibur_rep_c103408_632	23	-3.82	12.8	6.49	3.94
Carman 2012	3B	Excalibur_rep_c103408_632	22	-2.2	14.5	7.42	3.70
Winnipeg2012	3B	Excalibur_rep_c103408_632	21	-2.02	6.1	2.96*	3.76
6Siteyrs	4A	TA004912-0408	19	1.94	8.3	4.09	3.72
Carman 2011	4A	TA004912-0408	18	4.47	9.2	4.57	3.74
Winnipeg2013	4A	TA004912-0408	20	2.75	9.3	4.62	3.70
Carman 2012	5B	wsnp_Ex_c5915_10378807	13	1.87	7.7	3.81	3.70
6Siteyrs	6A	Kukri_c37301_385	116	1.77	9.6	4.78	3.72
Carman 2011	6A	GENE-3911_120	103	3.4	6.0	2.92*	3.74
Winnipeg 2011	6A	RFL_Contig3175_1250	128	2.49	5.9	2.90*	3.94
Winnipeg2012	6A	Kukri_c37301_385	116	1.81	6.5	3.20	3.76
Winnipeg2013	6A	GENE-3911_120	108	2.06	6.0	2.92	3.70
6Siteyrs	6B	Tdurum_contig10149_284	27	-1.57	7.3	3.60	3.72
Carman 2011	6B	Tdurum_contig10149_284	29	-3.2	7.6	3.77	3.74
Carman 2012	6B	Tdurum_contig10149_284	24	-1.57	6.7	3.28	3.70
Winnipeg2013	6B	wsnp_CAP12_c1388_706924	24	-2.21	5.9	2.90*	3.70
Carman 2013	6D	BS00021881_51	20	-1.98	6.9	3.37	3.78

Height

Trial data set	Chr. [a]	Marker at the QTL peak [b]	Pos (cM) c	Add [d]	PVE [e]	LOD [f]	LOD α=0.05 [g]
2010	1A	IAAV2694	78	0.89	6.4	3.15	3.96
2010	4D	Tdurum_contig12204_384	3	1.05	6.4	3.13	3.96
2010	5D	BS00023151_51	100	-1.02	8.4	4.13	3.96
2010	7A	wsnp_Ex_c40247_47349166	126	1.01	8.0	3.96	3.96

Incidence

Trial data set	Chr. [a]	Marker at the QTL peak [b]	Pos (cM) [c]	Add [d]	PVE [e]	LOD [f]	LOD α=0.05 [g]
6 site years	2B	Kukri_c25281_99	67	3.75	24.0	12.98	3.73
Winnipeg2011	2B	Kukri_c25281_99	67	7.73	19.4	10.23	3.81
Carman 2012	2B	Kukri_c25281_99	67	3.98	18.0	9.39	3.69
Winnipeg2012	2B	Kukri_c25281_99	67	3.29	11.5	5.79	3.60
Carman 2013	2B	Kukri_c25281_99	67	2.42	8.7	4.33	3.29
Winnipeg2013	2B	Kukri_c25281_99	67	2.72	9.8	4.86	3.61
Carman 2011	2D	D_GB5Y7FA01EHPZX_186	3	4.55	11.4	5.71	3.80
Carman 2013	2D	wsnp_Ex_c8303_14001708	10	2.19	7.3	3.59	3.29
Carman 2012	3A	Ex_c66865_584	217	2.42	8.0	3.94	3.69
6Siteyrs	3B	Excalibur_rep_c103408_632	23	-2.4	11.3	5.66	3.73
Winnipeg2011	3B	RAC875_c18399_175	12	-8.31	13.8	7.03	3.81
Carman 2012	3B	RAC875_c17373_848	12	-3.96	11.4	5.72	3.69
6Siteyrs	4A	IAAV2591	6	2.36	6.6	3.25	3.69
Winnipeg2011	4A	IAAV2591	1	5.73	7.3	3.60	3.81
Carman 2013	4A	TA004912-0408	20	2.75	9.3	4.62	3.29
Carman 2011	4B	Ex_c45493_761	79	3.75	7.9	3.90	3.80
6 site years	5A	-	41	1.83	7.2	3.55	3.73
6 site years	5B	wsnp_Ex_c5915_10378807	10	2.54	9.1	4.53	3.73
Winnipeg2011	5B	wsnp_Ex_c5915_10378807	12	4.71	8.2	4.03	3.81
Carman 2012	5B	Excalibur_rep_c103855_406	10	3.45	8.9	4.40	3.69
Winnipeg2013	5B	IACX6359	10	3.68	10.8	5.40	3.61
6Siteyrs	6A	Kukri_c37301_385	116	2.11	9.1	4.53	3.73
Winnipeg2011	6A	Ku_c12129_728	131	5.36	6.1	2.99*	3.81
Carman 2012	6A	GENE-3780_131	116	2.1	6.1	3.00	3.69
Winnipeg2012	6A	Kukri_c37301_385	116	2.38	6.4	3.13	3.60
Carman 2013	6A	GENE-3911_120	108	2.06	6.0	2.92*	3.29

Trial data set	Chr. [a]	Marker at the QTL peak [b]	Pos (cM) [c]	Add [d]	PVE [e]	LOD [f]	LOD α=0.05 [g]
Winnipeg2013	6A	Kukri_c11266_1036	125	2.62	6.1	2.98*	3.61
6Siteyrs	6B	Tdurum_contig10149_284	24	-2.24	7.3	3.58	3.73
6Siteyrs	6B	BS00067133_51	52	-1.86	7.5	3.68	3.73
Carman 2012	6B	Tdurum_contig10149_284	24	-2.64	6.2	3.01	3.69
Carman 2013	6B	Tdurum_contig10149_284	24	-2.21	5.9	2.90*	3.29
Winnipeg2013	6B	wsnp_CAP12_c1388_706924	24	-3.6	11.0	5.50	3. 61
Carman 2011	7A	wsnp_Ex_c40247_47349166	127	-3.96	9.6	4.78	3.80
6Siteyrs	7B	None	35	2.24	7.3	3.60	3.73

Severity

Trial data set	Chr. [a]	Marker at the QTL peak [b]	Pos (cM) [c]	Add [d]	PVE [e]	LOD [f]	LOD α=0.05 [g]
Carman 2013	1A	wsnp_Ku_c4271_7774388	25	-3.22	8.5	4.19	3.77
Winnipeg2011	1B	RAC875_c75971_295	51	-4.09	8.7	4.30	3.91
6siteyr	2D	D_GB5Y7FA01EHPZX_186	2	2.55	14.2	7.23	4.08
Carman 2011	2D	D_GB5Y7FA01EHPZX_186	3	6.31	22.0	11.78	2.52
Winnipeg2013	2D	Excalibur_c55975_231	11	3.23	12.6	6.40	3. 85
Carman 2013	3A	wsnp_Ex_c18883_27772081	249	-2.21	6.2	3.04	3.77
Winnipeg2013	3A	Kukri_c51666_401	13	-2.79	7.8	3.82	3.85
6Siteyrs	3B	Excalibur_rep_c103408_632	23	-2.62	15.4	7.93	4.08
Carman 2012	3B	Excalibur_rep_c103408_632	22	-2.88	12.6	6.39	3.64
Winnipeg2012	3B	Excalibur_rep_c103408_632	21	-2.28	6.7	3.27	3.42
Carman 2011	4A	TA004912-0408	17	4.57	8.8	4.38	2.52
Carman 2013	4A	TA004912-0408	21	2.11	6.7	3.26	3.77
Winnipeg2013	4A	TA004912-0408	18	3.07	7.5	3.69	3.85
6 Site year	6A	Kukri_c37301_385	116	1.72	7.4	3.65	4.08
Carman 2011	6B	Tdurum_contig10149_284	29	-3.3	7.4	3.62	2.52
Winnipeg2011	7B	BS00111363_51	6	4.53	6.4	3.15	3.91

[a] Chr., Chromosome
[b] Marker at the peak of the QTL
[c] Pos, Position on linkage group in cM
[d] Add ,Additive effect (Additive effect of allele substitution of the trait in question. A negative sign indicates that 22A*13 allele decreased the trait and vice versa)
[e] %PVE, Phenotypic variation explained (R^2; %)
[f] LOD, Peak LOD score
g LOD α=0.05, Significant LOD score threshold at 5%
* Detected QTL with LOD<3.00

4.7 Discussion

4.7.1 FHB inheritance discussion

Wheat breeders focused on breeding for Type 2 resistance in the past. Recently, they have incorporated other types of resistance including resistance to kernel damage and DON accumulation. This study evaluated multiple components of FHB resistance in wheat, including resistance to initial infection, resistance to kernel damage, and resistance to DON accumulation. Other sources of FHB resistance can supplement the existing ones, especially from elite sources which are adapted to the wheat growing areas in question. Extensive use of single genes like *Fhb1* from Sumai 3 and its derivatives may increase selection pressure on the pathogen, thus reducing the effectiveness of the resistance. In addition, they may display poor agronomic traits and low end-use quality (Gervais et al. 2003; McCartney et al. 2004)

The analysis of variance indicated that genotypic effects were significant for all environments and for combined data for Carman and Winnipeg for six site years. The performance of the DH lines varied across years under different disease pressures, but most of the lines showed better resistance compared to the susceptible checks except four lines which had more disease than one of the susceptible check, Caledonia (data not shown). Different locations and years of testing are necessary to identify resistant genotypes, and the conditions simulated in the nurseries are essential to increase the heritability of the resistance (Capettini et al. 2003). The majority of the lines fell in intermediate phenotypic classes. The means of all FHB traits were normally distributed with transgressive segregants observed for all traits measured. This suggested that both parental lines carried resistance alleles that could be combined to produce genotypes with increased levels of resistance (Somers et al. 2003; Semagn et al. 2007). These findings are in agreement with other studies, indicating that FHB resistance is controlled

by multiple genes (Jiang et al. 2007; Bonin and Kolb 2009). This demonstrates that transgressive segregation can be exploited as an alternative approach to develop good FHB resistant wheat cultivars. More recently, Emerson, the first registered FHB resistant winter wheat variety for western Canadian production was developed by selection of a trangressive segregant (Graf et al. 2013), verifying this hypothesis. This means that FHB resistance can be accomplished by exploiting transgressive segregation in crosses using contrasting alleles at FHB QTL (Suzuki et al. 2012; Schweiger et al. 2013).

About 93 out of 218 DH lines had 10- 20% FHB index for the pooled data for all site years in the 22A*13/CDC Buteo population. The small differences for FHB traits between parental genotypes accounted for moderate genetic variation in the mapping population. The FHB reaction of the two parents in this cross was very close such that the parents were in the same phenotypic class for severity and FHB index. The parents differed in disease incidence, FDK, and DON in some environments. The small difference in infection between the two parents could be due to great genetic similarity between the two parents where Norstar was a key contributor for alleles in both parents. Possible seed mixture for CDC Buteo, could explain the 2011 results where 22A*13 was more resistant than CDC Buteo compared to 2012 and 2013 for both locations. CDC Buteo was observed to have a lower incidence rate compared to 22A*13 for individual and pooled data for all environments except in 2011, thus confirming that the resistance from CDC Buteo may be due to lower disease incidence (Type 1 resistance). This is in agreement with observations in the 9HBT population (chapter 3).

22A*13 had more DON accumulation than CDC Buteo in all sites except Carman in 2013. Low infection levels recorded in 2012 were primarily due to dry conditions during

flowering (mid-June), making it not conducive for infection by the pathogen, even under mist-irrigated conditions. The relative differences between parents for DON varied across site years.

The correlation coefficients were moderate to high for all FHB traits and were highly significant at P<0.0001 ranging from r = 0.58-0.91. The highest correlations were between FHB index and incidence followed by FHB index and severity when data was pooled across site years. Correlations of DON concentration with various FHB disease measurements have been reported to range from -0.58 to 0.99 (Paul et al. 2005), indicating that DON accumulation is a complex and variable trait. Paul et al. (2005) analyzed 163 studies and found more than 65% of all correlation coefficients between FHB intensity and DON to be >0.50. Our results showed that the strongest relationship for DON was between FDK and DON (r = 0.73), followed by FHB index and DON (r = 0.66) when data was pooled across four site years in which DON analyses were done. In a study consisting of 269 RILs from a cross between IL94-1653/ Patton, DON concentration correlated best with the ISK index, a weighted index combining incidence, severity, and kernel damage measurements (Bonin and Kolb 2009). The correlation between DON concentration and FDK percentage was low in 2006 (r = 0.38) and moderate in 2007 (r = 0.59). Different results were observed in this study with association between FDK and DON being moderately high (r = 0.60-0.73) in both sites in 2012 and 2013. The best association was between incidence and FHB index (r = 0.91) and FHB index and severity (r = 0.84). Fusarium head blight index is a product of incidence and severity hence the high correlations.

4.7.2 Quantitative trait loci discussion for 22A*13/CDC Buteo population

Grain yield and quality can be significantly reduced by FHB with the most serious consequence being the contamination of grain with mycotoxins, including DON. Somers et al. (2003) showed that Type 1 and Type 2 resistance, and resistance to DON accumulation were

controlled by different loci in the same population. The majority of resistance QTL identified confer Type 2 resistance (Buerstmayr et al. 2009). As previously reported, the D genome is known to be conserved (Janda et al. 2004; Linkiewicz et al. 2004) and very few markers (511) were reported for all chromosomes in D genome (Table 4.6). Similar results were reported for the 9HBT population. Major QTL for the cross 19H*16/CDC Buteo cross (Chapter 3) were found on chromosomes 1A, 4A, 4B (*Rht-B1*), 4D (*Rht-D1*), 6B and 7A for FHB incidence, severity, FHB index, FDK and DON. Cuthbert et al. (2006) and Liu et al. (2008) qualitatively mapped a resistance QTL from Sumai 3 on chromosome 3BS, *Fhb1*, and chromosome 6BS, *Fhb2* (Cuthbert et al. 2007).The 6B QTL was the most significant and consistently expressed QTL controlling FHB in the 2ABT population. The 6B QTL for DON and FDK was also identified for the 9HBT population at 2 cM and in the 2ABT population between 16 and 29 cM. The two QTL at 16 and 29 cM (13 cM apart) may result from a single gene controlling both traits. The 6B QTL for the 9HBT population (0-2 cM), and 6B QTL for the 2ABT population (between 16 and 29 cM) were effective in controlling FDK and DON accumulation, even though they mapped to different positions. These two QTL are the same since they reduce disease symptoms for the same traits and the differences in position may be related to the length of the maps on this chromosome (Appendices 3.2.1). However, the 6B QTL for the 9HBT population was not identified for incidence compared to that on 2ABT. This could be explained by the fact that the *Rht-B1* (4B) and *Rht-D1* (4D) played a major role for incidence on 9HBT and could have masked the presence of this QTL. Based on the pedigree, there was greater genetic similarity between CDC Buteo and 22A*13 than between CDC Buteo and 19H*16. Another major QTL from the 22A*13/CDC Buteo cross was on chromosome 3B. Both QTL on 3B and 6B accounted for about 12-37.8 % of the total phenotypic variation explained for all traits that were measured.

These two QTL are stable and they could be used in marker assisted breeding. Comparative mapping revealed that the 6B QTL could be *Fhb2*. It is located on short arm of chromosome 6B at pos 24 cM while on (Cuthbert et al. 2007) it is located on chromosome 6BS flanked by GWM133 and GWM644. Relative comparison could not be done because for 2ABT population, SNP markers were used compared to microsatellite markers for *Fhb2.* However, SSRs and SNPs could be BLASTed to the wheat genome sequence to allow comparison of location of 6B QTL and *Fhb2*.

Gosman et al. (2008) reported QTL on 1B, 3A, 6A, 2B and 7A in a cross with spring wheat line RL4137 derived from Frontana. For the 2ABT population, a stable QTL on chromosome 2B was observed in five and four environments for incidence and FHB index, respectively, and for the pooled data. The 22A*13 alleles increased FHB infection for both QTL. This QTL was also identified for DON accumulation in two out of four environments. The 2B QTL was shown not to control FDK and severity in this population and this QTL is different from the one on 2B for 9HBT population which played a major role in increasing FDK and DON accumulation and 9HBT one is on short arm (pos 2 cM) of chromosome 2B while that of 2ABT is on the long arm (pos 67 cM). In a study of 90 RILs from a cross RL4137/Timgalen, FHB QTL of greatest effect on 2B (*Qfhs.jic-2b*) was reported, explaining up to 24% of the phenotypic variance. In the same study a DON QTL on 2B co-localized with that for the major FHB QTL for AUDPC (Area under disease progress curve) and plant height (Srinivasachary et al. 2008). Steiner et al. (2004) reported stable QTL for field resistance on 3A and 5A and less stable QTL on 2B and 6B in a Frontana/Remus cross. For Type 2 resistance, these researchers found only a single QTL of small effect on 2B. Most of the chromosome 2B regions are covered by QTL intervals contributed by Chinese, US and European winter wheat resistance sources (Buerstmayr

et al. 2009). In a study of 89 RILs from a cross Mironovskaja 808 /AC Ron, Ye (2015) identified QTL on 2B and 4D. The 2B QTL explained 17-24.8% of phenotypic variation. This QTL was associated with severity (Type 2 resistance) in the greenhouse, field incidence, field severity and FHB index in at least one of the three field trials.

Two FHB resistance (incidence, severity, FHB index) QTL (pos 3 and 10) mapped to a very small region of chromosome arm 2D. Given the distance between them, it was hard to distinguish if they are two distinct QTL, or not. In a similar study, McCartney et al. (2016) identified additive effect QTL for FHB resistance on chromosomes 2D, 4A, 4D, 5B, and 7D in a RIL population from spring wheat cross Kenyon/86ISMN2137. Kenyon alleles decreased FHB symptoms for QTL in 2D, 5B, and 7D, and increased visual rating index for QTL in 4A and 4D. McCartney et al. (2016) identified three FHB resistance QTL on 2D (*QFhb.crc-2D.1*, *QFhb.crc-2D.2*, and *QFhb.crc-2D.3*) which mapped to a relatively small region of chromosome arm 2DS with Kenyon contributing resistant alleles (*rht8* and *Ppd-D1b* -day length sensitive alleles) in the three loci. *QFhb.crc-2D.2* mapped 1.3cM distal of the expected location of *Rht8* (position 19.4cM) based on upon the position of *Xgwm261*. *QFhb.crc-2D.3* mapped approximately 2.1cM distal of *QAnth.crc-2D* (the location of *Ppd-D1*). According to McCartney et al. (2016) *QFhb.crc-2D.2* and *QFhb.crc-2D.3* may be as a result of pleiotropic effects of *Rht8* and *Ppd-D1* and may be independent of each other. *QFhb.crc-2D.1* mapped about 8.9 cM distal of *Xgwm261*, which is close to11 cM distal of *Rht8* suggesting that *QFhb.crc-2D.1* may be a distinct FHB resistance QTL from *QFhb.crc-2D.2*. FHB resistance QTL have been previously detected near *Rht8* (Somers et al. 2003; Handa et al. 2008; Löffler et al. 2009). The 2D QTL in which the 22A*13 alleles increased FHB disease in our study mapped about 3-10cM and was not identified for height and DON and may be similar to *QFhb.crc-2D.4* (marker wsnp_Ex_c8303_14001708

is common for 2ABT (10 cM) and Kenyon/86ISMN2137 (87.9 cM) maps. Based on this, the 2D QTL in 2ABT study may correspond to the *QFhb.crc-2D.4* reported in McCartney et al. (2016). The difference in map location may be related to the length of the maps on this chromosome due to differences in genetic backgrounds.

A consistent and stable QTL for incidence and FHB index on chromosome 6A was expressed in five environments with the 22A*13 allele increasing FHB disease. The 6A QTL was not reported in the 9HBT population. The 4A QTL located at pos 17-20 cM for FHB index, incidence and severity was stable and was also one of the major QTL identified in 4A for 9HBT population. Type 1 resistance QTL that have been identified include those on chromosome 5A (Buerstmayr et al. 2003); 4B (Lin et al. 2006; Xue et al. 2010) and 4A (Steed et al. 2005). The *T. macha* 4A resistance was shown to confer Type 1 resistance as it was clearly observed from spray inoculations .This resistance was mapped as a single gene to 4AS using a double haploid (DH) population, where it co-segregated with the SSR marker Gwm165 and was named QFhs.jic-4AS. However, the limited number of recombinants (43 lines) combined with a lack of polymorphic distal flanking markers prevented accurate localisation of the QTL (Steed et al. 2005).

A stable QTL in the 2ABT population was identified on chromosome 5B for incidence and FDK, but was inconsistent for severity and FHB index. Kenyon's allele decreased FHB symptoms for *QFhb.crc-5B*, in a recombinant inbred line population of the spring wheat cross Kenyon/86ISMN2137 (McCartney et al. 2016). The 5B QTL in our study is in a different map location as a FHB resistance QTL detected in Kenyon, but may be in the same region as the 5B QTL identified from a RIL population derived from varieties Neixiang 188/Yanzhan 1 (Lv et al. 2014), which was reported at marker interval *Xwmc235–Xwmc28* at pos18.3 cM.

The 22A*13 alleles decreased FHB on chromosomes 1A, 3A pos 13 cM, 3B, 6B and 6D and the CDC Buteo alleles on chromosomes 2B, 2D, 4A and 5B also reduced FHB infection. CDC Buteo was shown to contain dwarfing alleles *Rht-B1b /Rht-D1a* when it was genotyped using two SNP markers for this gene in 9HBT population. The major 4B (*Rht-B1b*) and 4D (*Rht-D1a*) did not segregate in the 2ABT population since the 2ABT parents did not differ at these loci. There is a possibility of high genetic similarity between CDC Buteo and 22A*13 since Norstar cultivar (very tall) is a parent for both genotypes. The majority of wheat cultivars grown in Europe are short and carry the dwarfing alleles *Rht-D1b* or *Rht-B1b* (Gosman et al. 2007).

In conclusion, about 93 out of 218 DH lines had less than 20% FHB index for pooled data for all site years. The 22A*13 alleles reduced FHB disease on seven QTL in chromosomes 1B, 3A, 3B, 3D, 6B, 6D and 7A, but increased FHB symptoms on 12 QTL for the FHB traits investigated. The alleles in CDC Buteo reduced FHB symptoms on chromosomes 2B, 2D, 4A 5B and 6A. The 6B QTL at pos 17-29 cM was the most significant QTL reported for all of the evaluated FHB traits in most of the environments and in the pooled data for some environments. The 6B QTL for 2ABT population was very effective in controlling FDK and DON accumulation. Type 1 (resistance to initial infection), Type 3 (resistance to DON accumulation) and Type 4 (resistance to kernel infection) resistance types contributed to overall FHB resistance in the 2ABT population. Other common QTL detected included those on chromosomes 2B, 2D, 3B, 4A, 6A and 5B. CDC Buteo and 22A*13 showed *Fusarium* infection symptoms which were very close, such that the parents were in the same phenotypic class for severity and FHB index, but differed in disease incidence, FDK, and DON in some environments. Transgressive segregation demonstrated that more highly resistance genotypes could be selected from the population.

CHAPTER 5

MAPPING OF QUANTITATIVE TRAIT LOCI FOR ANTHER RETENTION AND THE ASSOCIATION BETWEEN ANTHER EXTRUSION/RETENTION, ANTHER SIZE AND FUSARIUM HEAD BLIGHT (FHB) RESISTANCE

Abstract

Fusarium head blight (FHB) is a destructive disease of wheat worldwide. Anther retention (AR) has a high correlation with FHB susceptibility. Two studies characterized AR and its effect on FHB resistance in a double haploid (DH) population from a cross DH99W19H*16 /CDC Buteo (9HBT). The objective was to map QTL controlling AR in 100 DH lines and determine anther extrusion/retention and its association with anther length (AL), plant height (PH) and FHB resistance. Anther extrusion (AE) refers to the process where anthers are pushed out of the floret during flowering, while anther retention (AR) means the anthers are retained in the floret. The DH population was evaluated for FHB resistance under field conditions for six site years in Carman and Winnipeg. Phenotyping for AR, AE and AL was done in growth rooms. The AR was evaluated by opening florets and determining the number of anthers retained. The first study results demonstrated that AR was negatively correlated with AE, AL, PH and FHB resistance and may be used as an indirect selection target for enhancing FHB resistance. Some DH lines had anthers retained within the floret or trapped between lemma and palea. Mapping of AR was done on 100 randomly selected genotypes from 228 DH lines from 9HBT. Genotyping was done using 90K SNP beadchip. The second study results revealed that AR was negatively correlated to FHB resistance with two major QTL mapping on exact positions of dwarfing genes *Rht-B1* and *Rht-D1* on chromosome 4B and 4D, respectively. Other QTL were detected on chromosomes 1B, 3D and 4A. The DH99W19H*16 alleles on 4B and 3D chromosomes reduced

the AR but increased AR on chromosomes 4D and 4A. All AR QTL co-localized with FHB QTL for 9HBT population (N= 228). CDC Buteo contains allele combination *Rht-B1b/Rht-D1a* while DH99W19H*16 contained *Rht-B1a/Rht-D1b.* The double wild genotypes, *Rht-B1a/Rht-D1a* were the tallest with lowest AR and low FHB disease while double mutant *Rht-B1b/Rht-D1b* were the shortest with the highest AR and highest FHB infection.

5.1 Introduction

5.1.1 Fusarium head blight

Fusarium head blight (FHB) is one of the most economically important diseases of cereals in humid and semi-humid areas causing severe epidemics on wheat (*Triticum aestivum* L.) worldwide. *Fusarium graminearum* Schwabe (teleomorph *Gibberela zeae* (Schwein and Petch) is the primary causal pathogen of FHB in Eastern Canada, Saskatchewan, Manitoba, the United States (Tekauz et al. 2000; Goswami and Kistler 2005) and many other countries (Bai and Shaner 1994; McMullen et al. 1997).Yield and test weight reduction, contamination with the mycotoxin deoxynivalenol (DON), and additional costs on seed cleaning have caused large economic losses for farmers and the industry (McMullen et al. 2012). The plant hosts of FHB include bread wheat (*Triticum aestivum* L.), durum wheat (*Triticum durum* Desf.), barley (*Hordeum vulgare* L.), oats (*Avena sativa* L.) as well as many grasses. Resistance to FHB is subject to several resistance mechanisms that may not be genetically linked (Miedaner 1997) and are modulated by polygenes and environmental factors (Buerstmayr et al. 2009; Liu et al. 2009; Löffler et al. 2009). FHB epidemics are monocyclic since spike infection generally takes place during anthesis and early stages of kernel development (Leonard and Bushnell 2003; Audenaert et al. 2009). The monocyclic nature of FHB is thought to limit the infection to the primary inoculum released during the growing season (Wegulo 2012).

Fusarium head blight resistance can either be active or passive resistance (Mesterhazy 1995). Apart from active physiological resistance, plant developmental and morphological characters, especially plant height, flowering time, spike morphology and environmental conditions modulate disease development (Buerstmayr et al. 2009). Passive resistance is modulated by morphological factors within the plant leading to avoidance of the disease.

Avoidance is conditioned by morphological and developmental characters like AE (anther extrusion) during flowering and taller plant height. Anther extrusion is the process where anthers are pushed out of the floret during flowering, while anther retention (AR) means the anthers are retained within the floret. It has been discovered that in wheat, good extrusion of anthers is associated with high resistance to FHB (Skinnes et al. 2008; Buerstmayr and Buerstmayr 2015; Buerstmayr and Buerstmayr 2016). Only a few studies on FHB resistance have included AR as a morphological trait (Skinnes et al. 2010; Lu et al. 2013; Buerstmayr and Buerstmayr 2015; Buerstmayr and Buerstmayr 2016). The correlation between increased susceptibility in the presence of retained anthers was reported early (Dickson et al. 1921) and have been confirmed in several studies (Graham and Browne 2009; Skinnes et al. 2010; Kubo et al. 2013; Lu et al. 2013; Buerstmayr and Buerstmayr 2015; Buerstmayr and Buerstmayr 2016; He et al. 2016). Anther retention plays a major role in FHB resistance because the retained anthers have been shown to increase FHB susceptibility in various studies (Skinnes et al. 2010; Buerstmayr and Buerstmayr 2015; Buerstmayr and Buerstmayr 2016). The results of a study conducted by Graham and Browne (2009) concluded that selection for AE among European wheat could improve FHB resistance, without negatively impacting agronomic traits. It was shown that high AE led to low infection rate, contributing to Type 1 resistance (Skinnes et al. 2010). The FHB reactions were negatively correlated with both AE and plant height after spray and spawn inoculation as reported by Kubo et al. (2013) and Lu et al. (2013).

Skinnes et al. (2010) suggested that anthers retained and trapped between glumes provide a substrate for saprophytes like *Fusarium* and that subsequent infection of living tissues can occur under conducive conditions. Buerstmayr et al. (2014) speculated that anthers are an easy to conquer nutritious tissue for Fusarium that stimulate fungal development and thus give

Fusarium an advantage for penetrating the floret. This corresponds with microscopic observations showing that when anthers were retained in the florets, the hyphal density on anthers was higher than that on the inner surfaces of glumes (Kang and Buchenauer 2000). Also Graham and Browne (2009) hypothesized that anther morphology might be very closely related to disease incidence (Type 1 resistance) and early stages of FHB development. They also suggested that with low AE, anthers trapped between lemma and palea provided dead tissue readily colonized by Fusarium. Anther extrusion is a passive resistance factor which is primarily relevant for Type 1 resistance of wheat (Skinnes et al. 2010; Buerstmayr and Buerstmayr 2015) and selection of lines with a high degree of anther extrusion should result in a correlated selection response towards increased FHB resistance. Other morphological factors leading to avoidance of FHB include cuticular wax on the spike which may decrease water availability to the pathogen and constrain fungal germination and penetration. Plant height, and thickness and strength of a plant stem may indirectly affect resistance to FHB, because the stubble-borne spores can easily reach the spikes of short or lodged plants.

Three possible mechanisms have been proposed for the association between plant height and FHB susceptibility namely disease escape, pleiotropy of reduced height (*Rht*) genes and tight linkage (Buerstmayr et al. 2009). Dwarfing genes *Rht-B1b and Rht-D1b* (formally *Rht1* and *Rht2*, respectively) were derived from the Japanese cultivar 'Norin 10' and contributed greatly to the Green Revolution (Borlaug 1968; Hedden 2003). Dwarfing genes are classified according to their sensitivity to externally applied gibberellins (GA). Genes *Rht-B1* and *Rht-D1* are GA-insensitive genes, while *Rht8* is a GA-sensitive gene. The alleles conferring the dwarf phenotype are *Rht-B1b* and *Rht-D1b* and the wild-type alleles are *Rht-B1a* and *Rht-D1a*. Variation in European winter wheat cultivars plant height is mainly controlled by *Rht-B1b* and *Rht-D1b*

(Würschum et al. 2015). More than 90% of modern U.S. wheat cultivars possess either *Rht-B1b* or *Rht-D1b*. Among U.S. soft winter wheat cultivars, *Rht-D1b* is more frequent, but in hard winter wheat cultivars, *Rht-B1b* is more frequent (Guedira et al. 2010). Elongation of the anther filament in Arabidopsis is stimulated by GA and repressed by DELLA proteins which are orthologous to wheat *Rht*-1gene products (Cheng et al. 2004). The GA insensitive mutants *Rht-B1b* and *Rht-D1b* in wheat have similar function in repressing anther elongation through over-expression of DELLA proteins, resulting in the phenotype of anther retention or low AE. The pleiotropic effects of *Rht-B1b* and *Rht-D1b* on Type I FHB susceptibility may be due to the presence of two dwarfing genes leading to low AE causing increased Type I FHB susceptibility.

The loci of semi-dwarfing genes *Rht-B1* and *Rht-D1* are associated with plant height, FHB reaction, and AR. In a RIL population from cross Hermann (*Rht-B1b, Rht-D1a*) / Skalmeje (*Rht-B1a, Rht-D1b*) (H/S) and a backcross population 20812 (*Rht-B1a, Rht-D1a*- recurrent parent) /winter wheat Hermann (*Rht-B1b, Rht-D1a*) or Toras (*Rht-B1a, Rht-D1b*) (H/T×20812), H/Tx20814 , the ranking of *Rht* sub-groups was constant in all experiments, with *Rht-B1a/Rht-D1a* < *Rht-B1b/Rht-D1a* < *Rht-B1a/Rht-D1b* < *Rht-B1b/Rht-D1b* for FHB disease severity and %AR (Buerstmayr and Buerstmayr 2016). The double wild-type genotypes *Rht-B1a* and *Rht-D1a* had the lowest FHB severity and the lowest number of retained anthers, while double mutant genotypes *Rht-B1b* and *Rht-D1b* had the highest degree of AR and high FHB severity. The mutant allele *Rht-D1b* had a higher FHB level than the mutant allele *Rht-B1b* in H/T×20812. The *Rht-D1b* had a stronger impact on %AR than *Rht-B1b* with double dwarfs having the highest number of retained anthers (Buerstmayr and Buerstmayr 2016). The observed difference on FHB severity between *Rht-B1b and Rht-D1b* may be partially associated with differences in the number of retained anthers within the florets.

In two double haploid population studies from the cross TRAP#1/BOW//Taigu derivative /Ocoroni F86 (TO, N=135), and Ivan/Soru#2/OcoroniF86 (IO, N=92), the results indicated that *Rht-B1b* and *Rht-D1b* collectively accounted for 0-41% of FHB infection and 13-23% of reduced AE (He et al. 2016). These results demonstrated the effects of *Rht-B1b* and *Rht-D1b* on Type 1 FHB infection and AE reduction. Also He et al. (2016) proposed that the impacts of *Rht-B1b* and *Rht-D1b* on Type 1 FHB susceptibility may partly be explained by their effects on reducing AE. The QTL for AE in TO and IO populations was reported on *Rht-B1*, *Rht-D1* locus and on chromosome 2AL, all associated with FHB resistance. A QTL on 2BL was detected in the TO population and on 2DS and 3BL in the IO population. The two dwarfing genes explained around 20% of AE reduction in both populations and *Rht-B1b* was always more strongly associated with reduced AE than *Rht-D1b* (He et al. 2016). These results were different from (Buerstmayr and Buerstmayr 2016) who reported that *Rht-D1b* had a stronger impact on AR% than *Rht-B1b*. The *Rht-B1*and *Rht-D1* accounted for close to 60% variation in the two populations for plant height, while additional QTL were found on 5AL (*Vrn-A1*) and 7B in the TO population and on 5BS in the IO population (He et al. 2016).

In a different study by Buerstmayr and Buerstmayr (2015) with parents lacking dwarfing genes, QTL on 4AL and 6BL of the three identified QTL for AR (4AL, 5AL and 6BL) overlapped with QTL for FHB severity. Additionally, Lu et al. (2013) reported five QTL from SHA3/CBRD × Naxos RIL population on chromosomes 2DL, 3DL, 4BS, 5BL and 7AL for AE and all of them coincided with QTL for FHB traits. Results of these studies indicate that most of the variability in AR and plant height appeared to be controlled by independent factors. To better understand the relationship between AR, anther size and FHB reaction in the cross

DHWW19H*16/CDC Buteo (abbreviated 9HBT population), several experiments were carried out. The DHWW19H*16 is abbreviated 19H*16 in this chapter.

The objectives of this study were to:

a) Determine the effect of anther retention on FHB reaction on 100 double haploid lines derived from a cross DHWW19H*16/CDC Buteo
b) To determine the anther size and its association with anther retention/extrusion and FHB reaction
c) To map quantitative trait loci (QTL) controlling anther retention on 100 double haploid (DH) lines derived from a cross 19H*16/CDC Buteo

5.2 Methodology

5.2.1 Experiment 1: Evaluation of anther retention/extrusion on ten DH lines and its relationship to anther size and FHB resistance

A preliminary study was conducted in February 2014 and July 2014 to explore the potential role of anther retention in FHB reaction. Ten genotypes (five resistant/moderately resistant and five susceptible lines) were selected based on combined data for FHB reaction after FHB screening for three consecutive years in Carman and Winnipeg in 2011, 2012 and 2013 field seasons (Chapter 3).The genotypes were selected from a DH population (n= 228) developed from the cross DH99W19H*16 /CDC Buteo.

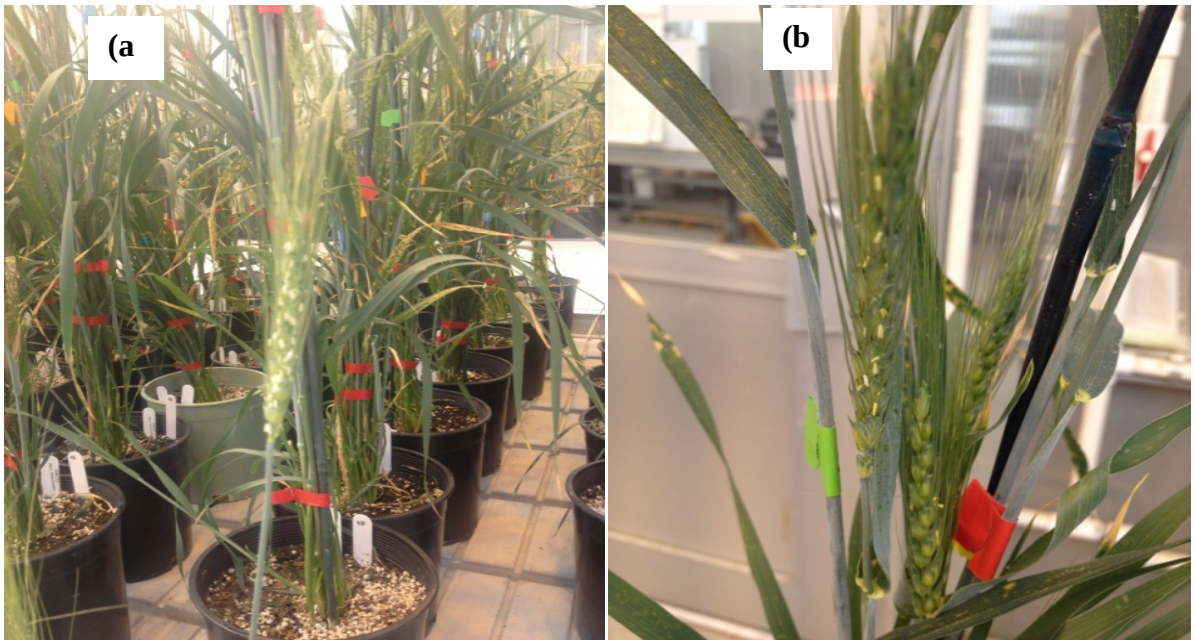
For the February 2014 trial, the parents, one resistant (FHB 148) and one susceptible (Hanover) check and 10 DH lines were included in the trial. The plants were seeded in root trainers and grown in the greenhouse until the two-leaf stage. They were then put into vernalization at 4 °C for 10-12 weeks after which they were placed in a greenhouse for one week at 14-16°C before transplanting to acclimatize them to avoid loss of vernalization. They were

then transplanted in February 2014 into one seedling per 20 inch pot at the Crop Technology Centre greenhouse (University of Manitoba) using a complete random design comprising of six replicates per genotype. They were placed on a greenhouse bench and supplemental light was set for16h daylight with temperature range from 18 to 25 °C. One month after transplanting, heading and flowering notes were taken from the first three spikes of each plant. Three primary spikes were tagged after heading and AE notes were taken as soon as flowering commenced. The AE notes were taken on a daily basis as flowering progressed in a spike until full anthesis was attained, or when no more florets flowered. The data were collected by counting the number of total florets and counting the number of flowering florets on a daily basis around noon. The percentage total of flowered florets was calculated by taking the number of flowered florets on a particular day divided by the total number of florets within that spike. This constituted the data for degree of anther extrusion, a test which can be used to measure degree of anther retention.

On the same spikes where AE data was taken (February 2014 trial), 7-10 days after flowering, the numbers of retained anthers were determined by randomly taking four primary florets (near the middle of the spike where flowering starts) within a spike and opening them up and determining the number of anthers retained in each floret. The anther retention (AR) data were taken on four florets and on three spikes per plant for six plants within a genotype (adopted with modifications from (Graham and Browne 2009) and Skinnes et al. 2010). Based on this trial it was established that AR gave more accurate and reliable data than AE. As a result, the AR protocol was adopted for the second anther retention trial (This procedure was modified according to Buerstmayr et al (2014).

The second trial was carried out in July 2014, at the Point growth rooms (University of Manitoba) using the same ten genotypes. The trial was laid out as a complete randomized design

with six plants per genotype (six spikes per plant and 10 florets from both sides of the spike near the middle were sampled with the protocol adopted from Buerstmayr et al, 2014 with modifications). Six primary spikes were tagged soon after the start of anthesis and four to six days after flowering, 10 primary florets were randomly picked (near the middle of the spike where flowering starts) and the floret was opened up and the number of retained anthers per floret recorded. Therefore, anther retention data were collected on ten florets per spike for six plants within a genotype.



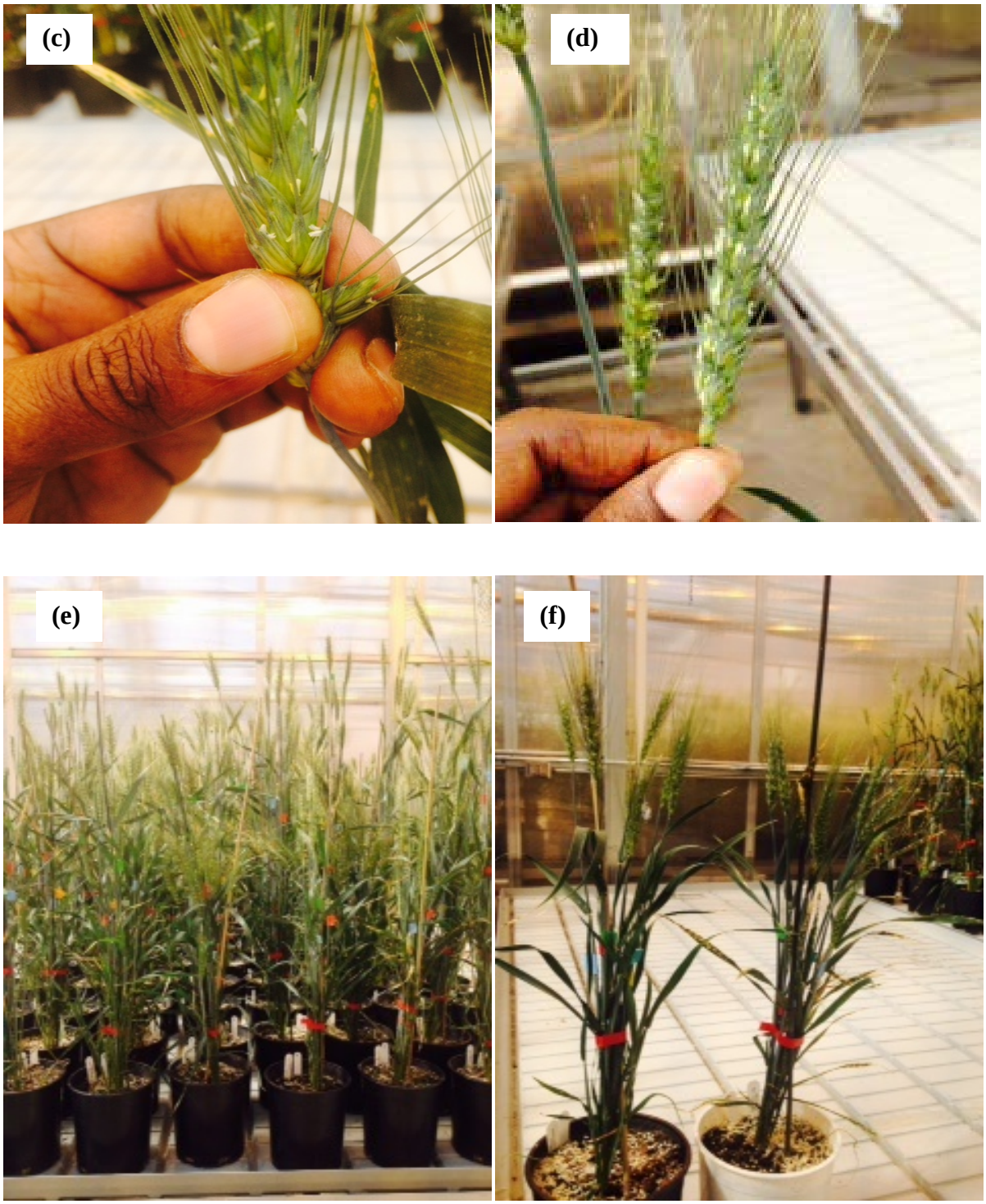


Figure 5.1 Anther extrusion and plant height variation amongst double haploid lines from the cross 19H*16/CDC Buteo (a) shows high anther extrusion b) low anther extrusion (c) and (d) anthers trapped between lemma and palea. (e) and (f) shows contrasting genotypes; tall vs semi-dwarf plants from different DH lines from this cross

5.2.2 Experiment 2: Effect of anther length on anther retention and FHB resistance on ten DH lines

The same DH lines, parents and checks used for the AR trial were also used to determine anther size and its relationship with AR and FHB reaction in a different experiment carried out in 2014 at the Point growth room. The plants were seeded in root trainers and grown in the greenhouse until the two leaf stage. They were then put into vernalization at 4 °C for 10-12 weeks after which they were placed in a greenhouse for one week at 14-16°C before transplanting to acclimatize them to avoid loss of vernalization. They were then transplanted in July 2014 into one seedling per 20 inch pot at the Point growth room (University of Manitoba) using a complete randomized design comprising of two replicates per genotype. Two plants per entry were transplanted separately and five spikes were selected for anther measurement. One month after transplanting and heading notes were taken from the first five spikes per plant for the anther retention trial. Giles and Bengtsson (1988) reported that there were differences in length among anthers at different stages of development, among anthers from different florets on the same spike and among anthers from the same floret. The following method was therefore used to minimize the random variation. Only non-dehisced anthers were collected from the primary and secondary florets near the middle of the spike from 3-7 spikelets (counting from approximately the middle of the spike where flowering starts) from both sides of the spike. The plants were monitored for anthesis twice a day to avoid anthers extruding from the florets.

Soon after flowering, when at least one floret had extruded anthers, the spikes were harvested either in the morning between 9:00-11:00 am or in the evening at 4:00-5:00 pm daily depending on when at least one floret had extruded anthers. Immediately after harvest, 18-21 anthers from each primary or secondary floret were emasculated from the centre of the spike

and fixed using Carnoy's solution 1(Farmers solution 1) consisting of acetic acid to 95% alcohol in a ratio of 1:3.The anthers were fixed for at least 1-4 hrs after which they were stored at 4 $^{\circ}$C for anther size measurements to be taken on a later date. Some of the lines did not readily extrude anthers, so at the time of flowering, the florets were opened to check for anthesis. The anther length was determined by calibrating a dissecting Zeiss microscope using a 1mm micrometer (known length) mounted in a 10X objective on a stereomicroscope. The anther images were captured using an Infinity 2 Scientific digital Camera and Infinity Camera Software (Lumenera infinity Analyze, Release 6.1.0) and the images were saved as Jpeg images. Representative random samples of 11 anthers were taken from each spike and a computer program ImageJ (([NIH image J software (U.S. NIH, Bethesda, MD] http://imagej.nih.gov/ij/) was used to process them by measuring the size of each anther twice and then averaging the length. The accuracy obtained was 0.001 mm by use of a micrometer mounted on a stereomicroscope.

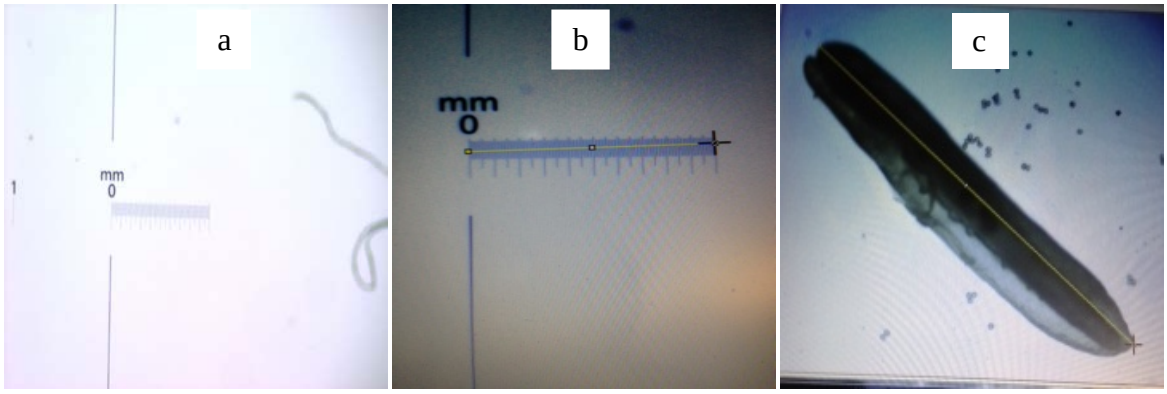


Figure 5. 2 A photo of a micrometer (a) and how it was used to calibrate and measure (b) the anther size (c) using a stereomicroscope attached to a camera. Calibration of a dissecting Zeiss microscope using a 1mm micrometer (known length) mounted in a 10X objective on a stereomicroscope for all anthers measured was done.

5.2.3 Experiment 3: Quantitative trait loci mapping for anther retention and relationship between anther retention and FHB resistance in 100 DH lines from the 9HBT population

A sample of 100 DH lines from the cross 19H*16 /CDC Buteo were randomly selected and used in the mapping study of anther retention. Six checks, namely Freedom and Caledonia (susceptible), 43I*18 and Freedom (intermediate) and FHB148 and DH00W32C*17 (resistant) and the parents were also included. Two different trials were carried out consisting of two plants per genotype, five spikes/genotype and eight florets per spike. Due to the labor intensive process of tagging and taking AR data, the two plants (two replicates trial with five spikes /genotype) were transplanted at intervals of one week and then the whole experiment was repeated again. The data were collected as described in anther retention trial section and analyses done using Proc GLM of SAS version 93 SAS Institute Inc. 2008.

5.2.3.4 Data analyses

The model for degree of anther extrusion (February 2014 trial) was; Model AE= rep genotype rep*genotype spike(genotype*rep) with all factors considered random. The anther length model was length = genotype rep*genotype spike(genotype*rep) with all factors considered random. The model for the pooled data for anther retention trials (February and July 2014) was Model AR= siteyr rep(siteyr) genotype siteyr*genotype with all factors considered random. Proc GLM of SAS version 9.3 was used for analyses of these two experiments. For the pooled data for AR mapping experiment the Proc GLM of SAS version 9.3 was used with the Model AR= siteyr rep(siteyr) genotype siteyr*genotype with all factors being random. The genotyping data from the 9HBT population combined with phenotypic data for AR was used for QTL analysis in Qgene (Joehanes and Nelson 2008). Linkage maps were drawn using Mapchart (Voorrips 2002).

5.3.2.5 Effect of reduced height (*Rht*) genes on anther retention, height and FHB traits

The 100 DHs were grouped into genotypic groups based on their *Rht* allele combinations in homozygous conditions (*Rht-B1b/Rht-D1a, Rht-B1a/Rht-D1a, Rht-B1b/Rht-D1b, and Rht-B1a/Rht-D1b)* to see if there is any difference in AR, height and FHB traits in these groups. The marker data from the *Rht-B1* and *Rht-D1* genes and pooled data for FHB traits (incidence, severity, FHB index, FDK and DON) for six site years for 100 DH lines (chapter 3) was used for this classification. The analysis of variance of each *Rht* allele combination was calculated using Proc GLM of SAS (SAS v9.3, SAS) with all factors considered random and means difference of the groups were compared using least significant difference (lsd) at $P<0.05$. The different *Rht* genotypic groups were correlated with the available height data (2010, one season) and FHB traits (Pooled data for Carman and Winnipeg for FHB traits for 2011, 2012 and 2013 FHB nurseries).

5.3 Results

5.3.1 Anther retention/extrusion and anther length results for the ten DH lines and its relationship with FHB resistance

There were significant differences between the ten genotypes in terms of extrusion/anther retention (Tables 5.1 and 5.2). The mean degree of AE was 67.9%. The degree of AE was observed to be lower in more susceptible genotypes except 9HBT374 (very susceptible, but had an intermediate anther extrusion) but higher in moderately resistant lines (Table 5.3). The higher the number of extruded anthers, the greater was the FHB resistance. A similar trend was observed on checks. Moderately resistant lines such as 9HBT5 and 9HBT537 had higher AE compared to the susceptible lines such as 9HBT371 and 9HBT261 (Table. 5.3). The entries with high anther retention had either anthers still attached to the receptacles or trapped between

lemma and palea (Fig.5.1 c and d) during flowering and they were the most susceptible to FHB. Some of the susceptible lines started flowering as the spike emerged from the flag leaf.

The analysis of variance for anther length showed that the genotypes were highly significant (Table 5.1). The mean anther length was 3.2mm. The anther length varied amongst the DH line with more susceptible genotypes having smaller sized anthers (Fig 5.3) than the moderately resistance lines (Table 5.3). CDC Buteo had an intermediate anther length of 3.4mm. The largest anther size was 4.1mm and the smallest was 2.6mm. It was observed that the longer the anther size, the greater the FHB resistance except for one line (9HBT141) which had medium sized anthers (Table 5.3), but was highly FHB susceptible. Greater anther length was associated with low AR leading to lower disease incidence, severity, FDK and DON (Table 5.3).

Table 5.1 Analysis of variance tables for degree of anther extrusion (February 2014) and anther length (February 2015) for ten double haploid lines (five resistant to moderately resistant and five susceptible lines from the cross 19H*16/CDC Buteo)

	Anther extrusion			Anther length		
Source of variation	DF	Mean Square	Pr > F	DF	Mean Square	Pr > F
Rep	5	275.2	0.9			
Genotype	9	1980.4	0.007	9	43.7	<.0001
Rep*Genotype	45	329.6	1	8	1.1	0.1
Spike(Rep*Genotype)	117	279.9	1	72	0.7	<.0001
Error	466	778.4		1003	0.03	

Standard error of the mean for anther extrusion = 3.3, anther length = 0.02

Table 5.2 Analysis of variance table for anther retention (AR) for pooled data for trials carried out on February and July 2014 at the Crop Technology Centre and Point growth rooms for ten genotypes (five resistant to moderately FHB resistant and five FHB susceptible double haploid lines from the cross 19H*16/CDC Buteo)

Source of variation	DF	Mean Square	Pr > F
Siteyr	1	18.0	0.4
Rep(Siteyr)	10	1.3	0.001
Genotype	9	237.1	0.001
Genotype*Siteyr	9	24.1	<.0001
Error	9	24.1	

Standard error of means = 0.03

Table 5.3 Degree of anther extrusion mean (% AE) and retention (AR-average number of retained anthers/floret) for greenhouse trial for ten 19H*16/CDC Buteo double haploid lines, parental lines and two checks: Anther length (AL in milimetres) means data are from a separate greenhouse trial: Disease incidence (INC), severity (SEV), Fusarium head blight index (FHBI) and Fusarium damaged kernels (FDK) means data are for Carman and Winnipeg for the pooled field data for the two sites for 2011,2012 and 2013 field seasons

Genotype	[1]AR	[2] Mean AE (%)	[3]AL	[4]FHB traits combined data for Carman and Winnipeg for 3 years				FHB classes
				INC	SEV	FHBI	FDK	
9HBT537	0.3	79.4	3.9	16.9	27.4	7.2	2.8	R
9HBT532	0.4	72.0	3.9	22.1	31.1	9.3	5.8	R
9HBT5	0.6	79.5	4.1	23.3	26.5	6.4	4.9	R
9HBT200	0.8	75.8	3.6	33.6	26.7	8.8	7.9	R
9HBT155	0.8	72.1	3.3	22.4	28.7	6.7	5.1	R
9HBT374	2.2	72.8	2.4	78.5	75.8	61.3	42.3	S
9HBT441	2.3	69.0	2.6	78.9	69.9	56.5	28.2	S
9HBT198	2.7	69.1	2.6	82.9	74.5	63.0	43.6	S
9HBT261	2.7	67.0	2.7	83.9	76.5	65.2	41.2	S
9HBT141	2.8	60.7	3.1	84.9	78.1	67.2	38.5	S
Parents								
Buteo	1.7	71.0	3.4	44.8	51.3	23.4	14.5	MR
19H*16	1.6	71.4	.	68.8	64.5	44	22.5	MS
Checks								
FHB148	0.8	77.5	3.2	18.6	31.3	8.1	3.6	R
Hanover	2.1	51.5	3.5	75.5	71.2	56	25.6	S

[1]Anther retention (AR) data is from two trials each consisting of six plants/entry and three to six spikes per plant

[2]Anther extrusion (AE) data is from one trial consisting of six plants per entry and three spikes per plant

[3]Anther length (AL) data is from one experiment consisting of two plants per entry and five spikes per entry

[4]All FHB traits data were from pooled field data from Carman and Winnipeg

Figure 5. 3 Photos showing different anther sizes for four DH lines with different FHB reaction. The anther size trial was carried out in February 2015 using 10 genotypes, two checks and parental lines. The scale of measurement was uniform for all DH lines. A dissecting Zeiss microscope was calibrated using a 1mm micrometer (known length) mounted in a 10X objective on a stereomicroscope. The anther images were captured using an Infinity 2 Scientific digital Camera and Infinity Camera Software

5.3.2 Phenotypic association between anther extrusion (February 2014), anther length (February 2015) and pooled data for anther retention (February and July 2014) and FHB traits for 10 genotypes

The level of significance between the correlations of anther morphology and FHB traits varied (Table 5.4). The AR was associated with increased FHB traits (Experiment 1 and 2 for ten genotypes) while a high degree of AE and longer AL were associated with increased FHB resistance with the lowest correlations varying from r = -0.53 to -0.71 (Table 5.4). Negative

correlations were reported between degree of AE and AR, incidence, severity, FHB index, FDK and DON with lowest positive association being between AE and AL. Anther retention was negatively correlated with anther length. The relationship between AR and FHB was highly positive and between AL and FHB was highly negative. This suggests that higher AR is associated with higher FHB symptoms while shorter anthers are associated with higher FHB. Anther retention and length can be used as indirect selection tools in breeding for resistance to FHB. The degree of anther extrusion had moderate correlation with FHB and is a hard trait to measure especially in the field conditions due to wind blowing extruded anthers. However, the anther length measurement is a tedious procedure which may not be feasible when many lines are to be evaluated, making AR an easier target trait for breeding for resistance to FHB in wheat.

Table 5.4 Correlation coefficients between anther retention(AR), degree of anther extrusion (AE), anther length (AL), disease incidence, disease severity, FHB index (FHBI), FDK (Fusarium damaged kernels) and deoxynivalenol (DON). The data for AE and AR are from same experiment, data for AL are from a different trial and data for FHB traits are from pooled field data for Winnipeg and Carman for 2011, 2012 and 2013 FHB nurseries

	AE	AL	Incidence	Severity	FHBI index	FDK	[1]DON CRM 2012	[2]DON CRM 2013	[3]DON WNP 2012	[4]DON WNP 2013
AR	-0.79**	-0.80**	0.93***	0.89***	0.90***	0.90***	0.87**	0.91***	0.76*	0.85**
AE		0.58ns	-0.70*	-0.71*	-0.71*	-0.66*	-0.60ns	-0.70*	-0.53ns	-0.67*
AL			-0.90***	-0.90***	-0.89**	-0.90***	-0.86**	-0.87**	-0.62ns	-0.87**

***<0.0001, **<0.001, *<0.01, ns- not significant
[1]DON Carman 2012, [2]DON Carman 2013, [3]DON Winnipeg 2012, [4]DON Winnipeg 2013
N=10

5.3.3 Mapping of QTL controlling anther retention in 100 DH lines and phenotypic association between anther retention and FHB traits

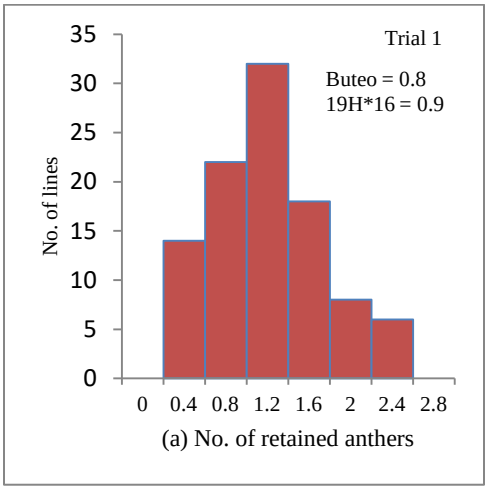
There were significant differences between the 100 genotypes in terms of retained anthers within the florets (Table 5.5). The population displayed continuous variation for AR with transgressive segregants towards the higher and the lower values for AR (Fig 5.4), an indication that both parents contributed alleles for AR. The number of anthers retained in trial one for CDC

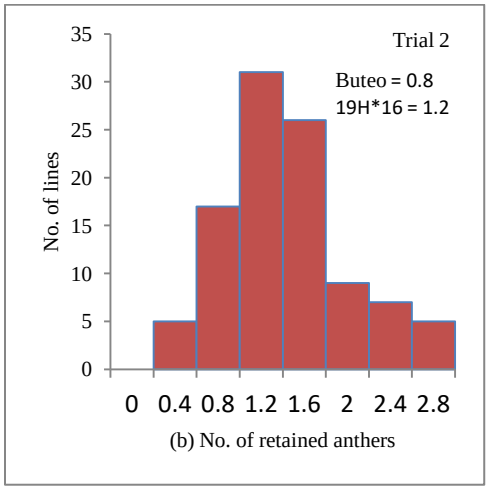
Buteo was 0.8 compared to 0.9 for 19H*16, while in trial 2 CDC Buteo had 0.8 while 19H*16 had 1.2 retained anthers. The mean AR was 1.0 with the AR varying from 0.2 to 2.3 in trial 1. While in the second trial, the mean was 1.2 and it varied from 0.04 to 2.7 amongst the 100 DH lines. After pooling the two data sets, CDC Buteo was found to have AR of 0.8 while 19H*16 had 1.0. The mean AR for the DH lines was 1.1 anthers and the AR ranged from 0.1 to 2.5 anthers out of three. The results suggested that lower anther retention leads to reduced FHB infection as shown in previous AR studies with 10 genotypes and also in the 100 DH lines (Tables 5.4 and 5.6).

Table 5.5 Analysis of variance table for quantitative trait loci mapped for anther retention pooled data for two trials carried out at Crop Centre Technology, University of Manitoba consisting of 100 double haploid lines from the cross 19H*16/CDC Buteo

Source of variation	DF	Mean Square	Pr > F
siteyr	1	136.8	<.0001
Rep(siteyr)	2	100.1	<.0001
Genotype	99	42.3	<.0001
Genotype*siteyr	99	5.4	<.0001
Error	15243	0.9	

Standard error of means = 0.07





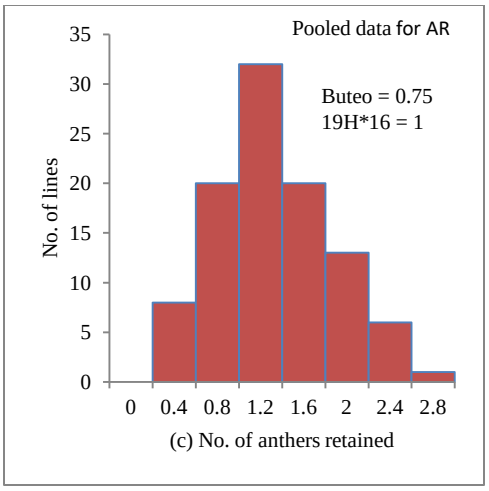


Figure 5. 4 Frequency distributions for anther retention for two greenhouse trials (Crop Technology Centre, University of Manitoba), Fig. a and b consist of two replicates each and Fig. c is pooled data for two anther retention trials for 100 DH lines from the cross 19H*16/CDC Buteo

5.3.3.1 Correlation between anther retention and FHB traits for 100 DH lines

Similar results were reported for correlation between AR and FHB traits in 100 DH lines as those between AR and FHB traits for 10 genotypes, where AR positively correlated with all FHB traits, but negatively correlated with height (Table 5.6). The correlations were significant and high except for height where only 30 genotypes were used. The correlations between AR and incidence, FHB index, FDK and DON were very similar suggesting that AR is a useful trait in determining FHB reaction in wheat in this population. The highest correlation was between AR and FDK (r = 0.67) followed by that between AR and incidence (r = 0.66).These results suggested that AR is a stable trait which can be used in selection for FHB resistance.

Table 5. 6 Correlation coefficients between anther retention (AR) and FHB traits (incidence, severity, Fusarium head blight index (FHB index), deoxynivalenol (DON) and plant height for 100 DH lines. The FHB traits data are from pooled field data for two Carman and Winnipeg field trials for 2011, 2012, 2013 Fusarium head blight nurseries while anther retention data is from means of the two greenhouse trials (2015)

Trait	[1]INC	[2]SEV	[3]FHBI	[4]FDK	[5]DON CRM2012	[6]DON WNP2012	[7]DON CRM2013	[8]DON WNP2013	[9]Height (cm)
[10]AR	0.66***	0.63***	0.66***	0.67***	0.60***	0.53***	0.65***	0.57***	-0.24***
INC		0.92***	0.96***	0.90***	0.76***	0.68***	0.76***	0.83***	-0.63***
SEV			0.97***	0.89***	0.78***	0.66***	0.80***	0.81***	-0.46*
FHBI				0.93***	0.82***	0.71***	0.80***	0.85***	-0.50**
FDK					0.80***	0.76***	0.89***	0.92***	-0.50**

***<0.0001, **<0.001, *<0.01, ns- not significant
[1]Incidence, [2]Severity, [3]FHB index, [4]Fusarium damaged kernels, [5]Height N=30 (one season data), [6]DON (Deoxynivalenol) Carman2012, [7]DONWinnipeg2012, [8]DONCarman2013, [9]DONWinnipeg2013, [10]Anther retention

5.3.4 Mapping anther retention QTL

Five major QTL for anther retention were identified in the population of 100 DH lines (Table 5.7 and appendix 3.2). The major QTL for AR were detected on chromosomes 4D and 4B, each explaining 32.9 and 27.1% phenotypic variation, respectively, and they mapped on the exact location of *Rht-D1* and *Rht-B1*, respectively. The 1B, 3D and 4A each explained phenotypic variations of 19.4%, 15.8% and 12.2%, respectively. The 4A QTL was not significant but was reported in this trial because it played a role in inheritance of FHB resistance in this population (Chapter 3). The 19H*16 alleles on 4B and 3D decreased anthers retained within the floret, while 19H*16 alleles on 1B, 4A and 4D increased anther retention. This suggests that both parents contributed alleles which reduced anther retention. All AR QTL coincided with QTL reported for FHB traits.

Table 5. 7 Anther retention (AR) quantitative trait loci (QTL) detected using multiple interval mapping (MIM)for 19H*16/CDC Buteo pooled data for two trials carried out in 2015 for 100 DH lines grown in the greenhouse at Crop Research Centre, University of Manitoba

Trait	Chr. [a]	Marker at the QTL peak [b]	Pos (cM)	Add [d]	PVE (R^2) [e]	LOD [f]	LOD α=0.05 [g]
[h]AR	1B	BS00022153_51	34	0.19	19.4	4.64	3.57
	3D	wsnp_Ra_c17636_26538543	42	-0.16	15.8	3.71	3.57
	4A	Kukri_c2706_1424	32	0.13	12.2	2.80*	3.57
	4B	Kukri_c26905 392	4	-0.23	27.1	6.79	3.57
	4D	wMAS000002	17	0.27	32.9	8.58	3.57

[a] Chr., Chromosome
[b] Marker at the peak of the QTL
[c] Pos, Position on linkage group in cM
[d] Add, Additive effect (Additive effect of allele substitution of the trait in question. A negative sign indicates that 19H*16 allele decreased the trait and vice versa)
[e] PVE, Phenotypic variation explained (R^2; %)
[f] LOD, Peak LOD score
g LOD α=0.05, Significant LOD score threshold at 5%
* Detected QTL with LOD<3.00, that was previously detected in whole population QTL analysis in Chapter 3

5.3. *Rht* genes and their correlation to anther retention, plant height and FHB traits

The means of the *Rht* genotypic groups and ranking are summarized in Table 5.8. The classification of the 100 9HBT DH lines according to *Rht* (Reduced height) genes resulted in four clusters comprising of combinations (*Rht-B1b/Rht-D1a* and *Rht-B1a/Rht-D1b*), double mutants (*Rht-B1b/Rht-D1b*) , double wild-type (*Rht-B1a/Rht-D1a*) and two other classes consisting of six genotypes which had missing data (data is not shown for six genotypes).The ranking of these clusters was consistent for AR and plant height and all FHB traits (incidence, severity, FHB index, FDK and DON) with *Rht-B1a/Rht-D1a< Rht-B1b/Rht-D1a < Rht-B1a/Rht-D1b <Rht-B1b/Rht-D1b* (Table 5.8). The double wild-type genotypes (*Rht-B1a/Rht-D1a)* had the lowest AR (Table 5.8) and lowest disease incidence, severity, FHB index and FDK and DON (Table 5.8) while the double mutant genotypes *(Rht-B1b/Rht-D1b)* had the highest values. However, the mutant allele *Rht-D1b* had higher retained anthers and FHB infection than *Rht-*

B1b. There was significant difference in incidence, FHB index and FDK between *Rht-B1b/Rht-D1a and Rht-B1a/Rht-D1b* with the latter having higher disease ratings.

For plant height, only a few lines were available for classification (N=27, Table 5.8). The double mutant genotypes were shown to be shorter (61.7 cm) than the double wild genotypes (102.5 cm). The allele combination *Rht-B1a/Rht-D1b (*was shorter than its counterpart *Rht-B1b/Rht-D1a* by 5.5 cm and had more retained anthers though the difference in AR and plant height was not significant. The total phenotypic variation explained by these two genes was 60% for AR with the largest proportion of phenotypic variation explained by *Rht-D1* (Table 5.3). The shortest genotypes (double mutants) had the greatest number of retained anthers.

Table 5.8 Means of reduced height (*Rht*) genotypic groups and least significance difference (LSD) for anther retention (AR) for combined data for two green house trials, plant height (one season data), and pooled data for six site years (Carman and Winnipeg, 2011, 2012 and 2013) for incidence, severity, Fusarium head blight (FHB index)**,** Fusarium damaged kernel (FDK) and four site years (Carman and Winnipeg 2011 and 2012) for deoxynivalenol (DON) in100 DH lines from the cross 19H*16/CDC Buteo

Rht genotypes	N	AR 2015	Incidence	Severity	FHB index	FDK	DON
B1a/D1a	25	0.7 a*	38.9 a	43 a	19.5 a	11.1 a	13.3 a
B1b/D1a	24	1.1 b	52.3 b	51.4 b	27.7 b	16.1 b	20.6 b
B1a/D1b	27	1.2 b	56.4 c	52.8 b	30.89 c	17.6 c	21.7 b
B1b/D1b	16	1.7 c	74.1 d	63.5 c	48.6 d	32 d	39.1 c

Rht genotypes	N	Plant height (cm)
B1a/D1a	6	102.5 a
B1b/D1a	11	89.1 b
B1a/D1b	7	83.6 b
B1b/D1b	3	61.7 c

* Numbers followed by a different letter in a column are significantly different based on LSD ($p \leq 0.05$)

5.3.6 Discussion and conclusion

Escapes from infection have been discussed as a possible cause for FHB resistance. CDC Buteo (moderately resistant parent) was shown to have lower AR than the moderately

susceptible parent 19H*16 which was statistically insignificant. The results of this study suggest that the fewer anthers retained, the higher is the FHB resistance. Results from the two experiments (10 and 100 DH lines) were an indication that AR is a stable trait which can be used in FHB breeding for selection of FHB resistant genotypes. Similar results were reported by Buerstmayr and Buerstmayr (2015), who suggested that selection of lines with low AR should result in correlated selection response towards increased FHB resistance. There are suggestions from breeders that AE is a stable character (Salvatore 1978; Lu et al. 2013; Buerstmayr and Buerstmayr 2015), while others have concluded that the trait is highly influenced by environmental factors, especially drought (Sage and De Isturiz 1974; Abdel-Ghani et al. 2005, Stråbø 2014).

Generally, the lines with low or no anther retention had low FHB symptoms compared to partially extruded anthers that had relatively severe FHB symptoms. Similar results were reported by Buerstmayr and Buerstmayr (2015) who observed that partially extruded anthers were considered to be a source of FHB infection. Phenotypes with rapid anther extrusion and ejection may contribute to the avoidance of FHB infection. Transgressive segregants were reported in this study which suggests both parents contributed alleles for low AR. Transgressive segregation for FHB severity and for AR were also reported in various studies (Skinnes et al. 2010; Lu et al. 2013; Buerstmayr and Buerstmayr 2015). The 19H*16 alleles on chromosomes 4B and 3D decreased AR in this population while the alleles from corresponding loci from CDC Buteo increased AR. Also the 19H*16 alleles for 1B, 4A and 4D QTL increased AR relative to the CDC Buteo alleles. The 4B and 4D QTL mapped on the exact locations of dwarfing genes *Rht-B1* and *Rht-D1*, respectively.

Data on height were collected only in one season, but they revealed a negative correlation with AR. There was a strong relationship between plant height and dwarfing genes *Rht-B1b* and *Rht-D1a.* The marker data for the 9HBT population revealed that CDC Buteo contained allele combination *Rht-B1b/Rht-D1a* while 19H*16 contained *Rht-B1a/Rht-D1b* (Chapter 3). The wild-type genotypes *Rht-B1a / Rht-D1a* were the tallest, had low FHB disease and the lowest number of retained anthers compared to double mutants that were the shortest and had the highest FHB disease and the highest number of retained anthers. Some of the shorter genotypes, *Rht-B1a /Rht-D1b* and *Rht-B1b/Rht-D1a,* had higher AR compared to tall ones, which had lower AR, as shown when the height data for the 100 AR mapping lines were classified into respective *Rht* groups based on height. The shorter genotypes were also shown to be the most susceptible to FHB disease. However, there were some recombinant semi-dwarf lines with low FHB symptoms from all the *Rht* genotypic groups except the double mutants with low AR. Similar results were reported by Buerstmayr and Buerstmayr (2016) and He et al. (2016) where double dwarf mutants (*Rht-B1b*/*Rht-D1b*) were shown to be the shortest and had the highest AR and the highest FHB infection. All studies that evaluated the correlation between AR and FHB resistance observed decreased FHB symptoms in genotypes with low AR (Graham and Browne 2009; Skinnes et al. 2010; Kubo et al. 2013; Lu et al. 2013; Buerstmayr and Buerstmayr 2015). Colonization of spikes starts often on anthers retained within the florets because they are an easy to conquer nutritious tissue for Fusarium that stimulate fungal development and thus give Fusarium an advantage for penetrating the floret (Miller et al. 2004; Buerstmayr and Buerstmayr 2015). Anther retention could have resulted also from shorter lodicules and stamen filaments in shorter genotypes which contain dwarfing genes (*Rht-B1* and *Rht-D1*) which are insensitive to gibberelic acid leading to high FHB symptoms. Gibberelic acid is responsible for cell elongation in plants.

The shorter lodicules and stamen filaments lead to anthers being retained within the floret or trapped between lemma and palea.

There are many floral traits which are associated with the extent of anther extrusion which include openness of florets, duration of flower opening, length of anthers and filaments, tenacity and form of glumes. A high positive correlation was observed between AE and both anther length and duration of floral opening (Singh et al. 2007). Similar results were obtained in this study where AR was negatively correlated with anther size. This could be explained by the fact that the larger the anther size, the greater the possibility of the anther being extruded out of the floret compared to the shorter anther which may remain inside the floret or be trapped between lemma and palea. The anther length varied from 2.4-4.1mm in ten DH lines from 19H*16/CDC Buteo cross. In a similar study where anther lengths of 61 cultivars of common wheat were measured just before flowering, anther length varied from 3.0 to 5.1 mm, (Komaki and Tsunewaki 1981).

The allele combination *Rht-B1b* /*Rht-D1a was* shown to have lower FHB infection and AR compared to *Rht-B1a* /*Rht-D1b*. Semi-dwarf genotypes were identified in our study with low AR and decreased FHB disease. These semi-dwarf genotypes should be evaluated for suitability for cultivar registration or used in breeding for FHB resistance. These results agree with those of Buerstmayr and Buerstmayr (2016) where they concluded that semi dwarf progeny with very good FHB resistance can be obtained from *Rht-B1b* and lines with moderate to good FHB resistance when using *Rht-D1b* and backcrossing with tall genotypes. However, these results disagree with those of He et al. (2016), who concluded that the two dwarfing genes in a study, which collectively explained around 20% of AE reduction in both populations that *Rht-B1b* was always more strongly associated with reduced AE than *Rht-D1b*.

Other QTL which were reported included QTL 1B and 4A in this AR study. Semagn et al. (2007) and Skinnes et al. (2010) showed that AE was also controlled by a minor QTL at 1BL. Buerstmayr and Buerstmayr (2015) detected a QTL on 4AL which overlapped with QTL for FHB reaction. In this study, the 4A QTL for AR (not significant at LOD=3 .may be due to a small mapping population of 100 DH lines) also overlapped with a second QTL detected on 4A for FHB reaction from field data. The coincident QTL for AE and FHB was found on chromosome 4A which increased AR and FHB susceptibility (Graham and Browne 2009). However, the *Rht-B1* and *Rht-D1* dwarfing genes were shown to be crucial in AR and FHB resistance in this study.

High correlation coefficients ranging from +/- 0.58 to +/-0.90 were obtained between AE, AR and FHB reaction in experiments 1 and 2. Similar observations were made where AR was positively correlated with FHB severity (r = 0.63) reflecting the lower FHB severity on plants with high AE in a study of 171 RILs from Arina (resistant) /Capo (moderately resistant cross) (Buerstmayr and Buerstmayr 2015). The relationship between AE and AR and FHB found in this investigation are in agreement with previous studies (Taylor 2004; Skinnes et al. 2008; Skinnes et al. 2010). In conclusion, CDC Buteo is a source of resistance which can be used in breeding for FHB resistance and low AR for the Canadian Prairies. Additionally, the other parent, 19H*16 also could contribute important alleles for FHB resistance and low AR.

CHAPTER 6

GENERAL DISCUSSION

Fusarium head blight (FHB) caused mainly by *Fusarium graminearum* is a devastating disease of wheat, barley, corn and oat in Western Canada. FHB causes yield reductions and contamination of grain with trichothecene mycotoxins such as DON which is a major health concern for humans and animals. Rating of visual symptoms of infected spikes (disease incidence and severity) is the most common method of phenotyping plants for FHB symptoms in wheat. The levels of FDK and the DON may also be used to measure resistance to FHB, thus focusing on different types of resistance. There are many mechanisms for managing FHB with pyramiding genetic host resistance being considered to be the most practical approach for minimizing economic losses from FHB. Conventional breeding programs were traditionally used, but they are limited by lack of effective resistance genes. Identification of QTL conferring FHB resistance followed by marker-assisted selection (MAS) is an efficient approach to breed FHB-resistant varieties.

Numerous sources of FHB resistance that have been genetically mapped to chromosomes are from Asia, North America, South America, and Europe, but the most common resistance source is Sumai 3 and its derivatives. To date, few cultivars with adequate resistance have been available to wheat farmers. Two large DH population from the crosses 19H*16/CDC Buteo (9HBT) and 22A*13/CDC Buteo (2ABT) were phenotyped in Carman and Winnipeg (Manitoba) under artificially inoculated FHB nurseries for three years. The disease performance of the parents as well as the range of values observed for the DH population indicated there was substantial genetic variation for all FHB traits evaluated. Consistent FHB responses of both parents and DH lines were observed during the six field trials. The resistant to moderately

resistant lines remained resistance across the environments and susceptible ones showed susceptibility across different site years. The majority of the DH lines fell in the intermediate category. Pooled analyses for FHB traits measured showed that site years were significantly different. This could be explained by different environmental factors including rainfall and temperature across the locations and years. Environmental factors play a major role in disease triangle, hand in hand with the host and the pathogen. The FHB disease infection was higher in Carman than Winnipeg for both populations and all the traits measured. All FHB traits evaluated for FHB resistance scores for the DH population showed a continuous distribution with transgressive segregation, particularly, some lines exhibiting lower FHB symptoms than the moderately resistant parent. In the 9HBT study, we identified six lines which had a FHB index of less than 10% and 52 lines with FHB index of 10-20%. For the 2ABT population, 93 lines out of 218 had a FHB index of between 10-20%. The lower disease was observed to be a result of lower incidence levels compared to severity levels. Double haploid lines with lower DON accumulation and FDK than CDC Buteo were also reported for both populations. Evaluation of Type 2 resistance in the greenhouse for the 9HBT population revealed non-significant differences between the 81 DH lines used in the study (data not shown). Different types of resistance were observed in these populations namely Type 1 and 2 (resistance to initial infection and spread within the spike), Type 3 (resistance to DON accumulation), Type 4 (resistance to kernel infection) and Type 5 (tolerance). The different types of resistance contributed to the overall FHB resistance in some genotypes. This confirms that CDC Buteo is a source of resistance to FHB disease and the results also imply that other parents in these crosses can contribute to FHB resistance.

The QTL identified for 9HBT FHB resistance were located on 13 chromosomes with QTL consistently reported on chromosomes 2B, 4A, 4B, 4D, 6B and 7A. Amongst them, 4B and 4D showed the largest effects and were detected in all environments and FHB traits except for severity in Winnipeg and Carman in 2012. These QTL explained the largest proportion of the phenotypic variation that ranged from 7.4 -72%. The two QTL were associated with plant height and are dwarfing genes *Rht-B1* and *Rht-D1*, respectively. CDC Buteo was confirmed to contain the *Rht-B1b/Rht-D1a* allele combination, while 19H *16 contains *RhtB1a/Rht-D1b*. The major QTL from the 9HBT population which reduced FHB symptoms were those on 4B and 6B from 19H*16 alleles and 2B, 3B, 4A, 4D, 5B and 7A from CDC Buteo alleles. The 6B QTL from 19H*16 allele explaining phenotypic variation of 5.6-36.9% was consistently identified and stable, and was shown to reduce disease severity, FHB index, FDK and DON. It was only reported in one location for incidence at pos 38 cM. These QTL can be used in MAS or the moderately resistant genotypes should be evaluated for suitability for cultivar registration. Conclusive studies were not carried out to determine if the 6BS QTL is *Fhb2*, although comparative mapping showed that it could be *Fhb2*.

The 9HBT population segregated for two *Rht* genes, *Rht-BI* and *Rht-D1*, which had major effects on disease incidence, severity, FHB index, FDK and DON and may have masked other QTL. Data adjustment by fixing for each dwarfing gene separately to determine the contribution of the dwarfing genes on the final phenotype was partial and these analyses did not detect any new QTL.

The 2ABT population segregated for 19 major QTL with 22A*13 alleles on chromosomes 3B and 6B reducing incidence, FHB index, FDK and DON and the CDC Buteo alleles reducing FHB disease on chromosomes 2B, 2D, 4A, 5B and 6A in most of the

environments. Both QTL on 3B and 6B accounted for about 12-37.8 % of the total phenotypic variation explained. Comparative mapping suggested that the 6B QTL may not be *Fhb2*. The 22A*13 alleles also reduced FHB disease on chromosomes 1B, 3A, 3D, 6D and 7A, but were inconsistently reported. The 2B, 3B, 4A, 6A and 6B QTL were consistent and they could be used in marker assisted breeding. Height QTL were reported on chromosomes 1A, 4D, 5D and 7A, explaining phenotypic variation of 6.4-8.4%. The minor QTL for height detected on chromosome 5D from 22A*13 alleles reduced the height by 1.02cm, while that on chromosome 7A increased the height by 1.01cm.

High correlations were reported between FHB index and incidence (r= 0.98) and between FDK and DON (r=0.95) in the 9HBT population, an indication that a decrease in one FHB trait led to decreased symptoms in another. Height was negatively associated with all other traits, suggesting that taller genotypes have decreased symptoms compared to the shorter genotypes. Similar results were reported for 2ABT where the correlations between FHB index and incidence were the highest (r=0.91) and between FDK and DON (r=0.73) were third after severity and FHB index (r=0.84). However, the correlation between height and FHB traits were not significant in this population.

Validation of QTL conferring FHB resistance is a prerequisite for MAS. Comparison of QTL in the 9HBT and 2ABT populations was done to establish the common QTL associated with FHB resistance in CDC Buteo. There were some commonalities in the two studies in terms of QTL detected especially on chromosomes 2B and 6B. In contrast to many spring wheat studies, where QTL have been identified in an exotic genetic background, the FHB resistant QTL in this study already reside in an adapted genetic background with excellent agronomic performance. The most effective QTL for both populations was reported on chromosome 6B.

The 6B QTL in 9HBT (pos 2 cM, same locus as that for 2ABT pos 18-26 cM) was reported in almost all environments for all traits evaluated except incidence. The 6B QTL for 2ABT (pos 18-26 cM) was identified for FDK, incidence, FHB index and DON and severity four, three, two, two, and one environment/(s), respectively. The 3B QTL in the 9HBT population was inconsistently reported for all traits and was localized in different regions (pos 2 and 22 cM) with alleles from 19H*16 increasing FHB infection. For 3B QTL (Pos 22 cM), 22A*13 alleles reduced FHB disease and the QTL was identified for all traits across many environments.

The 2B QTL was detected for 9HBT (pos 1 cM) mainly for FDK in three environments and for 2ABT (pos 67 cM), it was expressed in most environments except for FDK and severity. The 4A QTL is a stable QTL that was expressed in more than four environments in both populations for all traits except for FDK, DON and height for 2ABT. Two QTL on chromosome 4A were observed at between 18 and 28 cM. The 4A QTL played a major role in incidence in 9HBT population with alleles from CDC Buteo reducing FHB disease.

The 5B QTL was inconsistently reported for 9HBT while for 2ABT the QTL was reported in three and four environments for FDK and incidence, respectively. The 7A QTL was reported in many environments for 9HBT for all traits evaluated, especially incidence and severity, but was reported in only one environment (incidence) for the 2ABT population on a different position. Other QTL expressed inconsistently in one or both populations were located on chromosomes 1A, 1B, 1D, 3A, 3D and 7B. The unique and stable QTL for 9HBT were located on chromosomes 4B and 4D for all traits, while for 2ABT they were located on chromosome 2D (incidence, severity and FHB index) and 6A (incidence and severity, FHB index, and FDK). Six QTL were common to both populations and were located on chromosomes 2B, 3B, 4A, 5B, 6B and 7A and were detected in various traits investigated. Only QTL on 2B,

4A, 6B and 7A had strong similarity in the magnitude of the effects between these two populations. This suggests that the these QTL can be used to identify breeding lines carrying alleles for increased resistance to FHB traits in addition to 4B and 4D.

The association of anther retention and FHB resistance in 9HBT population revealed that low AR was strongly associated with increased FHB resistance. Anther retention results suggested that it is a stable trait which can be used for indirect selection for reduced FHB symptoms. Alleles for dwarf plant height (*Rht-B1b* and *Rht-D1b*) had pleiotropic effects in reducing height and increasing FHB susceptibility. This is still controversial since many wheat breeders attribute it to close linkage. The 9HBT population was classified into four genotypic groups based on *Rht* genes. The ranking of all clusters was constant for all trials with *Rht-B1a/Rht-D1a*< *Rht-B1b/Rht-D1a*< *Rht-B1a/Rht-D1b*< *Rht-B1b/Rht-D1b* for incidence, severity, FHB index, FDK, DON, AR and height. The wild genotype was the tallest with the lowest disease infection and low AR compared to double mutants that were the shortest with the highest disease level and highest AR. The *Rht-B1b/Rht-D1a* and *Rht-B1a/Rht-D1b* combinations were shown to be almost of the same height but *Rht-B1b/Rht-D1a* had lower FHB and lower AR compared to *Rht-B1a/Rht-D1b*. The differences between these combinations were not always significant. In conclusion, CDC Buteo is a source of resistance to FHB disease and some of semi-dwarf genotypes from 9HBT may be converted to cultivars or used for breeding for FHB resistance. The other two parents (19H*16 and 22A*13) also could contribute important alleles for FHB resistance.

6.1 Conclusion

CDC Buteo, 19H*16 and 22A*13 are sources of resistance to FHB disease. The source of resistance in CDC Buteo, which is a moderately resistant elite cultivar, was revealed to be partly

contributed by the *Rht-D1a* allele and QTL on chromosomes 2B, 4A, and 7A.The 2ABT and 9HBT populations produced DH lines which could be converted to cultivars or used as a source of resistance for breeding for FHB resistance in the Prairies. Further studies should be done to see if 2B, 4A, 6B and 7A could be used in MAS.

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LIST OF APPENDICES

SUPPLEMENTAL DATA SHEET 1

Appendix1.1 Analysis of variance tables for disease incidence, severity, Fusarium head blight index (FHB index) and Fusarium damaged kernels (FDK) from the cross 19H*16/CDC Buteo for 228 double haploid lines for individual environments in Carman or Winnipeg 2011, 2012 and 2013 Fusarium head blight nurseries

Table 1a: Winnipeg FHB nursery in 2011

Source of variation	Incidence (%)		Severity (%)		FHB index (%)		FDK (%)	
	MS	P>F	MS	P>F	MS	P>F	MS	P>F
Genotype	2129.8	<.0001	1365.2	<.0001	1469.8	<.0001	227.5	<.0001
Rep	5170.1	<.0001	266.8	0.22	939.2	0.005	138.7	0.13
Error	303.6		177.2		174.8		58.8	

Table 1b: Carman FHB nursery in 2011

Source of variation	Incidence (%)		Severity (%)		FHB index (%)		FDK (%)	
	MS	P>F	MS	P>F	MS	P>F	MS	P>F
Genotype	933.8	<.0001	1107.7	<.0001	1264.3	<.0001	118.0	<.0001
Rep	3256.4	<.0001	2152.2	0.0001	2523.3	<.0001	789.0	<.0001
Error	250.1		230.4		222.6		30.5	

Table 1c: Winnipeg 2012 FHB nursery

Source of Variation	Incidence (%)		Severity (%)		FHB index (%)		FDK (%)	
	MS	P>F	MS	P>F	MS	P>F	MS	P>F
Genotype	763.2	<.0001	268.2	<.0001	428.3	<.0001	51.5	<.0001
Rep	796.1	0.008	77	0.55	285.4	0.04	11.4	0.20
Error	164.5		129.4		86		7	

Table 1d: Carman, 2012 FHB nursery

Source of variation	Incidence (%)		Severity (%)		FHB index (%)		FDK (%)	
	MS	P>F	MS	P>F	MS	P>F	MS	P>F
Genotype	589.6	<.0001	184.5	<.0001	231.2	<.0001	68.4	<.0001
Rep	232	0.18	623.4	0.0002	305.5	0.001	834.7	<.0001
Error	132.6		72.1		45.2		11.2	

Table 1e: Winnipeg, 2013 FHB nursery

Source of variation	Incidence (%)		Severity (%)		FHB index (%)		FDK (%)	
	MS	P>F	MS	P>F	MS	P>F	MS	P>F
Genotype	724.5	<.0001	388.9	<.0001	687.6	<.0001	322.4	<.0001
Rep	1281.2	<.0001	1322.6	<.0001	642.3	0.0008	209.7	0.003
Error	108.3		75.5		88.2		36.2	

Table 1f: Carman FHB nursery in 2013

Source of variation	Incidence (%)		Severity (%)		FHB index (%)		FDK (%)	
	MS	P>F	MS	P>F	MS	P>F	MS	P>F
Genotype	440	<.0001	345.3	<.0001	501.8	<.0001	799.2	<.0001
Rep	11700	<.0001	12992.0	<.0001	17598	<.0001	4876.8	<.0001
Error	164.3		120.4		165.7		70.5	

Appendix 1.2 Analysis of variance for disease incidence, severity, FHB index (FHB index) and Fusarium damaged kernels (FDK) for pooled data for Carman and Winnipeg 2011 FHB nurseries for the cross 19H*16/CDC Buteo consisting of 228 double haploid lines

	DF	Incidence (%)		Severity (%)		FHB index (%)		*FDK (%)		
Source of variation		MS	P>F	MS	P>F	MS	P>F	DF	MS	P>F
siteyear	1	136222	0.003	34048	0.004	44152	0.0052	1	1045.7	0.27
Rep(siteyear)	4	4046.7	<.0001	1183.2	0.0001	1638.9	<.0001	2	461.7	<.0001
Genotype	227	2567.2	<.0001	2143.7	<.0001	2417.1	<.0001	227	299.4	<.0001
siteyear*Genotype	224	503.82	<.0001	318.2	<.0001	305.9	<.0001	227	46	0.39
Error	902	277.81		203.3		199.2		453	44.6	

*****FDK degrees of freedom different from other traits because in 2011 only two replicates were used in FDK analysis

Appendix 1.3 Analysis of variance for disease incidence, severity, Fusarium head blight index (FHB index) and Fusarium damaged kernels (FDK) for pooled data for Carman and Winnipeg 2012 Fusarium head blight nurseries for the cross 19H*16/CDC Buteo consisting of 228 double haploid lines

Source of variation	DF	Incidence (%)		Severity (%)		FHB index (%)		*FDK (%)	
		MS	P>F	MS	P>F	MS	P>F	MS	P>F
siteyear	1	22543	0.001	19953	0.005	767.9	0.2	3728.2	0.04
Rep(siteyear)	4	415.9	0.07	715.4	0.0002	310.4	0.01	428.2	<.0001
Genotype	227	1314.7	<.0001	435.9	<.0001	746.4	<.0001	106.6	<.0001
siteyear*Genotype	227	221.7	0.07	168.8	0.007	136.2	0.0002	13.4	<.0001
Error	903	191.1		131.1		95.4		9.1	

Appendix 1.4 Analysis of variance for disease incidence, severity, Fusarium head blight index (FHB index) and Fusarium damaged kernels (FDK) for pooled data for Carman and Winnipeg 2013 Fusarium head blight nurseries for the cross 19H*16/CDC Buteo consisting of 228 double haploid lines

Source variation	DF	Incidence (%)		Severity (%)		FHB index (%)		FDK (%)	
		MS	P>F	MS	P>F	MS	P>F	MS	P>F
siteyear	1	3335.2	0.51	708.2	0.77	739.1	0.79	156581	0.001
Rep(siteyear)	4	6485.5	<.0001	7101.4	<.0001	9092.6	<.0001	2542.5	<.0001
Genotype	227	991.12	<.0001	594.6	<.0001	1039.3	<.0001	1006.5	<.0001
siteyear*Genotype	227	171.4	0.01	139.0	0.0002	148.7	0.06	115.4	<.0001
Error	904	136.0		97.6		126.6		53.3	

Appendix 1.5 Analysis of variance table for disease incidence, severity, Fusarium head blight index (FHB index) and Fusarium damaged kernels (FDK) for pooled data for 9HBT Winnipeg 2011, 2012 and 2013 Fusarium head bligh nurseries for the cross 19H*16/CDC Buteo consisting of 228 double haploid lines

Source of variation	DF	Incidence (%)		Severity (%)		FHB index (%)		FDK (%)		
		MS	P>F	MS	P>F	MS	P>F	DF	MS	P>F
siteyear	2	78619	0.0003	185741	<.0001	50612	<.0001	2	59597	<.0001
Rep(siteyear)	6	2415.8	<.0001	555.5	0.0002	622.3	<.0001	5	114.5	0.002
Genotype	227	2757.8	<.0001	1300.5	<.0001	1985.4	<.0001	227	484.2	<.0001
siteyear*Genotype	454	429.3	<.0001	357.7	<.0001	296.2	<.0001	454	66.4	<.0001
Error	1356	192.0		127.3		116.3		1132	29.1	

*****FDK degrees of freedom different from other traits because in 2011 only two replicates were used in FDK analysis

Appendix 1.6 Analysis of variance tables for pooled data for disease incidence, severity, Fusarium head blight index (FHB index) and Fusarium damaged kernels (FDK) for Carman for 2011, 2012 and 2013 Fusarium head blight nurseries for the cross 19H*16/CDC Buteo consisting of 228 double haploid lines

Source of variation	DF	Incidence (%)		Severity (%)		FHB index (%)		*FDK (%)		
		MS	P>F	MS	P>F	MS	P>F	DF	MS	P>F
siteyear	2	166022	0.0005	86148	0.004	519348	0.022	2	236604	<.0001
Rep(siteyear)	6	4921.1	<.0001	5455.4	<.0001	6760.9	<.0001	5	2446.8	<.0001
Genotype	227	1541.1	<.0001	1144.00	<.0001	1596.8	<.0001	227	626.5	<.0001
siteyear*Genotype	452	304.7	<.0001	318.5	<.0001	308.8	<.0001	454	148.4	<.0001
Error	1355	211		161.0		164.5		1128	38.7	

*****FDK degrees of freedom different from other traits because in 2011 only two replicates were used in FDK analysis

SUPPLEMENTAL DATA SHEET 2

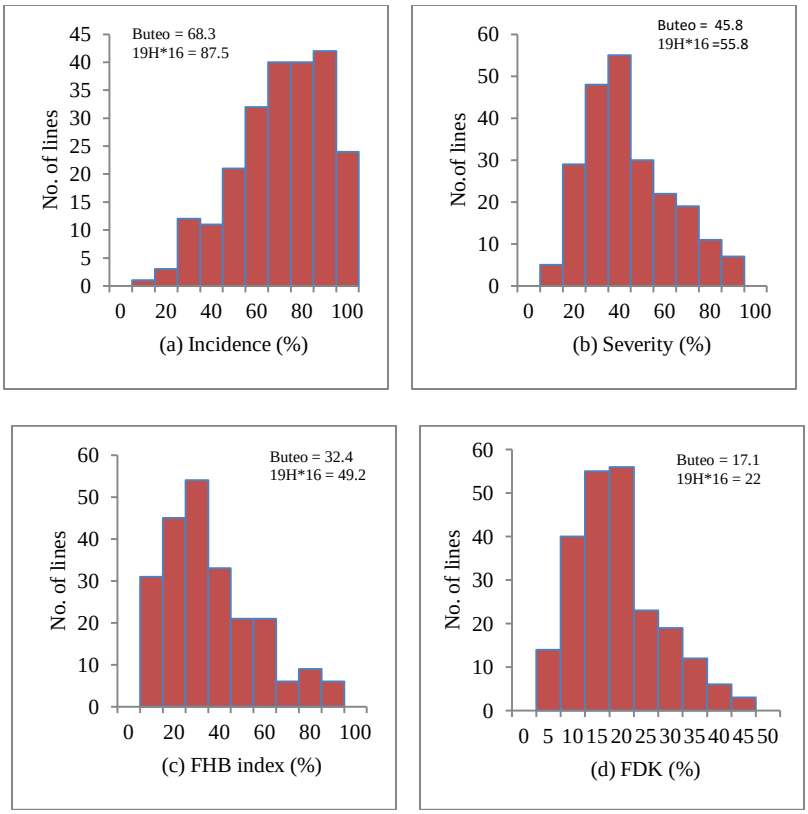
Traits variations and trait correlations

Appendix 2.1 Mean and range of disease incidence, severity, Fusarium head blight index (FHB index), Fusarium damaged kernels (FDK) and deoxynivalenol (DON) in Winnipeg or Carman, in 2011, 2012 and 2013 individual Fusarium head blight nurseries for the cross 19H*16/CDC Buteo consisting of 228 double haploid lines

		Winnipeg					Carman				
		Parents		DH population			Parents		DH Population		
Year	Trait	Buteo	19H*16	Mean	Min	Max	Buteo	19H*16	Mean	Min	Max
2011	Incidence	61.7	83.3	55.9	0.4	97.3	75.0	91.7	75.9	7.0	100
	severity	33.3	61.0	33.9	0.4	90.7	50.7	58.3	43.9	4.3	91.7
	FHB index	21.0	51.6	25.0	0.0	88	43.8	46.8	36.4	0.4	88.3
	FDK	8.0	28.8	15.6	0.8	50.8	26.3	15.3	17.7	2.8	39.0
2012	Incidence	18.0	40.0	36.7	7.0	81.7	28.8	68.3	42.3	17.5	82.8
	severity	46.7	60.8	55.1	27.2	77.5	39.7	52.5	40.2	19.5	67.8
	FHB index	8.4	22.9	21.5	2.33	58.1	11.6	35.5	18.0	4.7	53.5
	FDK	0.8	4.0	3.5	0.0	22.5	2.7	10.8	6.8	0.5	27.0
	*DON	1.4	1.5		0.0	18.0	2.8	10.0		0.7	37.5
2013	Incidence	33.3	63.8	54.5	9.3	85	52.2	67.5	57.7	26.3	87
	severity	62.5	80.5	66.5	28.3	87.5	56.7	65.8	65.0	25.8	89.2
	FHB index	20.9	51.5	37.9	3.1	72.6	32.0	46.0	39.4	6.9	74.3
	FDK	12.2	22.2	21.9	3.3	52.7	36.8	54.0	43.4	8.7	88.8
	*DON	17.5	18.0		1.7	77.5	42.5	65.0		8.5	136.5

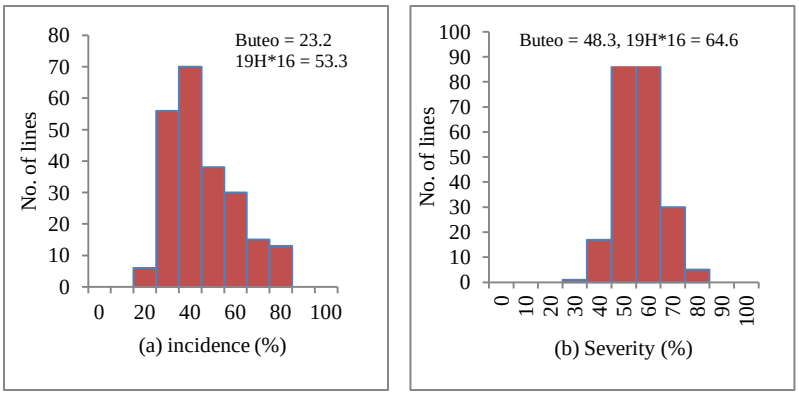
*****DON analyses were done on pooled samples across the replicates

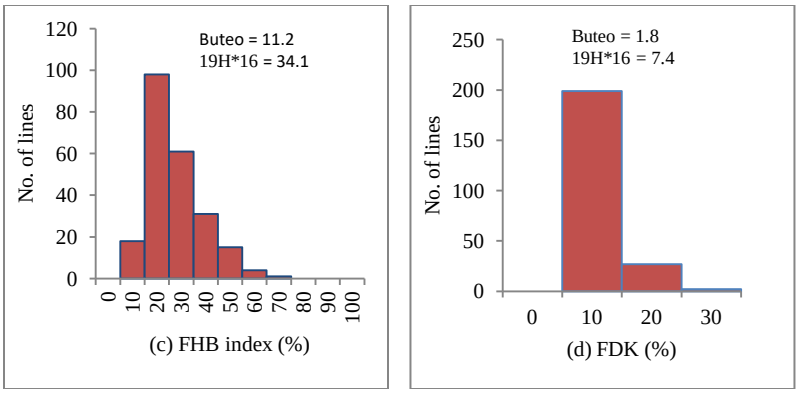
Appendix 2.2 Histograms for the cross 19H*16/CDC Buteo consisting of 228 double haploid lines after pooling Carman and Winnipeg 2011 Fusarium head blight nurseries data for disease incidence, severity, Fusarium head blight index (FHB index) and Fusarium damaged kernels (FDK)



Appendix 2.2 Frequency histograms showing the distribution of 228 9HBT lines derived from the cross of 19H*16/CDC Buteo for field incidence (a), severity (b), FHB index(c) and FDK (d). The data is for one year replicated Carman and Winnipeg, 2011. The heights of bars represent the number of lines in the population in each phenotypic class

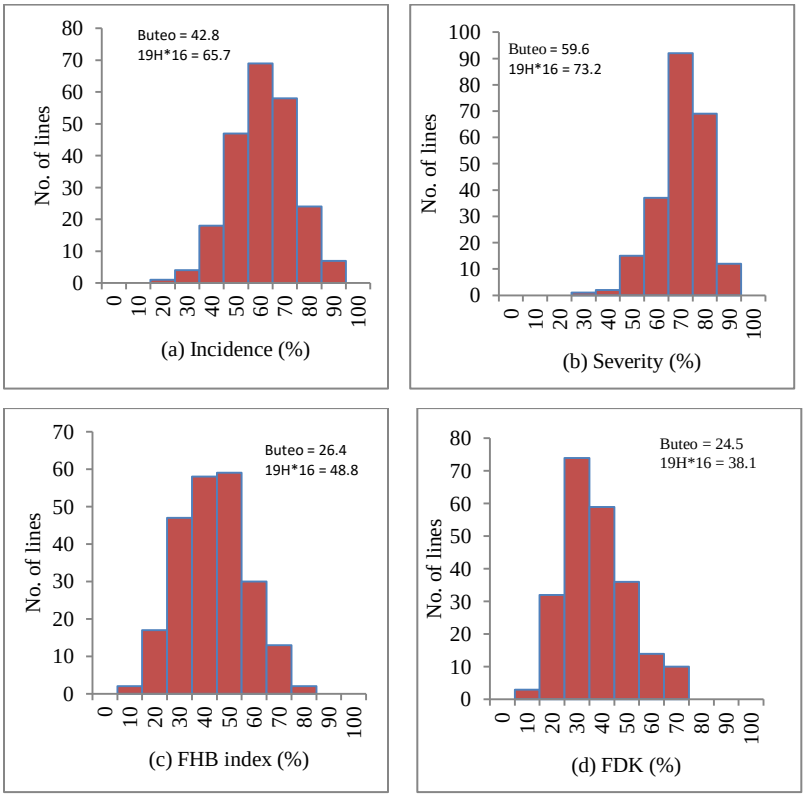
Appendix 2.3 Histograms for pooled Carman and Winnipeg 2012 Fusarium head blight nurseries data for incidence, severity, Fusarium head blight index (FHB index) and Fusarium damaged kernels (FDK) for the cross 19H*16/CDC Buteo consisting of 228 double haploid lines





Appendix 2. 3 Frequency histograms showing the distribution of 228 9HBT lines derived from the cross of 19H*16 x CDC Buteo for field incidence (a), severity (b), FHB index (c) and FDK (d). The data is for one year replicated Carman and Winnipeg, in 2012. The heights of bars represent the number of lines in the population in each phenotypic class

Appendix 2.4 Histograms for pooled Carman and Winnipeg 2013 Fusarium head blight nurseries data for incidence, severity, Fusarium head blight index (FHB index) and Fusarium damaged kernels (FDK) for the cross 19H*16/CDC Buteo consisting of 228 double haploid lines

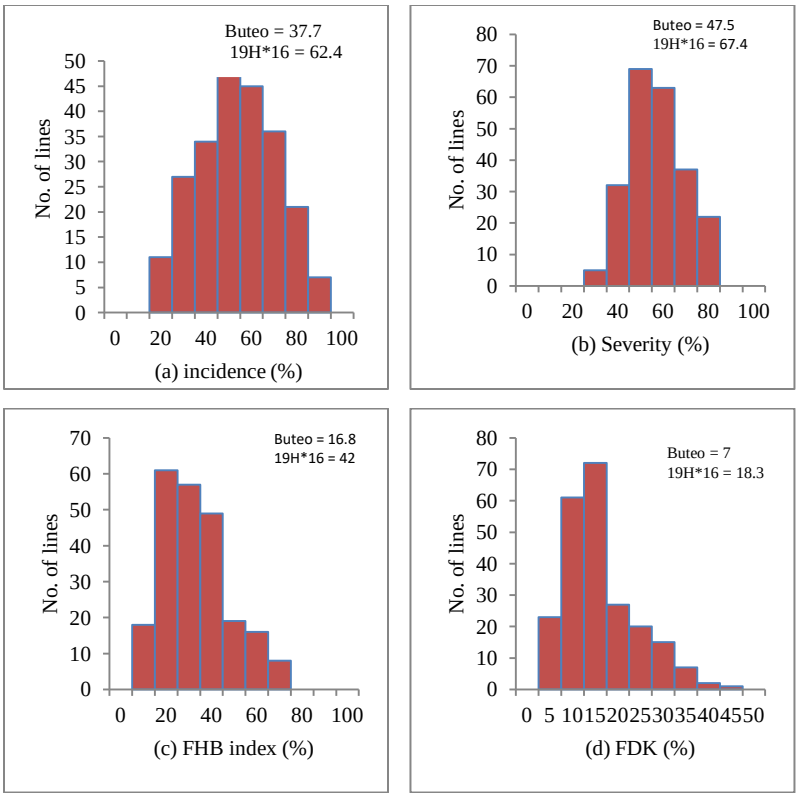


Appendix 2.4 Frequency histograms showing the distribution of 228 9HBT lines derived from the cross of 19H*16 x CDC Buteo for field incidence (a), severity (b), FHB index (c) and FDK (d). The data is for one year, replicated in Carman and Winnipeg in 2013. The heights of bars represent the number of lines in the population in each phenotypic class

Appendix 2.5 Mean and range of disease incidence, severity, Fusarium head blight index (FHB index), Fusarium damaged kernels (FDK) and deoxynivalenol (DON) after pooling Carman and Winnipeg data for each year forthe cross 19H*16/CDC Buteo consisting of 228 double haploid lines

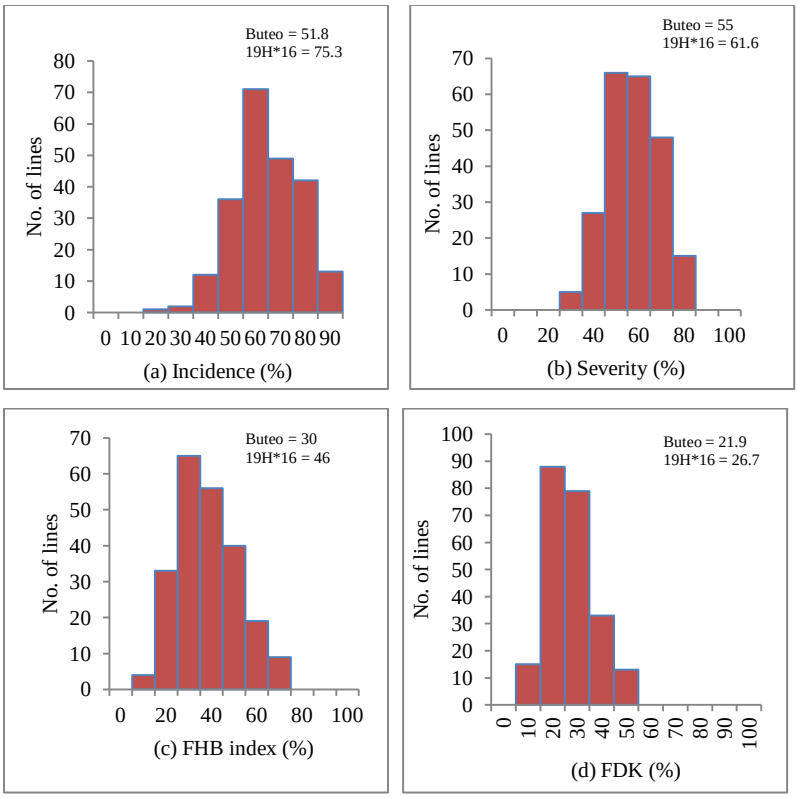
Carman and Winnipeg						
		Parents		DH Population		
Year	Trait	Buteo	19H*16	Mean	Min	Max
2011	Incidence	68.3	87.5	65.9	3.7	98
	severity	45.8	55.8	38.9	2.4	87.2
	FHB index	32.4	49.2	30.7	0.2	85.1
	FDK	17.1	22.0	16.6	1.8	42.0
2012	Incidence	23.2	53.3	40.7	11.3	79.6
	severity	48.3	64.6	51.2	29.3	78.8
	FHB index	11.2	34.1	22.3	3.5	60.7
	FDK	1.8	7.4	5.1	0.3	22.8
2013	Incidence	42.8	65.7	56.1	18.4	84.6
	severity	59.6	73.2	65.7	29.8	84.3
	FHB index	26.4	48.8	38.6	5.0	70.4
	FDK	24.5	38.1	32.6	6.0	68.5

Appendix 2.6 Histograms for pooled data for Winnipeg 2011, 2012 and 2013 Fusarium head blight nurseries for field incidence, severity, Fusarium head blight index (FHB index) and Fusarium damaged kernels (FDK) for the cross 19H*16/CDC Buteo consisting of 228 double haploid lines



Appendix 2.6 Frequency histograms showing the distribution of 228 9HBT lines derived from the cross of 19H*16 x CDC Buteo for field incidence (a), severity (b), FHB index (c) and FDK (d). The data is for Winnipeg, across 3 years (2011, 2012 and 2013). The heights of bars represent the number of lines in the population in each phenotypic class

Appendix 2.7 Histograms for pooled data for Carman 2011, 2012 and 2013 Fusarium head blight nurseries for field incidence, severity, Fusarium head blight index (FHB index) and Fusarium damaged kernels (FDK) for the cross 19H*16/CDC Buteo consisting of 228 double haploid lines



Appendix 2.7 Frequency histograms showing the distribution of 228 9HBT lines derived from the cross of 19H*16 x Buteo for field incidence (a), severity (b), FHB index (c) and FDK (d). The data is for Carman, across 3 years (2011, 2012 and 2013). The heights of bars represent the number of lines in the population in each phenotypic class

Appendix 2.8 Mean and range of disease incidence, severity, Fusarium head blight index (FHB index) and Fusarium damaged kernels (FDK) by pooling data across each site (Carman or Winnipeg separately) measured for three site years (2011, 2012, 2013) for the cross 19H*16/CDC Buteo consisting of 228 double haploid lines

	Winnipeg					Carman				
	Parents		DH Population			Parents		DH Population		
Trait	Buteo	19H*16	Mean	Min	Max	Buteo	19H*16	Mean	Min	Max
Incidence	37.7	62.4	49.1	10.7	85.9	51.8	75.3	54.9	17.3	87.9
severity	47.5	67.4	51.8	28.4	79.4	55.0	61.6	52.1	22.1	79.2
FHB index	16.8	42.0	28.1	4.0	68.0	30.0	46.0	32.9	5.1	69.4
FDK	7.0	18.3	13.4	1.5	41.2	21.9	26.7	23.2	4.0	49.0

Appendix 2.9-2.11 Correlation coefficients for 9HBT population between FHB traits (incidence, severity, Fusarium head blight index (FHB index) and Fusarium damaged kernels (FDK) measured in Winnipeg and Carman in 2011, 2012 and 2013 and for pooled data for Carman and

Winnipeg for three site years the cross 19H*16/CDC Buteo consisting of 228 double haploid lines

Appendix 2.9 Correlations (r) coefficients calculated separately for each site using field data measurements for incidence, severity, Fusarium head blight (FHB index), Fusarium damaged kernels (FDK) and/or deoxynivalenol (DON) measured from Carman and Winnipeg Fusarium head blight nurseries in 2011, 2012 and 2013

Carman (2011)				Winnipeg (2011)		
	SEV	FHBI	FDK*	SEV	FHBI	FDK
INC	0.72	0.80	0.68	0.86	0.85	0.80
SEV		0.98	0.76		0.99	0.87
FHBI			0.78			0.88

All correlations are significant at P=0.0001

Carman (2012)					Winnipeg (2012)			
	SEV	FHBI	FDK	DON	SEV	FHBI	FDK	DON
INC	0.64	0.93	0.84	0.81	0.64	0.97	0.74	0.66
SEV		0.84	0.56	0.58		0.77	0.37	0.39
FHBI			0.82	0.82			0.69	0.63
FDK				0.9				0.88

All correlations are significant at P=0.0001

Carman (2013)					Winnipeg (2013)			
	SEV	FHBI	FDK	DON	SEV	FHBI	FDK	DON
INC	0.73	0.95	0.79	0.64	0.80	0.97	0.84	0.82
SEV		0.89	0.68	0.58		0.90	0.77	0.73
FHBI			0.80	0.66			0.87	0.85
FDK				0.89				0.91

All correlations are significant at P=0.0001

The r value is the degree of relationship between the two traits

FHB traits N=228

*****In 2011, only two replicates were used for FDK analysis

Appendix 2.10 Correlation coefficients (r) for disease incidence, severity, Fusarium head blight (FHB index) and Fusarium damaged kernels (FDK) calculated using pooled field data measurements from two field locations (Winnipeg and Carman,) for individuals years (2011, 2012 and 2013 nurseries)

Carman &Winnipeg 2011				Carman &Winnipeg 2012				Carman &Winnipeg 2013			
	INC	SEV	FHBI	FDK*	INC	SEV	FHBI	FDK	INC	SEV	FHBI	FDK
INC		0.86	0.87	0.84		0.74	0.96	0.84		0.85	0.97	0.86
SEV			0.99	0.87			0.86	0.60			0.93	0.79
FHBI				0.89				0.82				0.88

All correlations are significant at P=0.0001
The r value is the degree of relationship between the two traits
FHB traits N=228
***** In 2011 only two replicates were used for FDK analysis

Appendix 2.11 Correlation coefficients (r) calculated for incidence, severity, Fusarium head blight (FHB index) and Fusarium damaged kernels (FDK) using pooled field data measurements for Winnipeg and Carman (separately) across three years (2011, 2012 and 2013)

Winnipeg (2011,2012,2013)				Carman (2011,2012,2013)		
	Severity	FHB index	FDK	Severity	FHB index	FDK
Incidence	0.91	0.96	0.87	0.84	0.93	0.86
Severity		0.96	0.84		0.96	0.82
FHB index			0.90			0.89

All correlations are significant at p=0.0001
The r value is the degree of relationship between the two traits
FHB traits N=228

SUPPLEMENTAL DATA 3

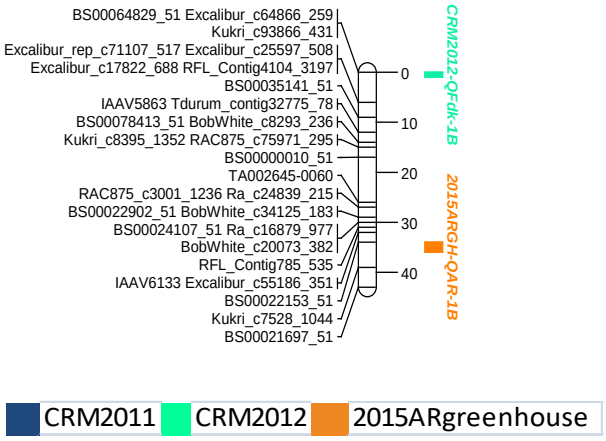
Appendix 3.1 A summary of resistance quantitative trait loci (QTL) detected using multiple interval mapping (MIM) for pooled data for incidence, severity, Fusarium head blight index (FHB index), Fusarium damaged kernels (FDK) and deoxynivalenol (DON) for Carman and Winnipeg 2011, 2012 and 2013 FHB nurseries(six environments) for 228 double haploid lines

Trait	Trial dataset	Chr. [a]	Marker at the QTL peak [b]	Interval (cM) [c]	Pos (cM) [d]	Add [e]	% PVE [f]	LOD [g]	LOD α=0.05 [h]
Incidence	6siteyrs	4A	Excalibur c23921 738	2.2	19	3.68	13.3	7.04	3.52
Incidence	6siteyrs	4B	Kukri_c26905 392	1.7	4	-7.45	38.2	23.81	3.52
Incidence	6siteyrs	4D	wMAS000002	12.0	12	9.32	47.6	32.00	3.52
Severity	6siteyrs	4A	Excalibur c23921 738	2.2	19	2.37	7.9	4.08	3.66
Severity	6siteyrs	4B	Kukri_c26905 392	1.7	4	-4.44	21.6	12.05	3.66
Severity	6siteyrs	4D	wMAS000002	12	11	6.41	35.0	21.36	3.66
Severity	6siteyrs	5B	wsnp Ex c60683 61038062	0.4	8	2.03	5.9	3.01	3.66
Severity	6siteyrs	6B	CAP8_c3629_486	0.4	0	-2.93	11.0	5.78	3.66
Severity	6siteyrs	7A	IAAV6131	4.0	54	2.47	8.3	4.28	3.66
FDK	6siteyrs	2B	wsnp Ra c4321 7860456	0.9	1	1.78	8.0	4.11	3.30
FDK	6siteyrs	4B	Kukri_c26905 392	1.7	4	-4.46	42.4	27.30	3.30
FDK	6siteyrs	4D	wMAS000002	11.9	11	5.74	54.2	38.63	3.30
FDK	6siteyrs	6B	CAP8_c3629_486	0.4	0	-2.11	14.6	7.79	3.30
FHB index	6siteyrs	4A	Excalibur c23921 738	2.2	19	2.90	8.1	4.19	3.43
FHB index	6siteyrs	4B	Kukri_c26905 392	1.7	4	-6.38	29.8	17.54	3.43
FHB index	6siteyrs	4D	wMAS000002	12.0	12	8.04	39.0	24.50	3.43
FHB index	6siteyrs	6B	CAP8_c3629_486	0.4	0	-2.69	7.8	4.01	3.43
DON	6siteyrs	4B	RAC875_c15872_141	1.7	5	-5.67	40.4	25.59	3.62
DON	6siteyrs	4D	wMAS000002	11.9	11	6.99	49.0	33.37	3.62
DON	6siteyrs	6B	CAP8_c3629_486	0.4	0	-3.81	24.1	13.63	3.62

[a] Chr., Chromosome
[b] Marker at the peak of the QTL
[c] Interval of the QTL (cM)
[d] Pos, Position on linkage group in cM
[e] Add ,Additive effect (Additive effect of allele substitution of the trait in question. A negative sign indicates that 19H*16 allele decreased the trait and vice versa)
[f] %PVE, Phenotypic variation explained (R^2; %)
[g] LOD, Peak LOD score
[h] LOD α=0.05, Significant LOD score threshold at 5%

Appendix 3.2 Linkage/genetic maps for cross 19H*16/CDC Buteo 228 double haploid lines, for specific chromosome (designated on top) and quantitative trait loci (QTL) position shown by different colored bars indicating different environments (Carman-CRM, or Winnipeg-WNP and 6Siteyrs- pooled data for CRM and WNP 2011,2012 and 2013) on the right of the linkage map. The QTL are for field incidence (QInc), severity (QSev), Fusarium head blight index (QFhb), Fusarium damaged kernels (QFdk), deoxynivalenol (QDON), height (QHt) and anther retention (QAr) for individual or pooled environment/s for 9HBT 228 double haploid population. For DON, the data are from four environments (Carman and Winnipeg 2012 and 2013) while AR data were from a greenhouse study with only 100 lines.

1B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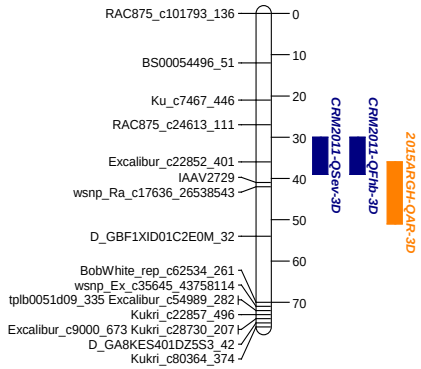
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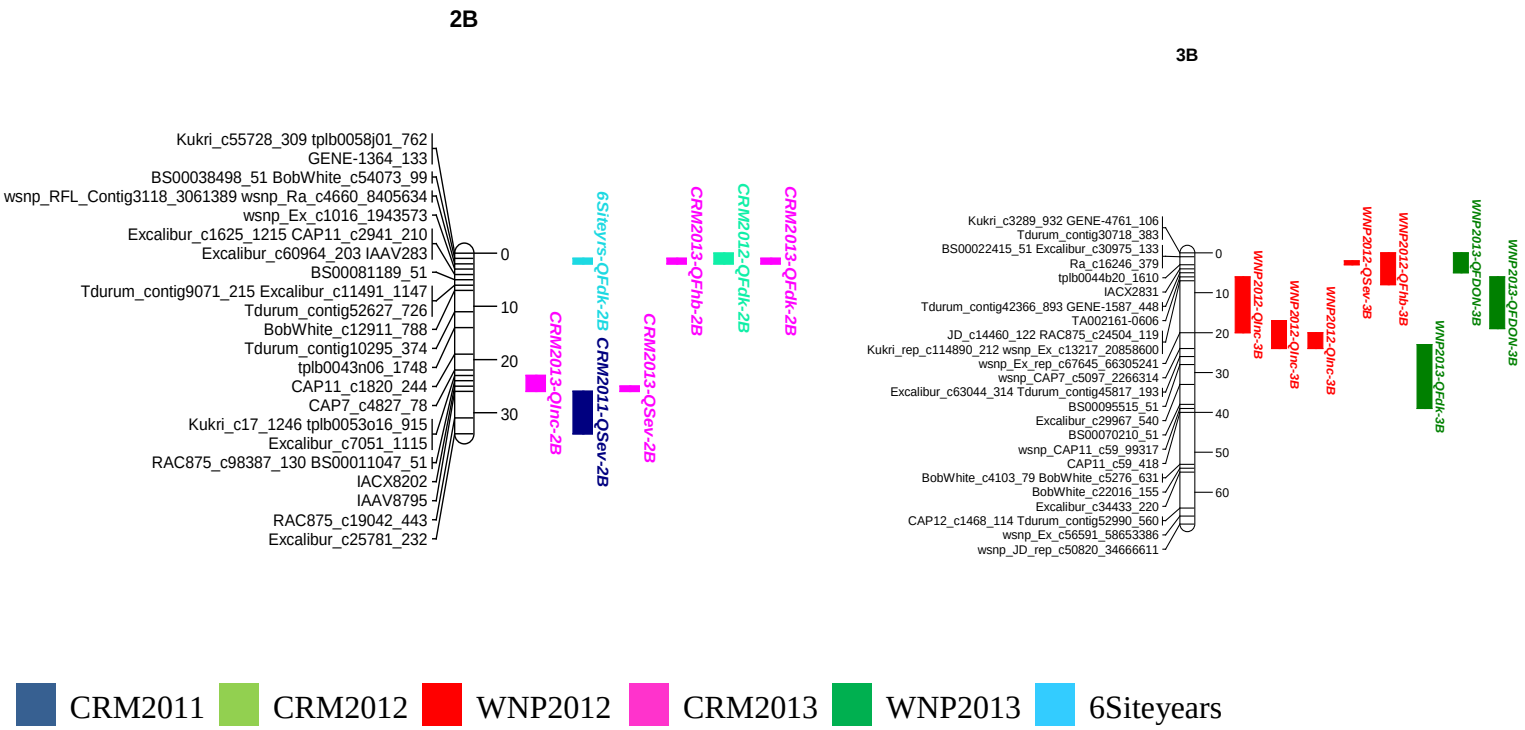
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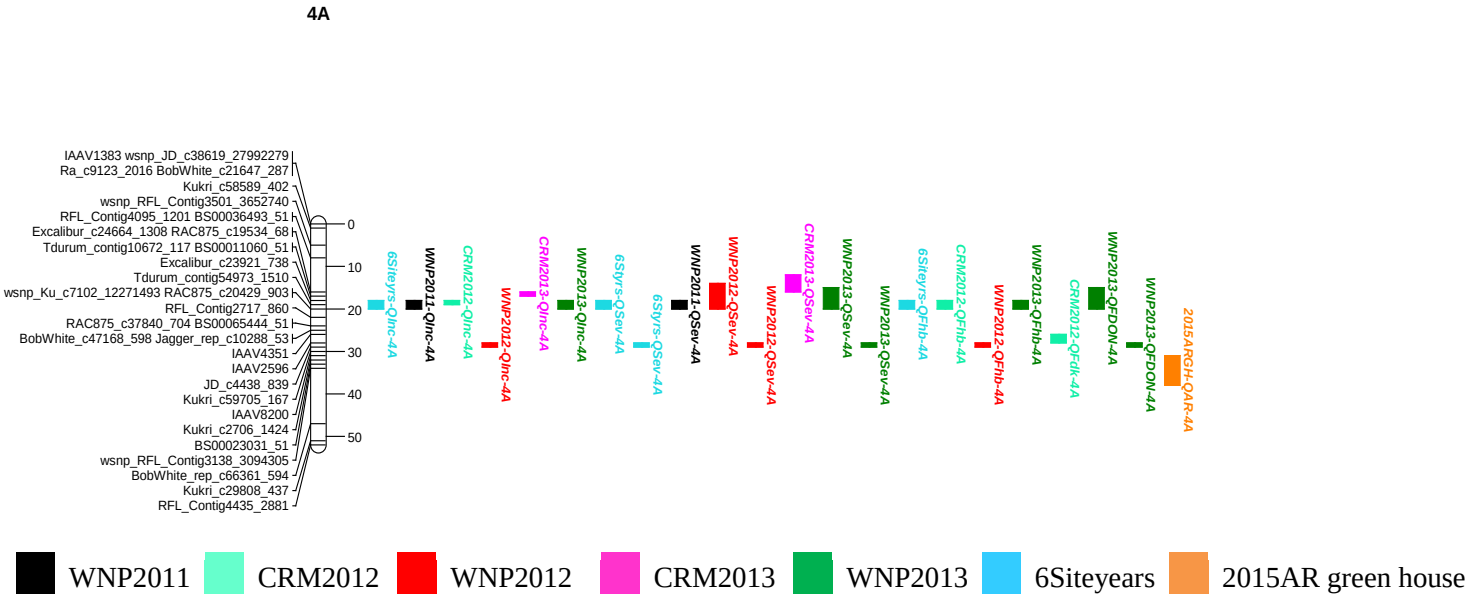
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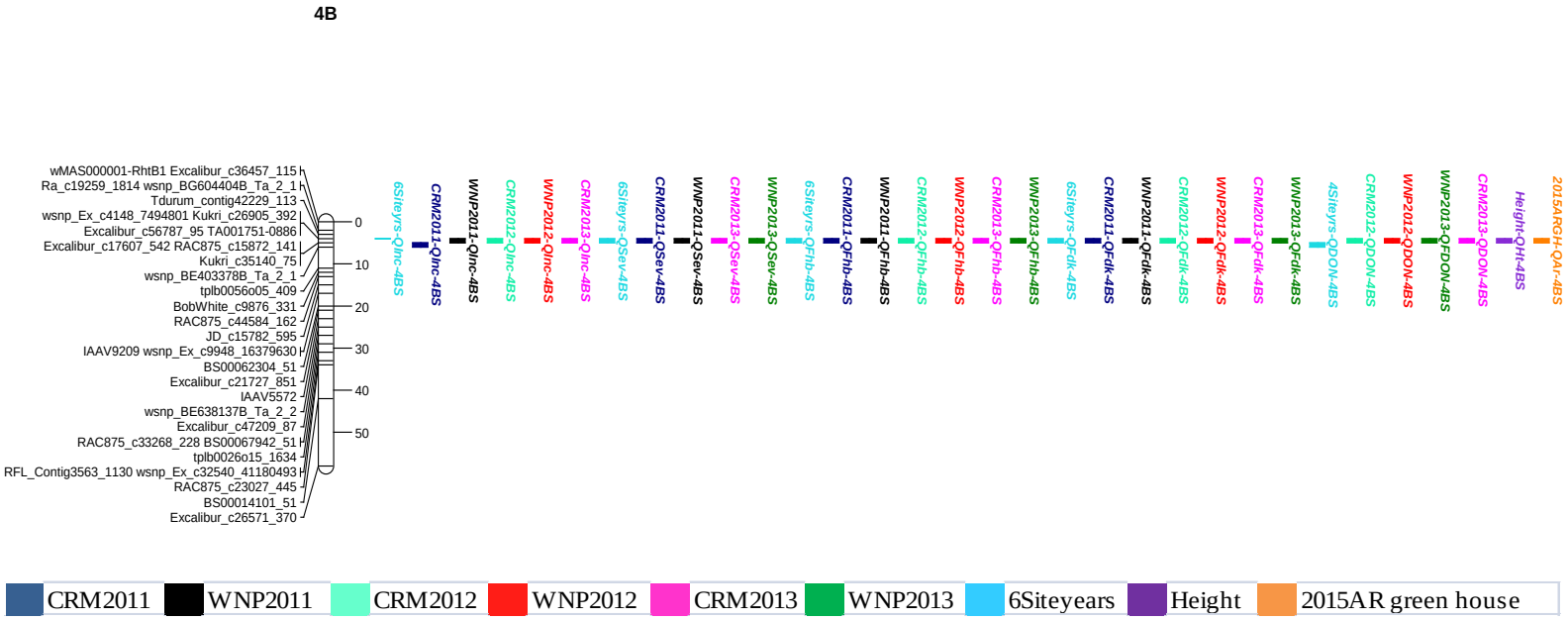
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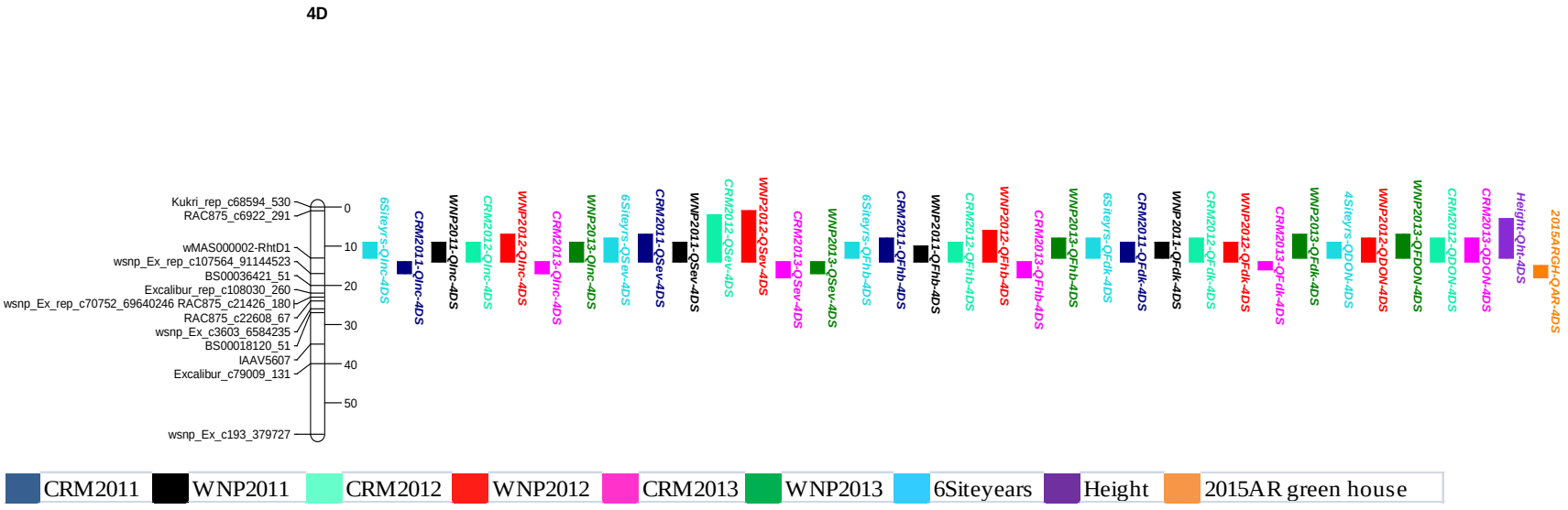
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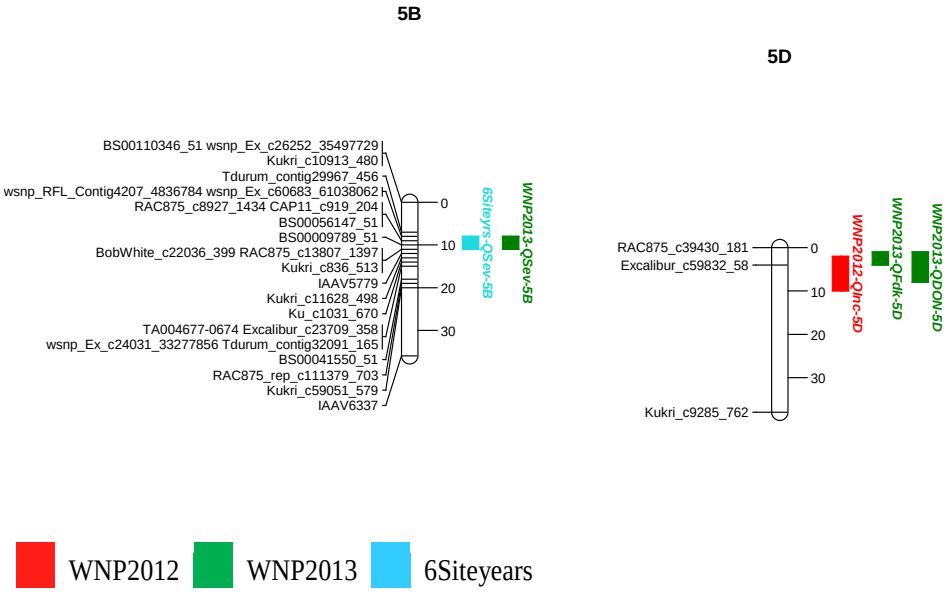
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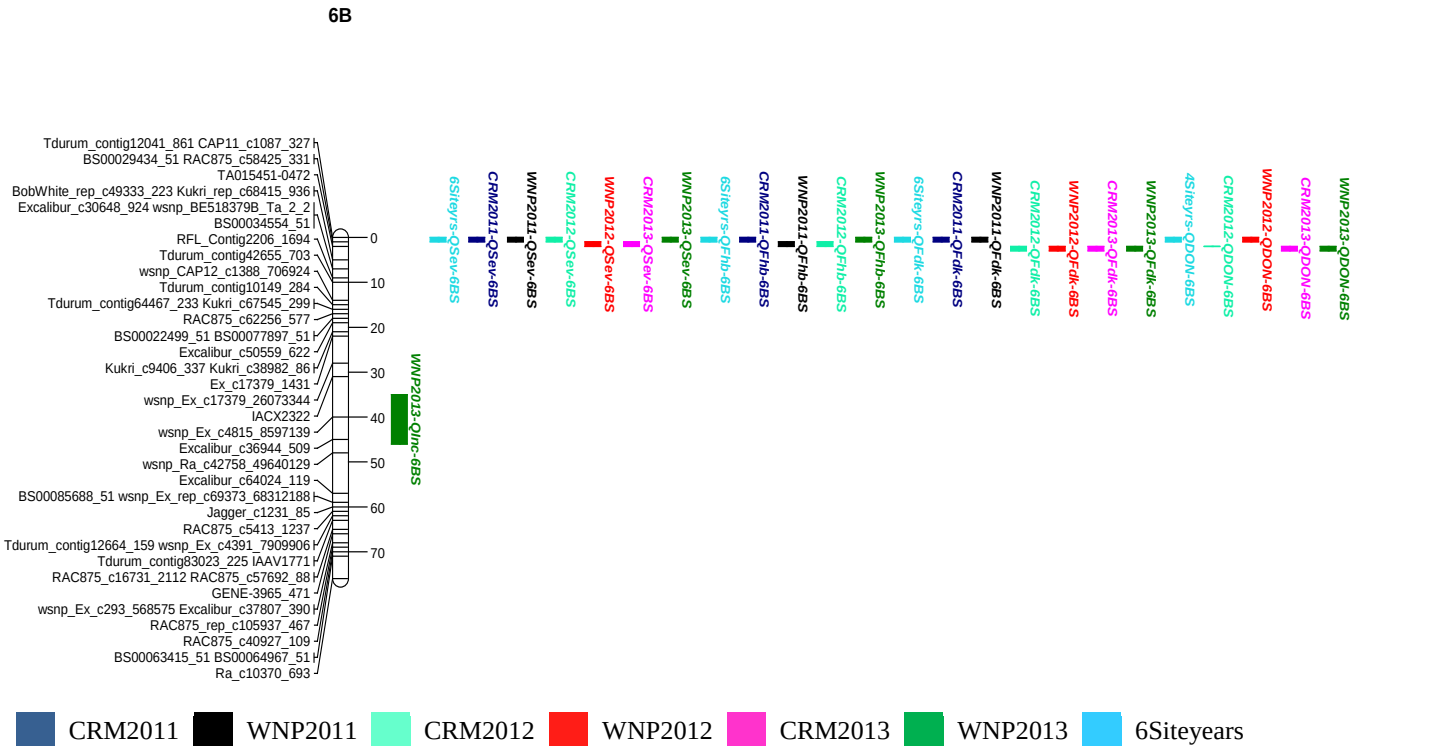
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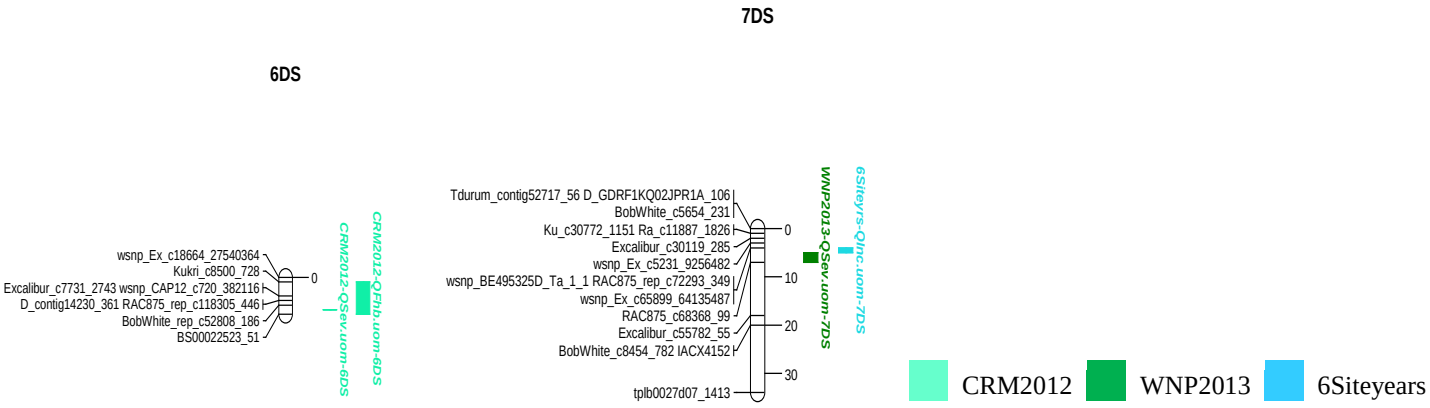
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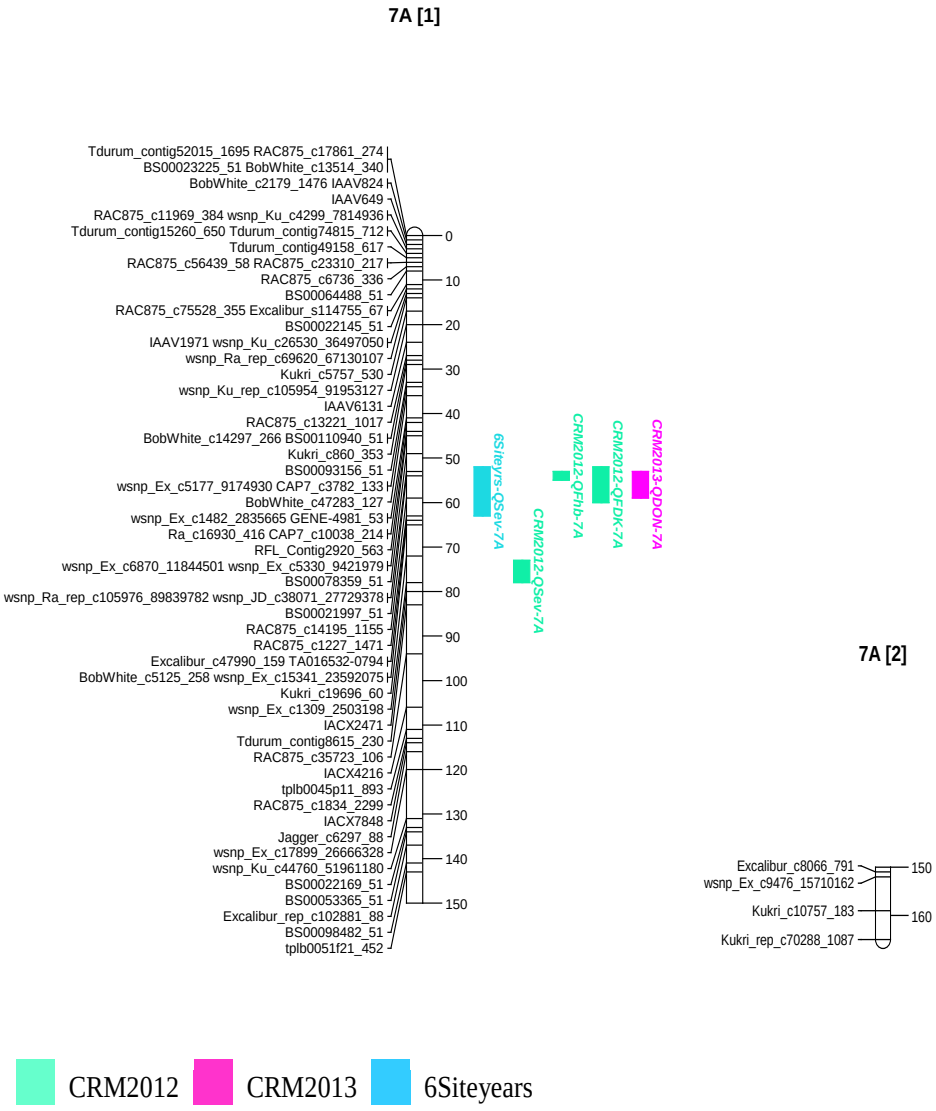
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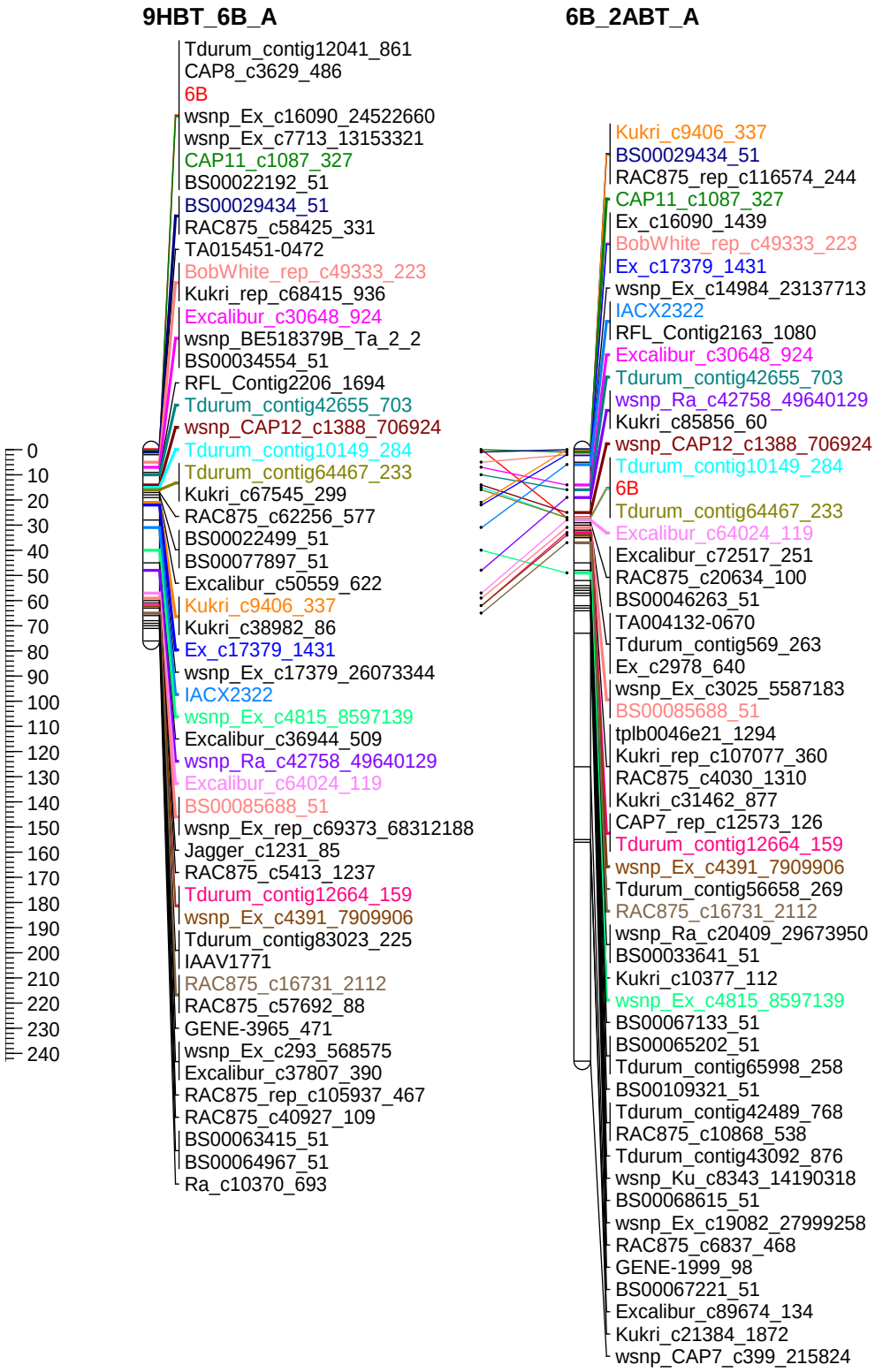
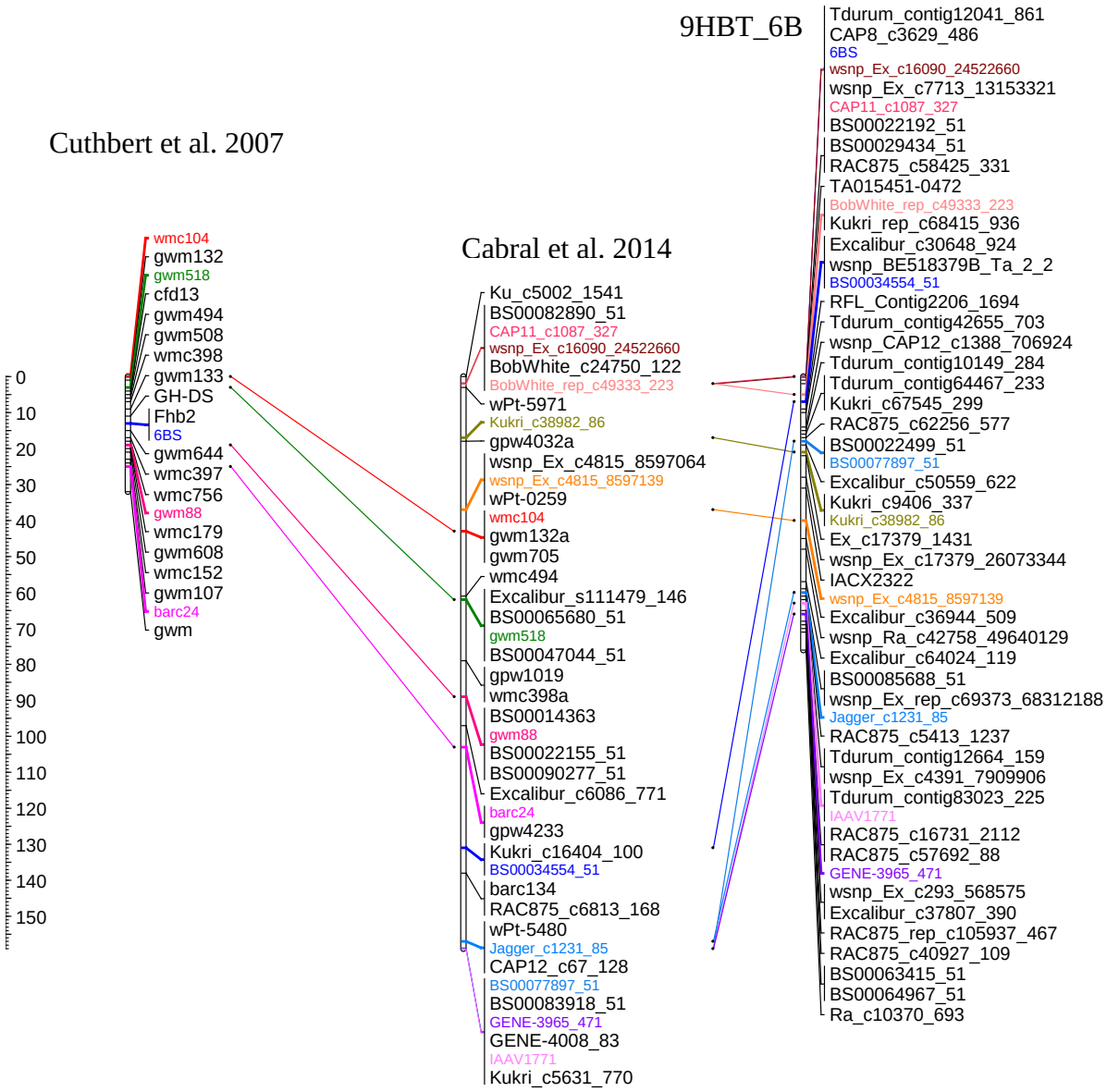
Appendix 3.2.1 Comparative mapping of 6B FHB QTL from the 9HBT and 2ABT (derived from CDC Buteo) with Fhb2 (Cuthbert et al. 2007)





Note: Cabral et al. 2014 chromosome 6B linkage map was constructed using only part of the markers reported by Cabral et al. 2014. The arrows and coloured markers show the common markers and indicate the direction of chromosomes from different studies to compare QTL locations

SUPPLEMENTAL DATA SHEET 4

Appendix 4.1 Analysis of variance table for disease incidence, severity, Fusarium head blight index (FHB index) and Fusarium damaged kernels (FDK) for individual Fusarium head blight nurseries for the cross 22A*13/CDC Buteo cross (218 double haploid lines) for Carman or Winnipeg for 2011, 2012 and 2013 cropping seasons

Table 2a: Winnipeg 2011 FHB nursery

Source of Variation	DF	Incidence (%)		Severity (%)		FHB index (%)		*FDK (%)		
		MS	Pr > F	MS	Pr > F	MS	Pr > F	DF	MS	Pr > F
Rep	2	3739.4	0.0017	1714.3	0.0048	1108.3	0.0007	1	38.7	0.151
Genotype	216	1083.2	<.0001	589.4	<.0001	401.7	<.0001	216	23.9	0.036
Error:	428	579.5		317.6		150.2		214	18.7	

b: Carman2011 FHB nursery

Source of variation	DF	Incidence (%)		Severity (%)		FHB index (%)		*FDK (%)		
		MS	Pr > F	MS	Pr > F	MS	Pr > F	DF	MS	Pr > F
Genotype	216	575.1	<.0001	572.0	<.0001	511.4	<.0001	216	32.5	<.0001
Rep	2	5292.5	<.0001	4142.7	<.0001	4388.7	<.0001	1	178.3	0.0010
Error	434	308.9		200.3		176.2		215	15.9	

c: Winnipeg FHB nursery in 2012

Source of variation	DF	Incidence (%)		Severity (%)		FHB index (%)		FDK (%)	
		MS	Pr > F	MS	Pr > F	MS	Pr > F	M S	Pr > F
Genotype	216	310.9	<.0001	186.2	0.0012	172.9	<.0001	3.1	<.0001
Rep	2	1,136.3	0.0004	198.7	0.2208	535.3	0.0014	13.5	0.0001
Error	435	140.5		131.1		80.3		1.5	

d: Carman nursery in 2012

Source of variation	DF	Incidence (%)		Severity (%)		FHB index (%)		FDK (%)		
		MS	Pr > F	MS	Pr > F	MS	Pr > F	DF	MS	Pr > F
Genotype	216	301.9	<.0001	136.6	<.0001	95.1	<.0001	216	7.5	<.0001
Rep	2	372.5	0.026	1629.6	<.0001	484.9	<.0001	2	31.6	0.0002
Error	427	101.2		68.6		32.7		425	3.7	

e: Winnipeg nursery in 2013

Source of variation	DF	Incidence (%)		Severity (%)		FHB index (%)		FDK (%)	
		MS	Pr > F	MS	Pr > F	MS	Pr > F	MS	Pr > F
Genotype	216	274.9	<.0001	282.4	<.0001	231.2	<.0001	59.6	<.0001
Rep	2	2,910.7	<.0001	123.2	0.27	1,017.7	<.0001	766	<.0001
Error	435	116.0		93.5		80.5		35	

f: Carman nursery in 2013

		Incidence (%)		Severity (%)		FHB index (%)		FDK (%)	
Source of variation	DF	MS	Pr > F	MS	Pr > F	MS	Pr > F	MS	Pr > F
Genotype	216	242.2	<.0001	234.0	<.0001	183.1	<.0001	110.6	<.0001
Rep	2	28638	<.0001	30261	<.0001	33140	<.0001	2207.4	<.0001
Error	423	152.8		117.6		98.5		53.3	

*****FDK degrees of freedom are different from other traits because in 2011 only two replicates were used in FDK analysis

Appendix 4.2-4.6 Histograms for 22A*13/CDC Buteo cross (218 double haploid lines) for pooled data for Carman and Winnipeg for 2011, 2012, 2013 Fusarium head blight nurseries and for pooled data for Carman or Winnipeg three site years

Appendix 4.2 Analysis of variance table for disease incidence, severity, Fusarium head blight index (FHB index) and Fusarium damaged kernels (FDK) after pooling data for 22A*13/CDC Buteo crossfor Carman and Winnipeg 2011 Fusarium head blight nurseries

		Incidence (%)		Severity (%)		FHB index (%)		*FDK (%)		
Source of variation	DF	MS	Pr > F	MS	Pr > F	MS	Pr > F	DF	MS	Pr > F
Siteyear	1	131574	0.005	2266.3	0.4	23886	0.04	1	3805.8	0.03
Rep(siteyear)	4	4481.0	<.0001	3011.8	<.0001	2700.3	<.0001	2	109.4	0.002
Genotype	217	1074.6	<.0001	834.3	<.0001	652.8	<.0001	217	37.1	<.0001
Siteyear*Genotype	216	582.2	0.005	333.1	0.008	251.4	<.0001	217	18.9	0.2
Error	866	444.6		258.9		162.2		431	17.2	

*****FDK degrees of freedom different from other traits because in 2011 only two replicates were used in FDK analysis

Appendix 4.3 Analysis of variance table for disease incidence, severity, Fusarium head blight index (FHB index) and Fusarium damaged kernels (FDK) after pooling data for Carman and Winnipeg 2012 Fusarium head blight nurseries for the cross 22A*13/CDC Buteo (218 double haploid lines)

		Incidence (%)		Severity (%)		FHB index (%)		*FDK (%)		
Source of variation	DF	MS	Pr > F	MS	Pr > F	MS	Pr > F	DF	MS	Pr>F
Siteyear	1	12668	0.01	109345	0.0004	4558.6	0.04	1	1149.6	0.002
Rep(siteyear)	4	754.4	<.0001	914.2	<.0001	510.0	<.0001	4	22.5	<.0001
Genotype	216	484.8	<.0001	212.9	<.0001	208.4	<.0001	216	7.7	<.0001
Siteyear*Genotype	216	129.2	0.3	110.3	0.2	59.4	0.3	216	2.9	0.1
Error	862	121.0		100.1		56.7		860	2.6	

Appendix 4.4 Analysis of variance table for disease incidence, severity, Fusarium head blight index (FHB index) and Fusarium damaged kernels (FDK) after pooling data for 22A*13/CDC Buteo cross (218 double haploid lines) for Carman and Winnipeg 2013 Fusarium head blight nurseries

		Incidence (%)		Severity (%)		FHB index (%)		*FDK (%)		
Source of variation	DF	MS	Pr > F	MS	Pr > F	MS	Pr > F	DF	MS	Pr > F
siteyear	1	6657.1	0.6	1685.3	0.76	1333.3	0.8	1	2233.4	0.3
BLOC(siteyear)	4	15774	<.0001	15192	<.0001	17079	<.0001	4	1486.7	<.0001
Genotype	216	332.0	<.0001	409.6	<.0001	302.6	<.0001	216	117.3	<.0001
Siteyear*Genotype	216	185.5	0.0009	105.6	0.5	111.3	0.02	216	53.5	0.03
Error: MS(Error)	858	134.1		105.4		89.4		859	44.0	

*****FDK degrees of freedom are different from other traits because in 2011 only two replicates were used in FDK analysis

Appendix 4.5 Analysis of variance table for disease incidence, severity, Fusarium head blight index (FHB index) and Fusarium damaged kernels (FDK) for Winnipeg after pooling data from Winnipeg 2011, 2012 and 2013 Fusarium head blight nurseries for 22A*13/CDC Buteo cross (218 double haploid lines)

		Incidence (%)		Severity (%)		FHB index (%)		*FDK (%)		
Source of variation	DF	MS	Pr > F	MS	Pr > F	MS	Pr > F	DF	MS	Pr > F
Siteyear	2	64888	0.001	152435	<.0001	27914	0.0005	2	3815	<.0001
Rep(siteyear)	6	2595.8	<.0001	728.6	0.0005	858.6	<.0001	5	319.5	<.0001
Genotype	216	946.3	<.0001	602.2	<.0001	532.7	<.0001	217	41.1	<.0001
Siteyear*Genotype	432	357.8	0.0006	231.1	0.0006	133.3	0.0004	432	22.3	0.0067
Error	1300	278.7		180.5		102.9		1085	18.3	

*****FDK degrees of freedom are different from other traits because in 2011 only two replicates were used in FDK analysis

Appendix 4.6 Analysis of variance for disease incidence, severity, Fusarium head blight index (FHB index) and Fusarium damaged kernels (FDK) for the cross 22A*13/CDC Buteo (218 double haploid lines) after pooling data from Carman 2011, 2012 and 2013 Fusarium head blight nurseries

		Incidence (%)		Severity (%)		FHB index (%)		*FDK (%)		
Source of variation	DF	MS	Pr > F	MS	Pr > F	MS	Pr > F	DF	MS	Pr > F
siteyear	2	167623	0.0048	102662	0.017	43976	0.099	2	41510	0.0006
Rep(siteyear)	6	11434	<.0001	12011	<.0001	12671	<.0001	5	931.7	<.0001
Genotype	216	589.1	<.0001	526.2	<.0001	446.06	<.0001	217	71.2	<.0001
siteyear*Genotype	432	263.5	<.0001	207.9	<.0001	170.9	<.0001	432	37.8	<.0001
Error: MS(Error)	1284	188.4		129.2		102.9		1065	25.9	

*****FDK degrees of freedom are different from other traits because in 2011 only two replicates were used in FDK analysis

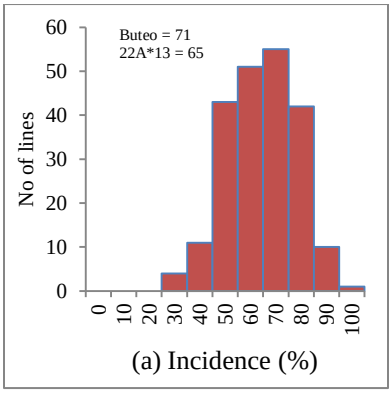
Traits variations and correlations

Appendix 4.7 Mean and range for field incidence, severity, Fusarium head blight index (FHB index) and Fusarium damaged kernels (FDK) across each site and year measured on the cross 22A*13/CDC Buteo for 2011, 2012 and 2013 Fusarium head blight nurseries in Carman or Winnipeg

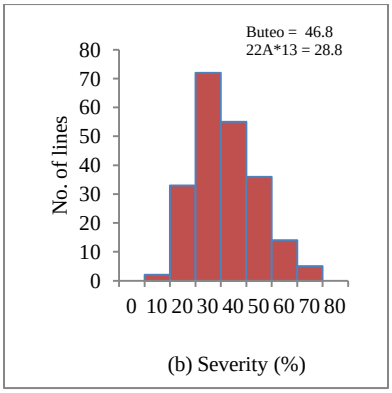
Winnipeg							Carman				
		Parents		DH population			Parents		DH Population		
Year	Trait	Buteo	22A*13	Mean	Min	Max	Buteo	22A*13	Mean	Min	Max
2011	Incidence	73.0	70.0	49.4	6.7	94.6	77.5	47.5	69.6	29.3	96.7
	Severity	43.0	25.0	30.5	6.7	81.6	60	22.5	33.4	3.7	70
	FHB index	33.0	17.5	16.5	0.8	69	46.5	10.75	25.2	1.4	65.7
	FDK	8.0	8.5	9.1	2.3	19.8	26.3	9.75	13.3	5.3	26.3
2012	Incidence	28.0	52.5	31.1	8.5	80.8	32	47.2	37.4	18.0	74.2
	Severity	47.5	61.7	55.0	29.7	72.5	35.8	40.0	36.6	17.0	52.5
	FHB index	16.0	33.6	18.1	2.9	54	11.4	18.8	14.3	5.5	34.6
	FDK	0.3	1.83	1.5	0.0	6.3	2.5	4.5	3.4	0.5	11.3
	DON	0.7	1.6				5.5	9.0			
2013	Incidence	40.8	47.3	47.2	22	85	54.2	51.7	51.9	29.2	75.5
	Severity	51.7	55.9	59.0	37	82.5	58.3	55.0	56.9	33.7	79.7
	FHB index	22.0	26.5	28.6	12.7	55.7	34.8	31.2	30.8	14.4	57.1
	FDK	15.3	12.3	16.8	7.3	32.5	24.5	14.8	19.5	7.3	38.8
	DON	28.0	39.0				52.5	42.5			

*DON analyses were done on pooled samples across the replicates

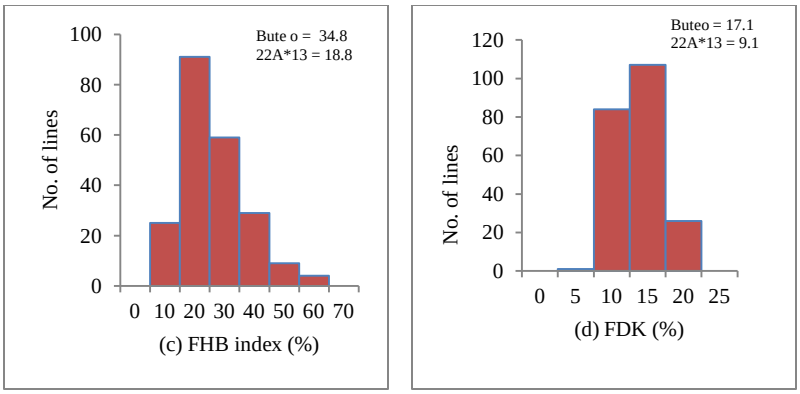
Appendix 4.8- 4.10 Histograms for the cross 22A*13/CDC Buteo (218 double haploid lines) for pooled data for Carman and/or Winnipeg 2011, 2012 and 2013 Fusarium head blight nurseries



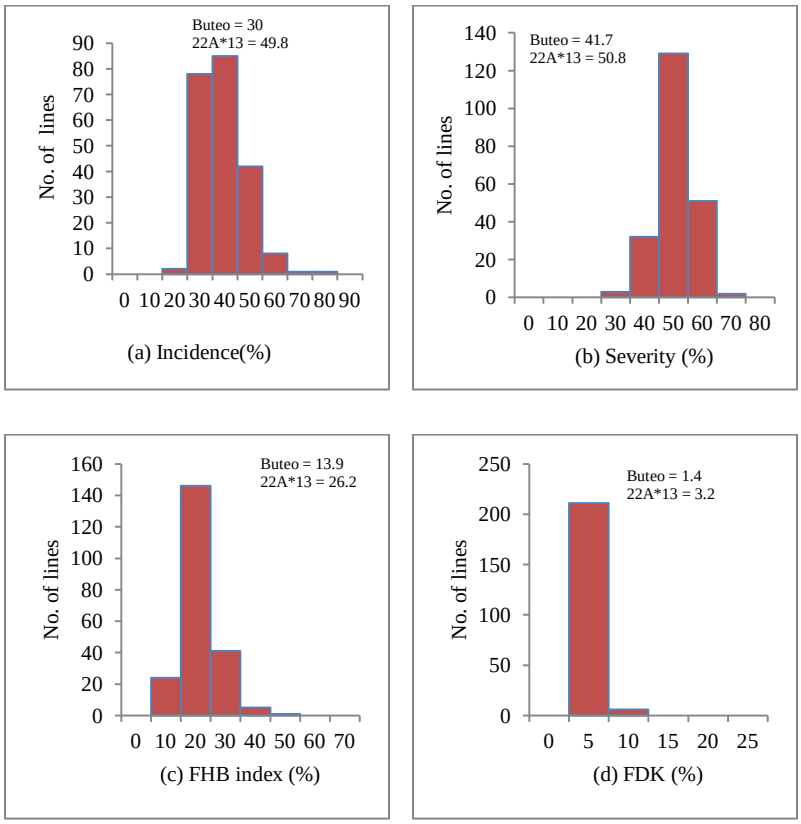
(a) Incidence (%)



(b) Severity (%)

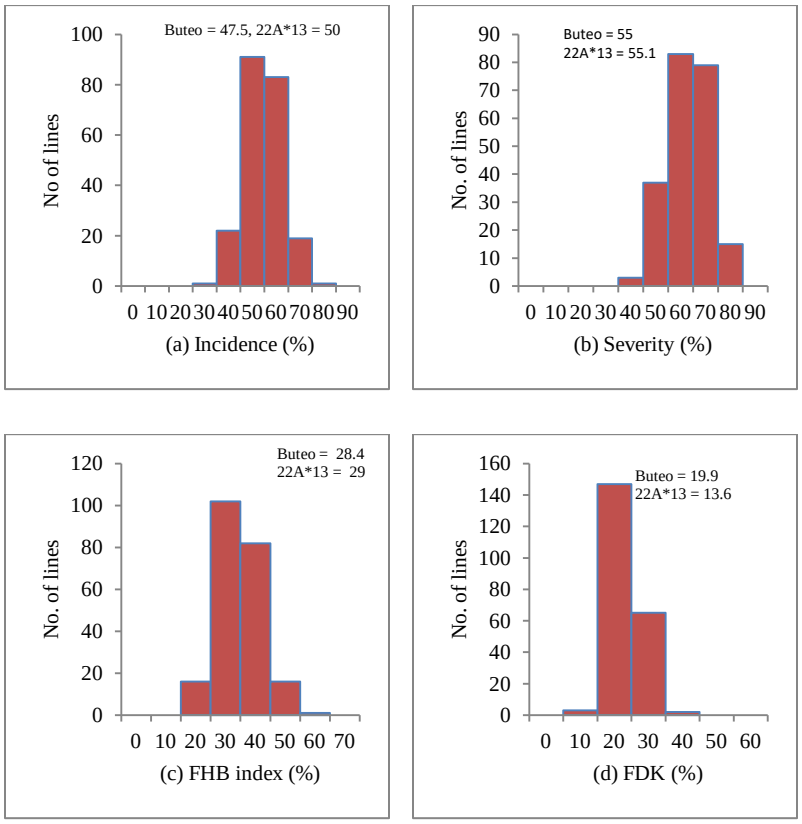


Appendix 4.8 Phenotypic distribution based on FHB infection of 218 DH from the cross 22 A*13/ CDC Buteo. Percent infection was measured from pooled replicated field disease nursery in Carman and Winnipeg 2011. Traits included), disease incidence (a), disease severity (b), Fusarium head blight index (FHB index = % incidence x % severity), and Fusarium damaged kernels (FDK) (d). Resistant and susceptible parent showed infection phenotypes within the respective modes of the distributions



Appendix 4.9 Phenotypic distribution based on FHB infection of 218 DH lines from the cross 22 A*13/ CDC Buteo. Percent infection was measured from combined replicated field disease nurseries in Carman and Winnipeg 2012. Traits included disease incidence (a), disease severity (b), Fusarium head blight index (c) (FHB index = % incidence x 5 severity), and Fusarium

damaged kernels (FDK) (d). Resistant and susceptible parent showed infection phenotypes within the respective modes of the distributions



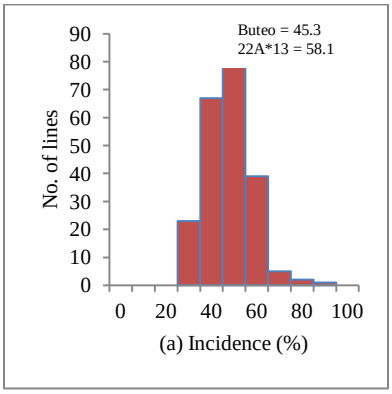
Appendix 4.10 Phenotypic distribution based on FHB infection of 218 DH lines from the cross 22 A*13/ CDC Buteo. Percent infection was measured from combined replicated field disease nursery in Carman and Winnipeg 2013. Traits included disease incidence (a), disease severity (b)FHB index (c) (FHB index= % incidence x % severity), and Fusarium damaged kernels (FDK). Resistant and susceptible parent showed infection phenotypes within the respective modes of the distributions

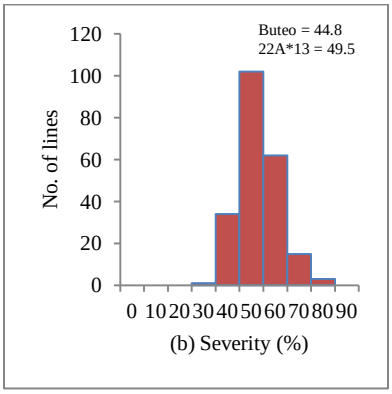
Appendix 4.11 Mean and range of the trait values by pooling data for Carman and Winnipeg Fusarium head blight nurseries for each year (2011,2012 and 2013) for four FHB variables (incidence, severity, Fusarium head blight index (FHB index), and Fusarium damaged kernels (FDK) measured for the cross 22A*13/CDC Buteo (218 double haploid lines)

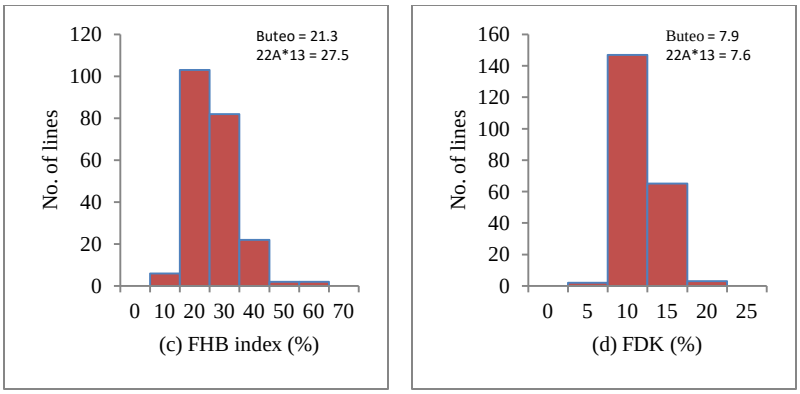
Carman and Winnipeg pooled data for 2011,2012 and 2013

		Parents		DH Lines		
Year	Trait	Buteo	22A*13	Mean	Min	Max
2011	Incidence	71.0	65.0	59.51	20.2	94
	Severity	46.8	28.8	31.9	7.3	66
	FHB index	34.8	18.8	20.8	2.0	55.4
	FDK	17.1	9.1	11.2	3.9	19.9
2012	Incidence	30.0	49.8	34.2	18.1	77.5
	Severity	41.7	50.8	45.8	28.8	61.7
	FHB index	13.9	26.2	16.2	5.3	41.5
	FDK	1.42	3.2	2.4	0.3	8.8
2013	Incidence	47.5	50.0	50.0	29.8	79.3
	Severity	55.0	55.1	58.0	37.6	75.6
	FHB index	28.4	29.0	29.7	14.3	52.5
	FDK	19.9	13.6	18.1	7.3	35.7

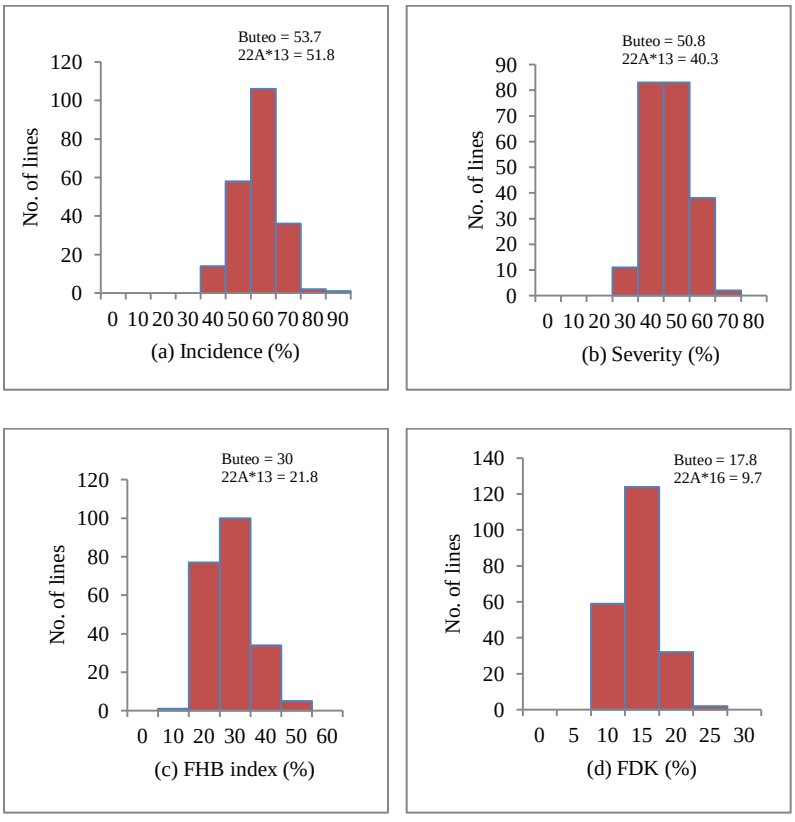
Appendix 4.12- 4.13 Histograms for the cross 22A*13/CDC Buteo (218 double haploid lines) pooled data for individual environments (Carman or Winnipeg) combined for three site years separately (2011, 2012 and 2013**)**







Appendix 4.12 Phenotypic distribution based on Fusarium head blight infection of 218 double haploid lines from the cross 22 A*13/ CDC Buteo. Percent infection was measured from combined replicated field disease nursery in Winnipeg for 2011, 2012 and 2013. Traits included disease incidence (a), disease severity (b), Fusarium head blight index (FHB index = % incidence x % severity), and Fusarium damaged kernels (FDK) (d). Resistant and susceptible parent showed infection phenotypes within the respective modes of the distributions



Appendix 4.13 Phenotypic distribution based on Fusarium head blight infection of 218 double haploid lines from the cross 22A*13/ CDC Buteo. Percent infection was measured from combined replicated field disease nursery in Carman for 2011, 2012 and 2013. Traits included disease incidence (a), disease severity (b), Fusarium head blight index (FHB index =% incidence

x % severity), and Fusarium damaged kernels (FDK) (d). Resistant and susceptible parent showed infection phenotypes within the respective modes of the distributions

Appendix 4.14 Mean and range of disease incidence, severity, Fusarium head blight index (FHB index) Fusarium damaged kernels (FDK) measured in Winnipeg or Carman after pooling data for three site years (2011,2012 and 2013) per location for the cross 22A*13/CDC Buteo (218 double haploid lines)

	Winnipeg					Carman				
	Parents		DH Population			Parents		DH Population		
Trait	Buteo	22A*13	Mean	Min	Max	Buteo	22A*13	Mean	Min	Max
Incidence	45.3	58.1	42.5	5.6	86.1	53.7	51.8	53.0	30.9	81.1
Severity	44.8	49.5	48.2	29.8	76.1	50.8	40.3	42.2	24.3	63.2
FHB index	21.3	27.5	21.1	7.9	54.4	30.0	21.8	23.4	9.9	45.6
FDK	7.9	7.6	9.2	4.5	16	17.8	9.7	11.9	6.1	23.5

Appendix 4.15 -14a-14c Correlations coefficients between disease incidence, severity, Fusarium head blight index (FHB index) and Fusarium damaged kernels (FDK) for the cross 22A*13/CDC Buteo (218 double haploid lines) for individual environments for Carman or Winnipeg 2011, 2012 or 2013 Fusarium head blight nurseries

14a: Winnipeg and Carman nurseries in 2011

	Winnipeg 2011			Carman 2011		
Trait	Severity	FHB index	FDK	Severity	FHB index	FDK
Incidence	0.32	0.70	0.60	0.61	0.74	0.38
Severity		0.83	0.35		0.96	0.45
FHB index			0.53			0.48

All correlations for all traits are significant at p>.0001

14b: Winnipeg and Carman nurseries in 2012

	Winnipeg 2012				Carman 2012			
Trait	Severity	FHB index	FDK	DON	Severity	FHB index	FDK	DON
Incidence	0.64	0.97	0.54	0.51	0.54	0.91	0.69	0.74
Severity		0.77	0.37	0.29		0.81	0.47	0.47
FHB index			0.55	0.48			0.69	0.72
FDK				0.64				0.79

All correlations for all traits are significant at p>.0001

14c: Winnipeg and Carman nursery in 2013

	Winnipeg 2013				Carman 2013			
Trait	Severity	FHB index	FDK	DON	Severity	FHB index	FDK	DON
Incidence	0.48	0.88	0.58	0.66	0.28	0.81	0.33	0.29
Severity		0.82	0.52	0.50		0.76	0.49	0.42
FHB index			0.64	0.68			0.52	0.44
FDK				0.72				0.62

All correlations for all traits are significant at p>.0001

Appendix 4.16 Correlations coefficients for the cross 22A*13/CDC Buteo (218 double haploid lines) between disease incidence, severity, Fusarium head blight index (FHB index) and Fusarium damaged kernels (FDK) after pooling data for both Carman and Winnipeg in each year (2011, 2012 or 2013)

Carman and Winnipeg 2011				Carman and Winnipeg 2012			Carman and Winnipeg 2013		
Trait	Severity	FHB index	FDK	Severity	FHB index	FDK	Severity	FHB index	FDK
Incidence	0.54	0.77	0.59	0.67	0.96	0.70	0.42	0.84	0.50
Severity		0.92	0.50		0.82	0.48		0.83	0.56
FHB index			0.60			0.68			0.63

All correlations for all traits are significant at <.0001

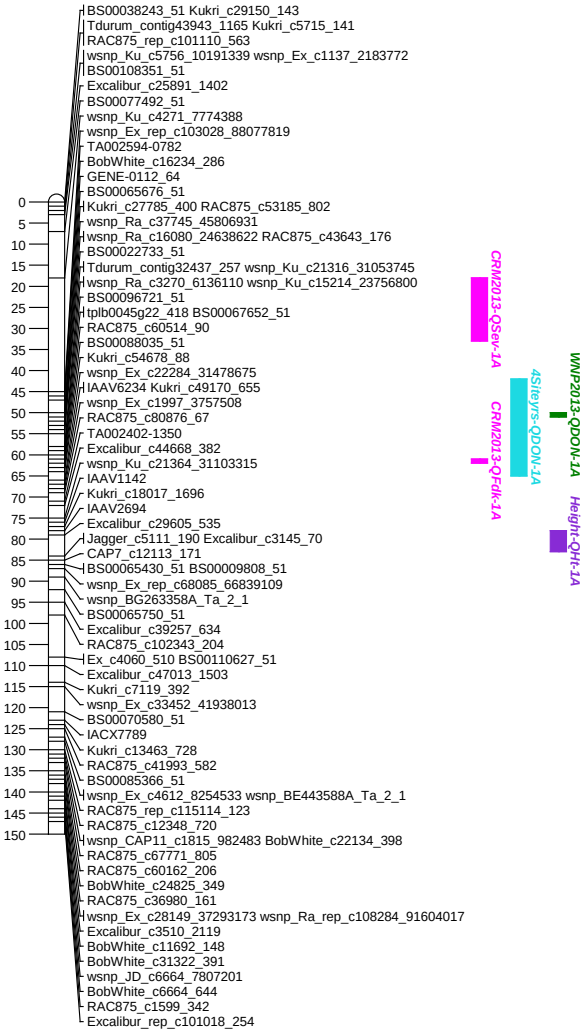
Appendix 4.17 Correlations coefficients for the cross 22A*13/CDC Buteo (218 double haploid lines) between disease incidence, severity, Fusarium head blight index (FHB index) and Fusarium damaged kernels (FDK) after pooling data for one location (Carman or Winnipeg) for three site years (2011, 2012 and 2013 Fusarium head blight nurseries)

Winnipeg 2011, 2012, 2013				Carman 2011, 2012, 2013		
	Severity	FHB index	FDK	Severity	FHB index	FDK
Incidence	0.60	0.88	0.61	0.58	0.83	0.50
Severity		0.87	0.50		0.91	0.62
FHB index			0.61			0.64

* All correlations for all traits are significant at <.0001

Appendix 4.18 Linkage maps for the cross 22A*13/CDC Buteo population(218 DH lines), specific chromosome (designated on top) and quantitative trait loci (QTL) position shown by different colored bars indicating different environments (Carman-CRM, or Winnipeg-WNP and 6Siteyrs- pooled data for Carman and Winnipeg 2011, 2012 and 2013) on the right of the linkage map. The QTL are for field incidence (QInc), severity (QSev), Fusarium head blight index (QFhb), Fusarium damaged kernels (QFdk), deoxynivalenol (QDON) and height (QHt) for individual or pooled environment/s. For DON, the data are from four environments (Carman and Winnipeg 2012 and 2013) and for height the data are from one environment

1A [1]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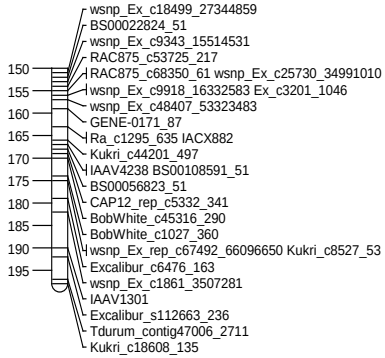
WNP2013

1A [2]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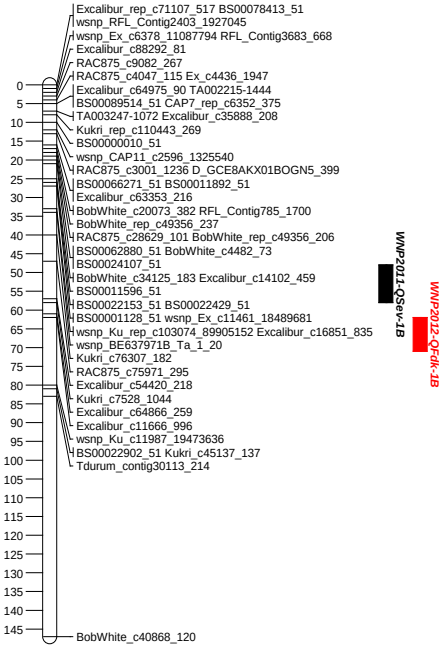
1B



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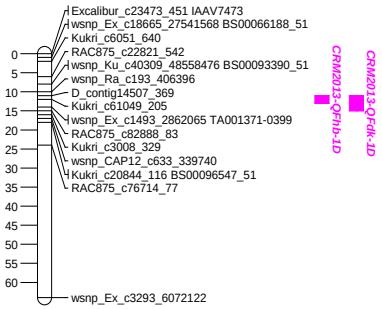
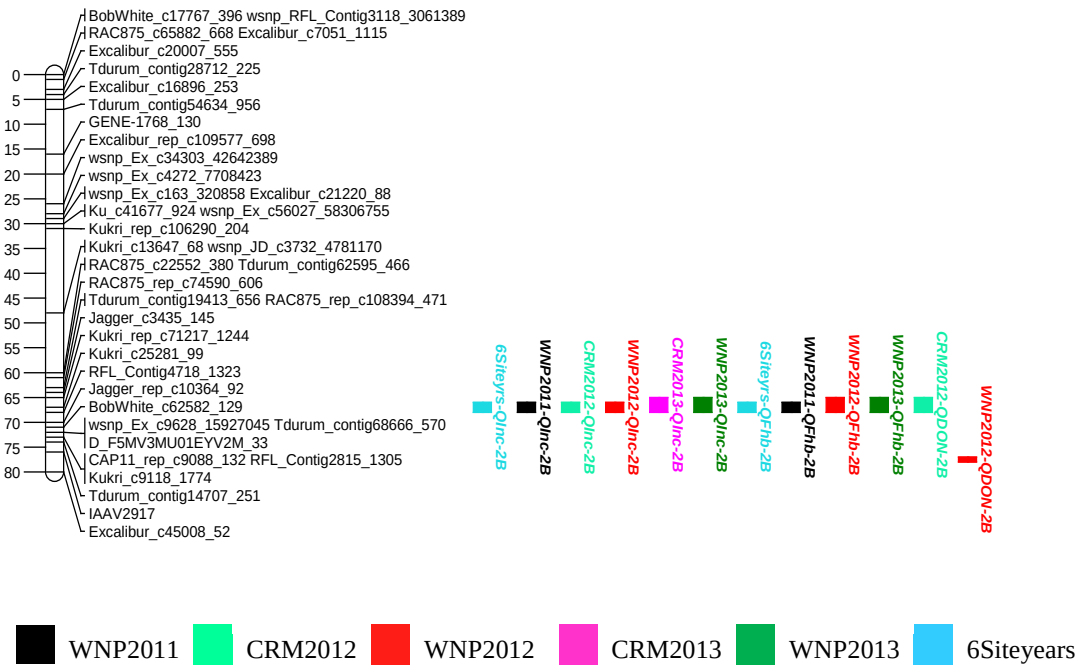


WNP2011 WNP2012 CRM2013

2B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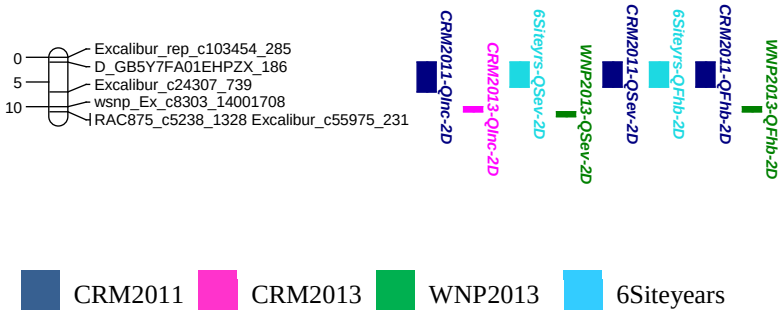
WNP2012
CRM2013
WNP2013
6Siteyears

2D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CRM2013-Qlnc-2D
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CRM2011-QSev-2D
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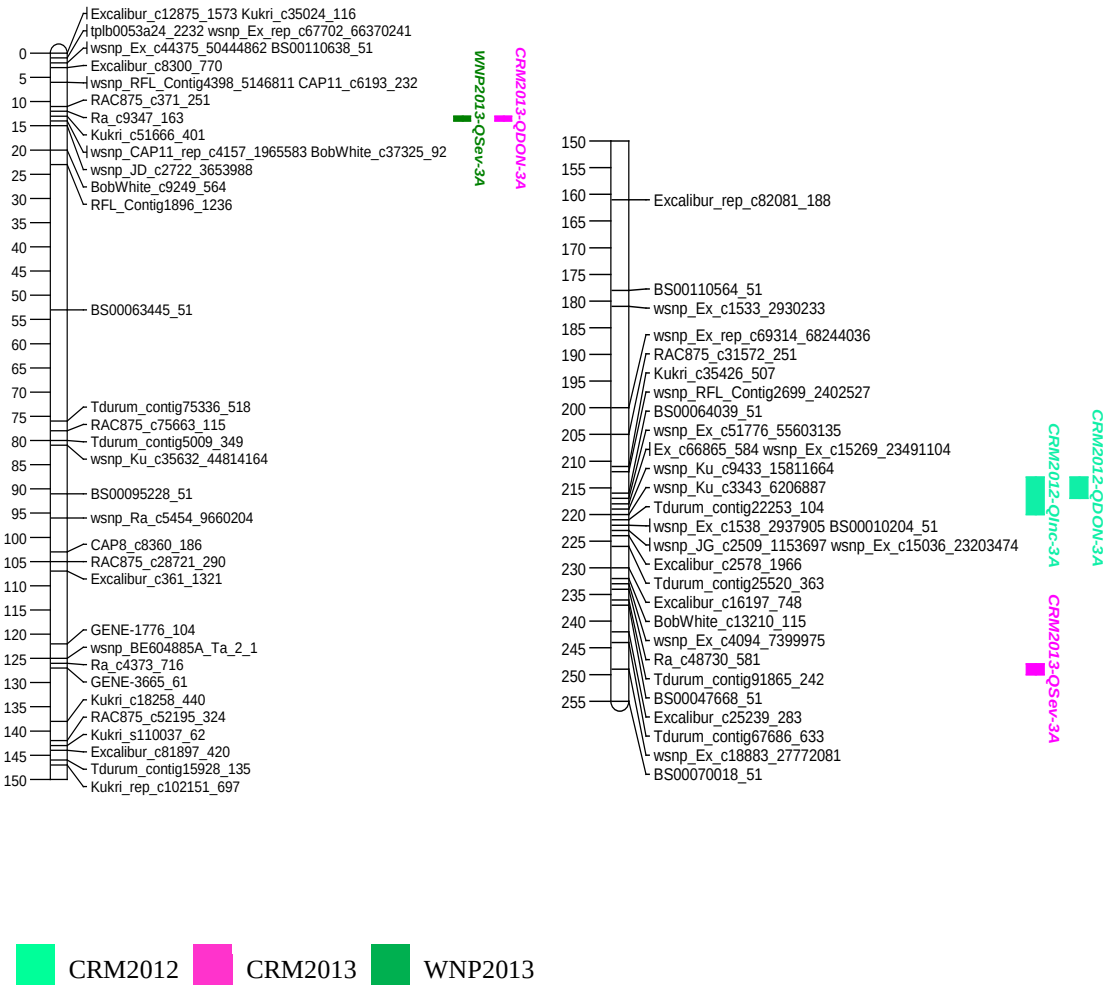
CRM2011
CRM2013
WNP2013
6Siteyears

3A [1]

3A [2]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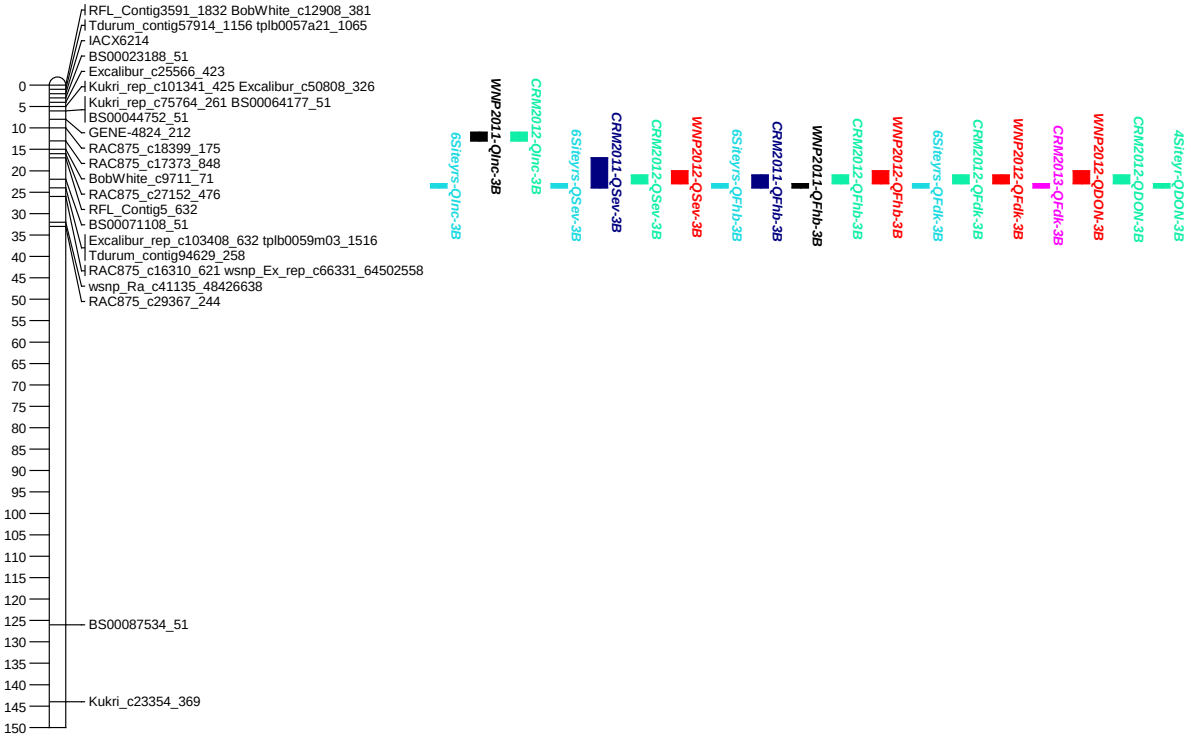
WNP2013

3B [1]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CRM2012-QSev-3B
WNP2012-QSev-3B
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CRM2011-QFhb-3B
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CRM2012-QFhb-3B
WNP2012-QFhb-3B
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CRM2012-QFdk-3B
WNP2012-QFdk-3B
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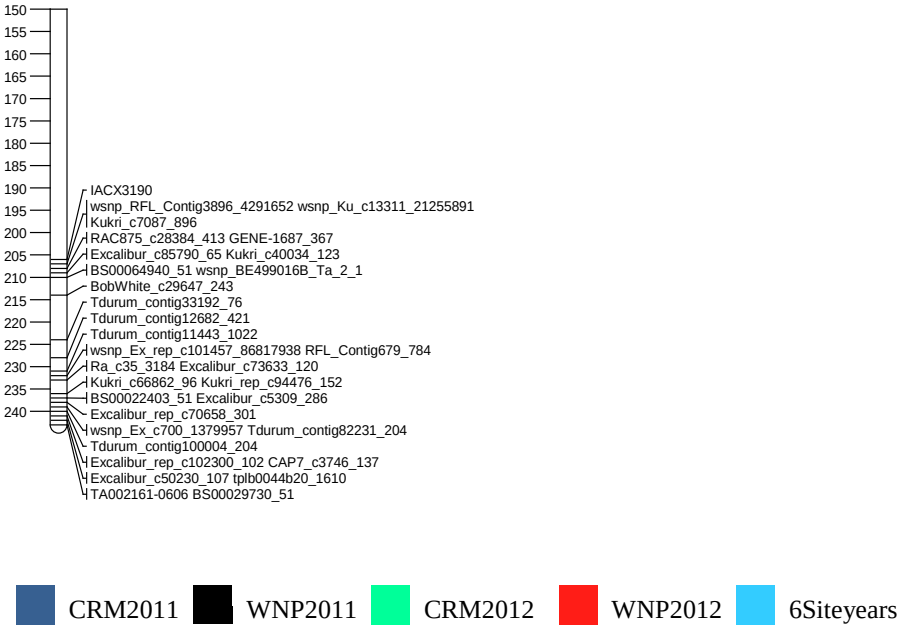
3B [2]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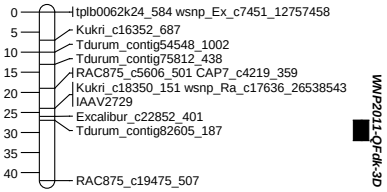
WNP2011
CRM2012
WNP2012
6Siteyears

3D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WNP2011

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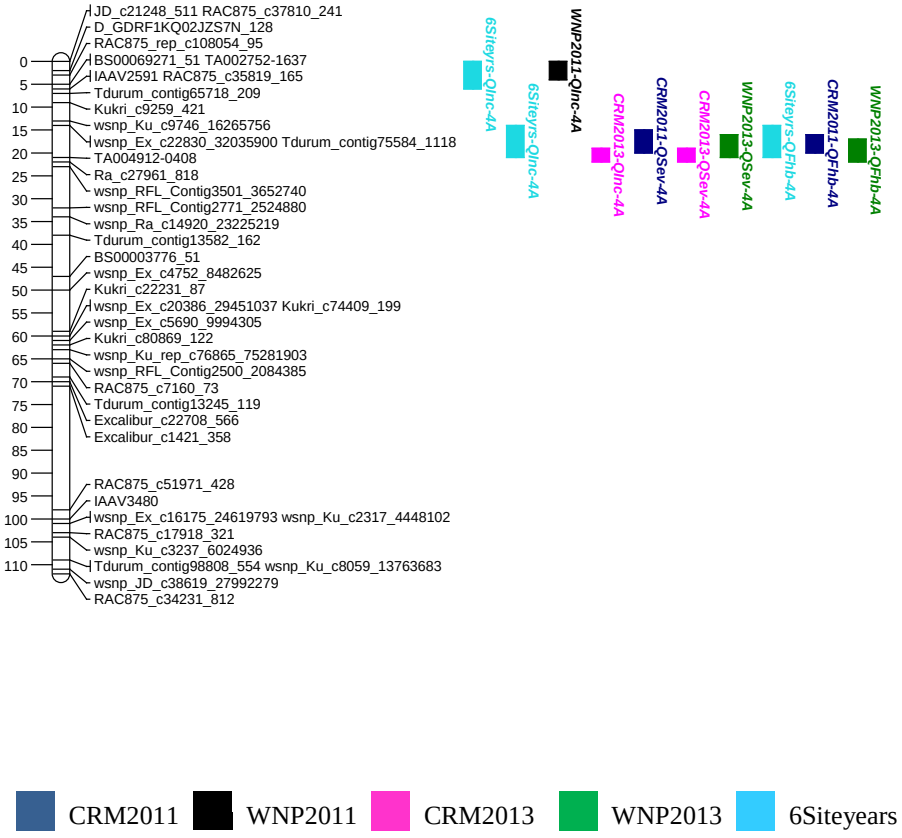
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CRM2013-QInc-4A
CRM2011-QSev-4A
CRM2013-QSev-4A
WNP2013-QSev-4A
6Siteyrs-QFhb-4A
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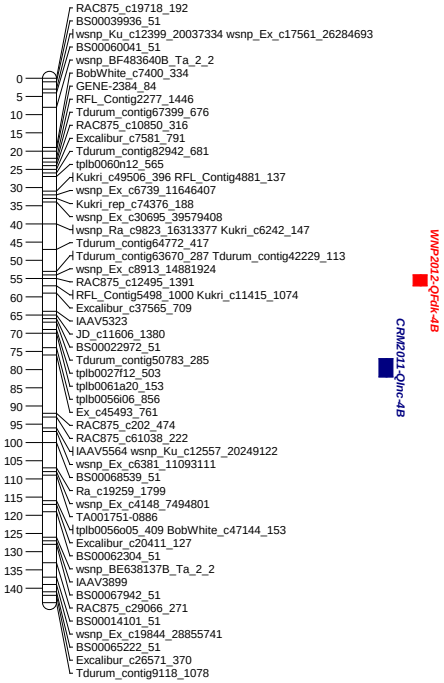
WNP2011
CRM2013
WNP2013
6Siteyears

4B



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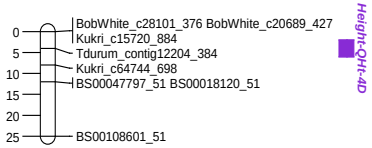

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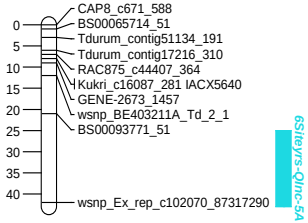
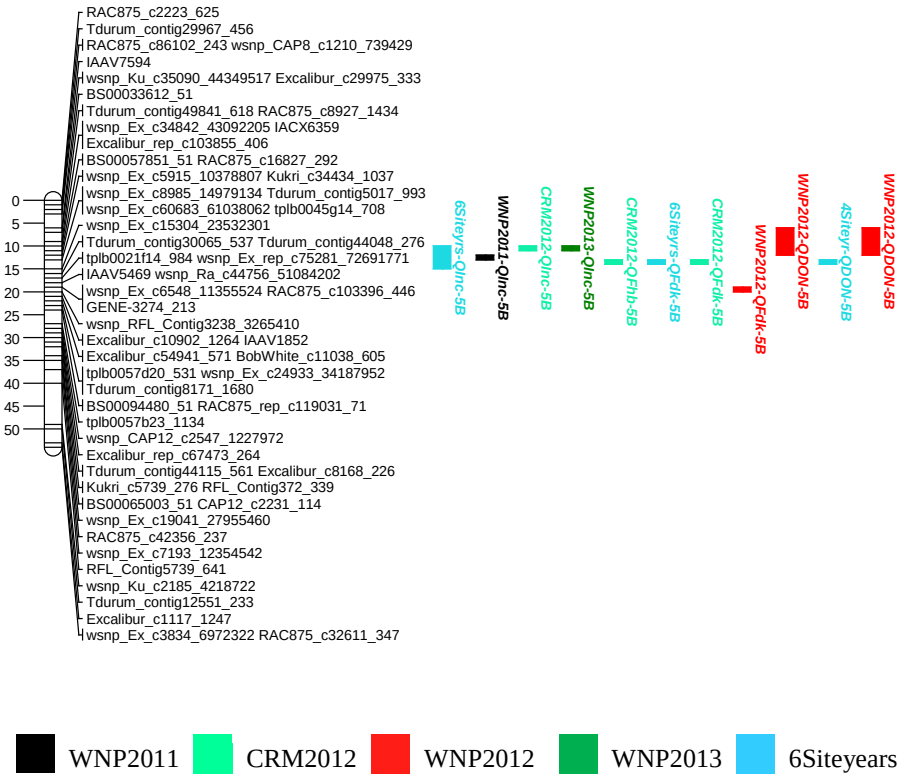


CRM2011 WNP2012 6 Siteyears Plant Height

5B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6Siteyrs-QFdk-5B
CRM2012-QFdk-5B
WNP2012-QFdk-5B
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CRM2012
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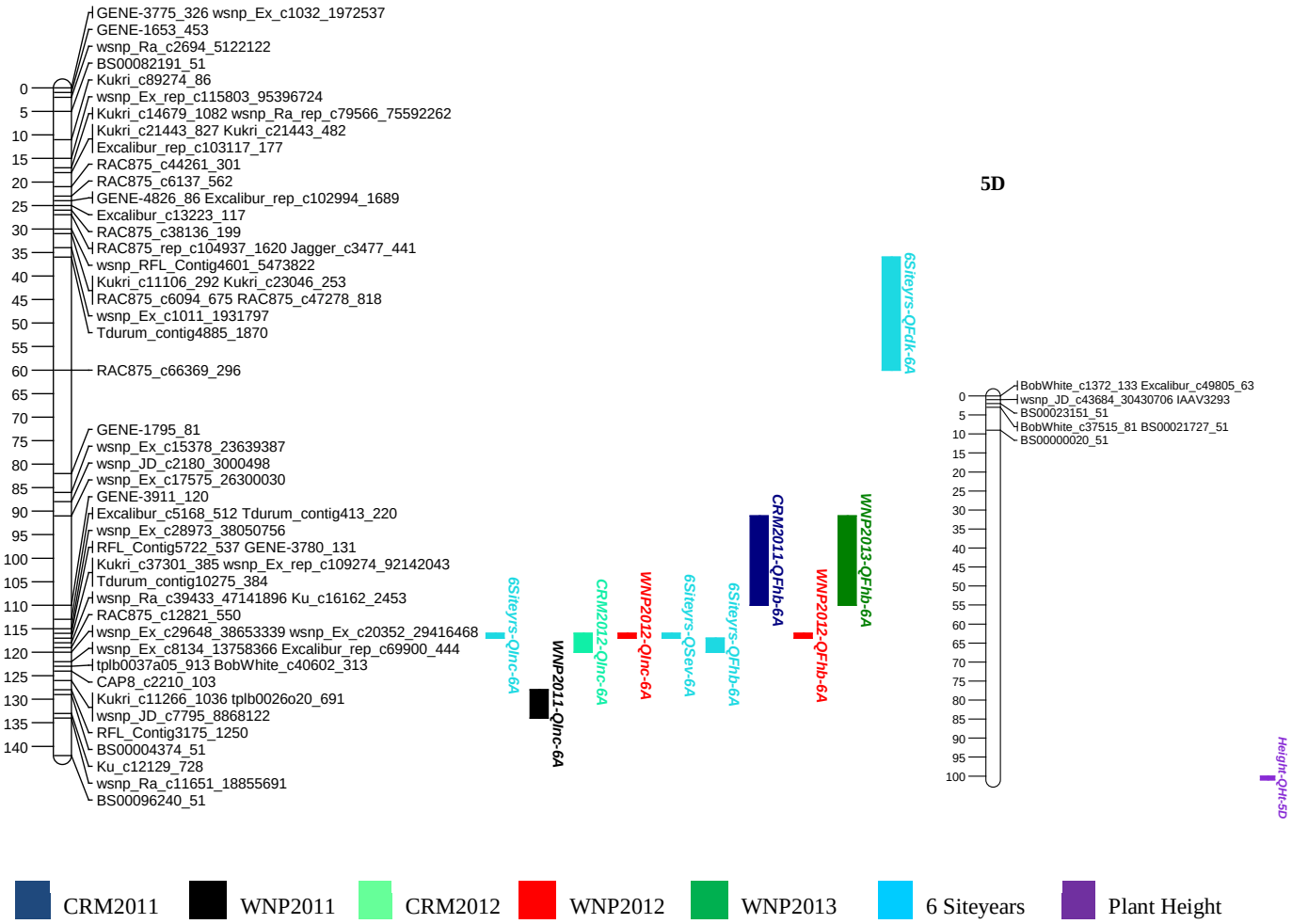
WNP2013
6Siteyears

6A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CRM2011-QFhb-6A
WNP2012-QFhb-6A
WNP2013-QFhb-6A
6Siteyrs-QFdk-6A
5D
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BobWhite_c37515_81 BS00021727_51
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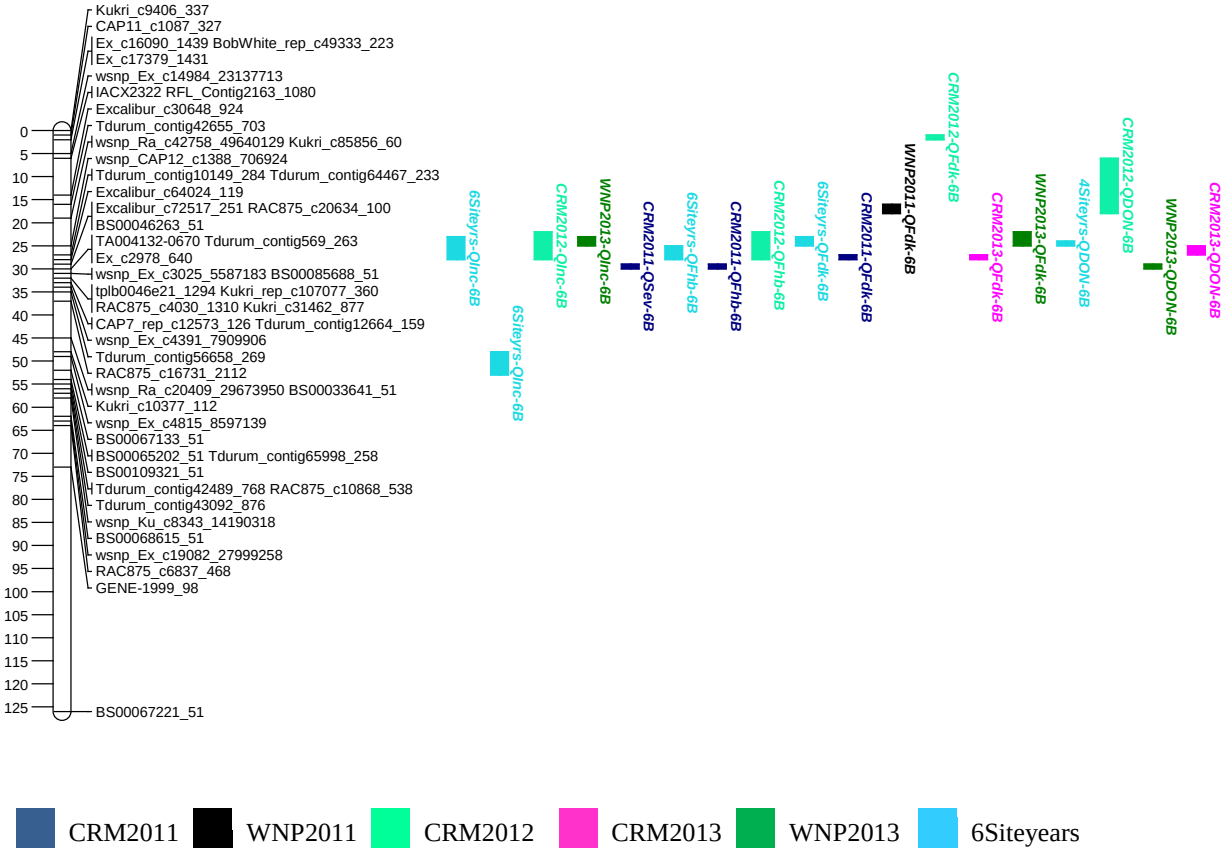
Height-QHk-5D
CRM2011
WNP2011
CRM2012
WNP2012
WNP2013
6 Siteyears
Plant Height

6B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6Siteyrs-QInc-6B
6Siteyrs-QInc-6B
CRM2012-QInc-6B
WNP2013-QInc-6B
CRM2011-QSev-6B
6Siteyrs-QFhb-6B
CRM2011-QFhb-6B
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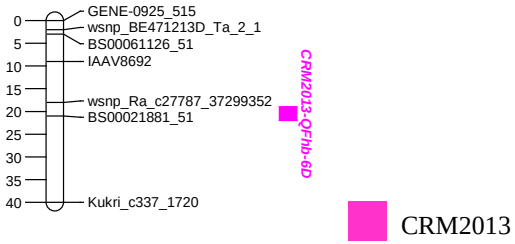
CRM2012
CRM2013
WNP2013
6Siteyears

6D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CRM2013

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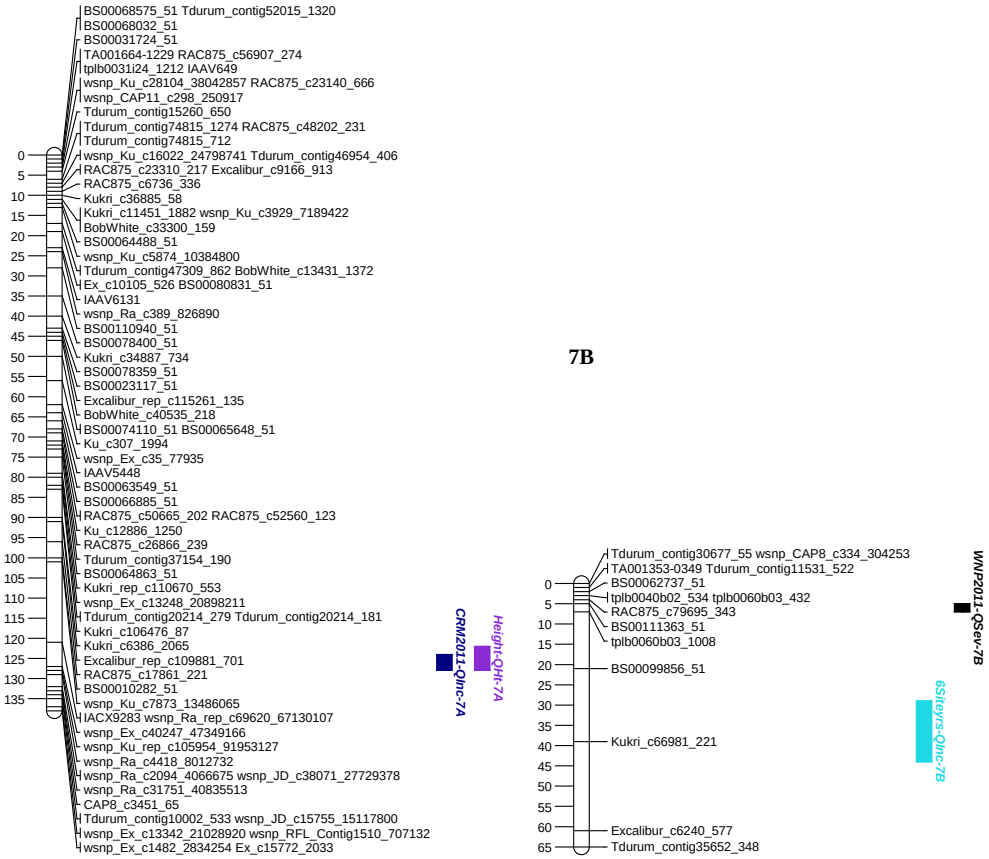

7B

CRM2011 WNP2011 6 Siteyears Plant Height