Combining Fusarium head blight resistance and barley yellow dwarf virus tolerance in spring wheat (*Triticum aestivum* L.)

By

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ABSTRACT

Fusarium head blight (FHB), a fungal disease caused principally by *Fusarium graminearum*, and barley yellow dwarf (BYD) caused by BYD luteoviruses are two serious fungal and viral diseases of wheat resulting in high economic losses annually.

Wuhan, a Chinese wheat cultivar resistant to FHB, and Maringa, a Brazilian cultivar tolerant to BYDV were inter-crossed and crossed with Roblin, a Canada western red spring wheat susceptible to both FHB and BYDV, to determine the genetic basis of resistance/tolerance and to combine the two traits. Four hundred ninety nine F_1-derived doubled haploid (DH) lines were generated from reciprocal crosses using corn pollen-mediated DH technology. The DH lines and the parents were evaluated for disease symptoms, reduction in height and spike mass for BYD and for disease incidence, disease severity and *Fusarium*-damaged kernels for FHB in field and controlled environments. A subset (20/150) of the best performing DH lines from Wuhan/Maringa populations for both BYD and FHB were further evaluated. Plants were point inoculated with *F. graminearum* in greenhouse experiments, and macroconidial spray inoculations and spread of corn inoculum were used in field environments to evaluate FHB. BYDV inoculations were performed by placing ten to fifteen viruliferous aphids (*Rhopalosiphum padi* infected with BYDV-PAV isolate 9301PAV), at the one to two leaf stage for both greenhouse and field trials.

The studies showed that both FHB and BYDV are quantitatively inherited. Transgressive segregants were observed and the broad sense heritability was high (0.90 to 0.97) for all traits evaluated. Results from independent testing of diseases on Wuhan/

Maringa populations showed fourteen DH lines were as, or more resistant than Wuhan for FHB and Maringa for BYDV tolerance and have combined both BYDV tolerance and FHB resistance.

Identifying such lines facilitates the pyramiding of independent genes to obtain adequate levels of enduring resistance. A further experiment was conducted on the 14 lines by inoculating them with BYDV and *F. graminearum* successively on the same plant. Six out of 14 selected DH lines demonstrated high resistance to FHB and tolerance to BYDV. These six lines can be used in FHB/BYDV resistance/tolerance breeding programs.

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FOREWARD

This thesis is written in manuscript style as out lined by the Faculty of Graduate Studies of University of Manitoba. The thesis is presented as three Chapters, each containing an abstract, introduction, materials and methods, results and discussion sections. A general review of literature precedes the chapters, and a general discussion follows the chapters. The thesis is written to conform to the requirements of the Canadian Journal of Plant Pathology.

CHAPTER 1

GENERAL INTRODUCTION

1.1 General introduction

Triticum aestivum L. em. Thell (bread wheat) is one of the most important food sources for humans in the world, especially in the temperate zone. Prairie farmers contribute approximately 23.8 million metric tons to world wheat production which remains at over six hundred eighty million metric tons (Statistics Canada 2009). A decrease in production in recent years has resulted from low commodity prices as well as disease pressures from fusarium head blight (FHB), barley yellow dwarf and other diseases.

Plant disease resistance that is conferred by single dominant genes (e.g. resistance to rusts) is genetically simple and has been analyzed extensively by the traditional methods of plant pathology, plant breeding and genetics. Polygenic disease resistance, based on the additive effects of several genes is genetically complex by contrast and is less well understood. An effective approach to the study of complex and polygenic forms of disease resistance is known as quantitative trait locus (QTL) mapping, which is based on linkage of DNA markers with the expression of quantitative traits. DNA-based molecular markers can be used to determine the chromosomal locations of genes of interest and to assist in the selection of desired genotypes, a process called marker-assisted selection (MAS) (Mohan et al., 1997).

Currently, molecular techniques offer new possibilities to augment resistance breeding by pyramiding resistance genes. However, it follows from many studies that a majority of the described resistance genes or QTLs have only a limited and variable (often not durable) impact on field resistance (Buerstmayr et al., 2009). Because multiple genes need to be transferred to achieve combined resistance, the advantage of

MAS may be lost and breeding costs may increase. The most advantageous strategy is still to detect germplasm possessing acceptable resistance to the majority of the most important diseases (in combination with high productivity, grain quality, resistance to abiotic stresses, etc.) and to improve single characters using appropriate crossing schemes or deliberate gene transfer (backcrossing) for which molecular markers may be very helpful.

Molecular markers can be used to select simultaneously for multiple traits in segregating populations and thereby decrease the number of backcrosses in a back cross program required to restore the adapted genetic background. It is believed that for some traits, molecular markers may be more cost effective than selection based on phenotype, especially if there is the ability to choose only the recombinant lines to decrease the number of genotypes that are screened. However, a review on QTL mapping and MAS for FHB resistance in wheat by Buerstmayr et al. (2009) suggested that it is obvious that even the major QTL are responsible only for a minor part of the phenotypic variation in FHB resistance. Hence it is essential in a breeding program to combine genotypic selection through MAS with phenotypic selection of genotypes revealing a sufficient level of FHB resistance in field tests. It is apparent that the latest improvement in FHB resistance was attained through the combination of favourable, not yet identified, genes via phenotypic selection (Kosova et al., 2009). Wilde et al. (2007) demonstrated that both phenotypic and MAS are efficient tools in practical breeding and have the potential to reduce FHB symptoms and grain deoxynivalenol (DON) content.

Fusarium head blight, a fungal disease caused principally by *Fusarium graminearum* Schwabe [teleomorph: *Gibberella zeae* (Schwein.) Petch is a serious economic threat to all classes of wheat (*Triticum aestivum* L. and *Triticum turgidum* L.

var *durum*) and other small grain cereals (Parry et al., 1995). The disease, FHB, is prevalent, particularly in the warm, and humid and semi-humid areas of the world (Parry et al., 1995; McMullen et al., 1997; Miedaner, 1997; Gilbert and Tekauz, 2000; Stack, 2003). Fusarium species produce mycotoxins in the grain that can harm animals and humans when contaminated grains are consumed.

The genetic variation for resistance to FHB has been reported to be very large and valuable sources of resistance have been detected among both spring and winter wheat cultivars. However, resistance to FHB has different components (Mesterhazy 1995; Wiśniewska et al., 2002) and there is a strong influence of environmental conditions on the response of wheat to FHB. Evaluation of the disease for breeding programs may be complicated because multi-environment tests and many characters are needed to fully describe the disease.

Barley yellow dwarf (BYD) is the most widely distributed and economically important virus disease of the cereal grains, (D'Arcy, 1995; Lister and Ranieri, 1995). This disease is caused by a group of related single stranded RNA viruses (Miller and Liu, 2002) called barley yellow dwarf viruses (BYDVs) species BYDV-PAV and BYDV-MAV (Oswald and Houston, 1951; Slykhuis, 1956; Watson and Mulligan 1960), genus *Luteoviruses,* family *Luteoviridae*, and cereal yellow dwarf viruses (CYDVs) species SGV, RMV and GPV, genus *Polerovirus*, family *Luteoviridae* (D'Arcy and Domier 2005). The virus group is distributed throughout the wheat growing areas of the world and can cause significant yield losses (Carrigan et al., 1980; El and Hill, 1990; Haber, 1990; Veskrna et al., 2009). The disease BYDV can affect several other species in the family Poaceae, including barley, oats, rye, triticale, rice and many grass species (D'Arcy, 1995).

Crop damage caused by BYD is variable, but present every year in all small grain growing regions of North America. The disease has been more obvious over the past decade because of the widespread control of rusts and its presence is no longer masked. Further, success in breeding for tolerance to BYDVs could have added to its increased presence in the crop environment as tolerant crops do not prevent virus multiplication. Cooper and Jones (1983) defined resistance in the context of BYDV as reduced viral replication in infected plants. Tolerance is defined as reduction or absence of pathogen-induced damage, and the presence of the pathogen in large or small amounts is not relevant to the concept of tolerance (Comeau and Haber, 2002). Tolerance, a desirable plant response to disease pressure, has been widely used to describe field resistance (Burnett et al., 1995; Comeau and Haber, 2002) and has been similarly used in this study. Tolerant cultivars do not readily succumb to BYD but they can maintain a relatively high virus content during most of the growing season yielding significantly better than susceptible cultivars but providing a suitable host for aphid vectors for a longer period than susceptible cultivars.

Resistance to BYDV is considered to be the most effective means of controlling damage caused to cereal crops. Sip et al. (2004) reported that damage to cereal crops by BYDV appeared to be particularly high under conditions of double stresses. Tolerance to BYDV in wheat is mainly based on the *Bdv1* gene (Singh et al., 1993), which is thought to have originated from the Brazilian spring wheat cultivar Frontana, one of the parents of the cultivar Maringa. This cultivar is considered to be a durable source because of its deployment and long-lasting effectiveness in numerous CIMMYT wheats worldwide (Sip et al., 2005).

Breeding for combined resistance to diseases has become an important strategy of disease resistance breeding. Understanding the sources of FHB resistance and BYDV tolerance aids in the genetic mapping of QTLs, and will provide information of value to wheat breeding programs.

There would be a great benefit to the field of disease resistance breeding if BYDV tolerance and FHB resistance were combined in spring wheat lines. The wheat accession Sumai 3 is widely used for FHB resistance in wheat breeding programs, but additional new sources of resistance to FHB as well as to other diseases, need to be identified and exploited to enable a strategy for pyramiding independent genes or QTL to obtain adequate levels of enduring resistance. The Chinese wheat cultivar Wuhan may provide additional resistance genes for FHB, and useful BYDV tolerance has been identified in the Brazilian wheat line, Maringa. This research aimed to integrate these two traits and develop spring wheat (*Triticum aestivum* L.) germplasm that combines FHB resistance with tolerance to BYDV infection.

The objectives of this study were: 1) To study the mode of inheritance of FHB resistance in the wheat cultivar Wuhan, 2) To study the inheritance of BYDV tolerance in the wheat cultivar Maringa, 3) To combine the two traits, FHB resistance and BYDV tolerance, in spring wheat lines.

CHAPTER 2

LITERATURE REVIEW

2.0 Literature review

2.1 Fusarium head blight

Fusarium head blight (FHB) also known as scab (Schroeder and Christensen, 1963; McMullen et al., 1997; Bai et al., 2000), or *Fusarium* ear blight (Parry et al., 1995), a fungal disease caused principally by *Fusarium graminearum* Schwabe [teleomorph: *Gibberella zeae* (Schwein.) Petch] in North America is a serious, worldwide economic threat to all classes of wheat (*Triticum aestivum* L. and *Triticum turgidum* L. var durum) and other small grain cereals. The disease is prevalent particularly in the warm, and humid and semi-humid areas of the world (Parry et al., 1995; McMullen et al., 1997; Miedaner, 1997; Gilbert and Tekauz, 2000; Dardis and Walsh, 2002; Stack, 2003). In North America, the first instance of FHB was reported by Weed in 1890 in Ohio (Stack, 2003). Since then there were regular, but infrequent epidemics until the early 1990s when the area affected and number of crops (wheat, barley and corn) devastated by the disease have been found with increasing frequency (Sutton, 1982; Gilbert and Tekauz, 2000; Tekauz et al., 2000; Stack, 2003).

In eastern Canada, FHB of wheat and ear and stalk rot of maize (*Zea mays* L.) have been long recognized as serious diseases (Sutton, 1982) with increasing importance in recent years. Although, *F. graminearum* was first identified on corn residue in Manitoba in 1923 (Bisby and Baily, 1923), no serious outbreaks were reported until 1984 (Clear and Abramson, 1986). In the late 1980s, *F. graminearum* was detected in wheat samples in Manitoba, however, *F. avenaceum* (Corda ex Fries) Sacc. (teleomorph: *Gibberella avenacea* R. J Cook) was reported to be the most common *Fusarium* species (Clear and Patrick, 1990). In Manitoba, FHB was reported at epidemic levels in 1993

due to prolonged wet and warm weather (Gilbert et al., 1994). Based on seed surveys conducted by the Grain Research Laboratory (1994-2003), it is evident that FHB was mostly restricted in western Canada to Manitoba and eastern Saskatchewan until 1994; however, the disease had spread to all areas of the prairies by 2002. Currently FHB, caused by several *Fusarium* species such as *F. graminearum*, *F. avenaceum* and *F. culmorum* (W.G.Smith) has been observed further west into Saskatchewan and Alberta (Clear and Patrick, 2000).

2.1.1 Economic importance of FHB on wheat

FHB's ever-increasing importance is attributed to its exceptional ability to influence every aspect of the grain industry. The economic impact of FHB can be three-fold causing direct and indirect losses. This disease not only lowers grain yield it also adversely affects market value (grade) and end-use quality. Direct losses are due to floret sterility, reduced seed filling, shriveled (tombstone) kernels known as *Fusarium*-damaged kernels (FDK, Gilbert and Tekauz, 2000) contributing to low test weight and losses at the farm gate. Indirect losses are due to poor seed germination and poor stand establishment in the field resulting from planting FHB-infested seeds. It has been reported that the fungal mycelia growing inside the grain thrives on the starch granules and storage proteins resulting in poor grain quality (Bechtel et al., 1985), which in turn affects baking and milling quality. The overall loss is further amplified by the presence of fungal toxins such as the trichothecene - deoxynivalenol (DON) and the estrogenic toxin zearalenone in the infected grain (Sutton, 1982; Gilbert et al., 2000). The accumulation of mycotoxins in the infected grain causes a variety of detrimental effects on humans and livestock (Snijders, 1990b; McMullen et al., 1997).

McMullen et al. (1997) reported that the FHB epidemic in 1993 incurred the greatest loss due to disease in a single year (over $1 billion) in the northern wheat growing areas of the United States. The disease is now viewed as a national research problem in Canada, USA and many other countries. Research to reduce grain losses due to FHB has become an important objective for plant breeders and plant pathologists globally.

2.1.2 The pathogens causing FHB

Link, a German mycologist, named the group of fungi having fusiform spores as the genus *Fusarium* in 1809 (Stack, 2003). *Fusarium graminearum* was one of the fungi included in his collection which at that time was not known. *Fusarium graminearum* was first described by Schwabe in 1838. Since then, other mycologists have described many more *Fusarium* species.

Several species of *Fusarium* including *F. acuminatum* (Ellis and Everhart), *F. avenaceum, F. culmorum*, *F. equiseti* (Corda), *F. graminearum, F. crookwellense* (L.W. Burgess, P.E. Nelson & Toussoun), *F. poae* (Peck), *F. sporotrichioides* (Sherb) have been reported to cause FHB in spring wheat in North America (Sutton, 1982; Stack and McMullen 1985; Wilcoxson et al., 1988; Clear and Patrick, 2000). The principal *Fusarium* species in North America are *F. graminearum, F. culmorum*, *F. avenaceum, F. crookwellense* and *F. poae*. However *F. graminearum* is the predominant species causing FHB on wheat (McMullen et al., 1997; Clear and Patrick, 2000; Stack, 2003). Although there are differences in aggressiveness among isolates, there is no evidence of host-species specificity in populations of this pathogen (van Eeuwijk et al., 1995; Dusabenyagasani et al., 1997). Three *Fusarium* species that are predominant in Asia,

Australia, Europe, and North and South America, are *F. graminearum*, *F culmorum* and *F. avenaceum* (Parry et al., 1995; Stack, 2003). Their geographical distribution is related to their temperature requirements. *Fusarium graminearum* is distributed in the warmer regions of the world including parts of the USA, Canada, Australia and Central Europe, whereas *F. culmorum* is more commonly isolated in cooler regions such as Northwestern Europe. *Fusarium avenaceum* has been reported from a range of climatic zones, but contributes little globally to the disease compared to *F. graminearum*.

The fungus is well established in the cereal growing areas of eastern Canada. In the west it is found in the black soil zone, where the highest rainfall occurs. Clear and Patrick (2000), reported *F. graminearum* to be the most prevalent species of fungi isolated from *Fusarium*-damaged kernels of wheat in surveys conducted between 1993 and 1998. This situation is different from earlier reports by Gordon. Gordon (1933, 1944, and 1952) reported the presence of *F. graminearum* in Manitoba fields being none to rare in the cereal samples collected. Wong et al. (1992) reported that *F. poae*, *F. culmorum* and *F. sporotrichioides* were as common as *F. graminearum* during the late 1980s.

Most *Fusarium* species are facultative, nonspecific parasites, meaning they are pathogenic on cereals and grasses without showing specialization for any one host (van Eeuwijk et al., 1995). *Fusarium* consists of anamorphic Hypocreaceous Ascomycetes (Ascomycota: Hypocreales: Hypocreaceae) in the genera *Gibberella* and *Nectaria* (Kendrick, 2000; Liddell, 2003). The fungus can be soil-borne, airborne, or carried in plant residues, and can be recovered from any part of a plant from the root to the flower.

The complexity and the extent of the genus *Fusarium* has been reviewed and our current knowledge of the genus has been described aptly by Leslie et al. (2001) as the 'tip of the iceberg'; much of what is described is based only on what is easily seen,

leaving a large margin of error on the species concept. Currently, *Fusarium* identification is conducted on three different species concepts – morphology (spore – shape and sizes), biology (cross-fertility: viability and fertility of progeny) and phylogeny, a molecular approach (Summerell et al., 2002; Bushnell et al., 2003; O'Donnell et al., 2004; O'Donnell et al., 2008).

Since *F. graminearum* is the primary causal agent of FHB on wheat, this species has been studied in greater detail than other species, especially in North America (Shaner, 2003). At one time *F. graminearum* was divided into two groups based on the inability or ability of cultures to form perithecia, respectively (Purss, 1969 and 1971; Burgess et al., 1975; Francis and Burgess, 1977). Isolates of Group 1 are heterothallic. They rarely form perithecia in nature and do not form them in culture on carnation leaf agar (CLA) from a single macroconidium (Nelson et al., 1983). Group 1 has been renamed *F. pseudograminearum* (teleomorph: *Gibberella coronicola*) (Aoki and O'Donnell, 1999). The Group 2 isolates readily form abundant perithecia in nature and on CLA. They are homothallic. They are propagated by airborne or splash-dispersed spores and cause disease on aerial plant parts, whereas Group 1 *F. pseudograminearum* is associated with crown rot disease (Burgess et al., 1994). *Fusarium graminearum* was the name retained for isolates of Group 2 (O'Donnell et al., 2000; Bushnell et al., 2003).

Phylogenetic analyses using DNA sequences of six nuclear genes from isolates of *F. graminearum* collected from around the world revealed seven biogeographically structured lineages within the *F.graminearum* clade (*Fg* clade) suggesting the presence of phylogenetically distinct species (O'Donnell et al. 2000). Ward et al. (2002) analysed a 19-kb region of the trichothecene gene cluster from 39 isolates of *F. graminearum* representing the global genetic diversity of species in the *Fg* clade, and reported one

additional lineage bringing the total to eight lineages in total within the *Fg* clade. Later, O'Donnell et al. (2004) investigated species limits within the *Fg* clade through phylogenetic analyses of DNA sequences from portions of 11 nuclear genes (13.6 kb) and accounted for nine phylogenetically distinct species within the *Fg* clade which included the eight previously reported and a new one. Likewise Starkey et al. (2007) found two new species within *F. graminearum* species complex: *Fusarium vorosii* and *Fusarium gerlachii* by investigating more isolates of the *Fg* clade. In recent years, O'Donnell et al. (2008) and Yli-Mattila et al. (2009) have reported more new species of *Fusarium*. These new species have different geographic distributions, differ in production of trichothecene mycotoxins, and may differ in their ability to cause disease on particular crops (Cumagun et al. 2004; O'Donnell et al. 2000; O'Donnell et al. 2004). Currently *F. graminearum,* is considered to be a monophyletic species complex consisting of at least 13 separate phylogenetic species with different geographic distributions and mycotoxin production (Cumagun et al., 2004; O'Donnell et al., 2004).

2.1.3 Symptoms and disease cycle

The symptoms of FHB on a wheat spike are characterized by the premature bleaching of a single or multiple spikelets, after anthesis, when other spikelets are still green. Bleached symptoms may be seen on the tip, base, or throughout the spike (Shaner, 2003). Initial infections appear as small water-soaked brownish or discoloured spots which can spread in all directions from the point of infection (Atanasoff, 1920; Pugh et al., 1933; Bushnell et al., 2003). Further, symptoms may occur only on a few spikes in a field or on many, and with time, the premature bleaching of the spike will extend to most spikelets in susceptible germplasm. In wet and warm weather, a pink mass of

sporodochia may be visible. In severe cases, the entire spike becomes bleached and the rachis turns black. Infected spikelets are often sterile and infection of the rachis may result in seeds above the point of infection not filling and grains from blighted spikes are shriveled (Schroeder and Christensen 1963; Bai, 1995; Bailey et al., 2003). Symptoms of FHB are restricted to the point of inoculation or infection in resistant genotypes, but spread to adjacent spikelets and through the spike causing the entire spike to become bleached in susceptible genotypes (Wang and Miller, 1988).

The source of FHB inoculum is mainly from crop residues (Clear and Abramson, 1986; Bai and Shaner, 1994) although the residue type may vary in different parts of the world. Parry et al. (1995) suggested that alternative hosts, such as different species of grasses and broad-leaved weeds, may also provide an important source of inoculum. It has been demonstrated that *F.graminearum* survives between wheat rotations as well as between wheat and maize or wheat and other cereal rotations on dead or living host tissues (Bai and Shaner, 1994; Stack, 1999). A high frequency of *F. graminearum* has been recovered from rotation of wheat with soybean in several parts of Brazil (Fernandez and Fernandes, 1990). In southern China, rice stubble is a major source of FHB inoculum on wheat (Bai and Shaner, 1994). Ascospores (sexual spores), macroconidia (asexual spores), chlamydospores and hyphal fragments can all serve as inoculum sources (Stack and McMullen, 1985).

Ascospores are considered the primary inoculum for FHB caused by *F. graminearum* (*G. zeae*) (Sutton, 1982; Shaner, 2003). Tschanz et al. (1976) reported that optimum temperatures for perithecia production range from 15 to 28^0 C. Optimum temperatures for ascospore formation are from 25 to 28^0 C (Sutton, 1982) while the optimum temperature for ascospore dispersal is 16.5^0 C (Tschanz et al.,1976). Tschanz et

al. (1976) also reported that at least four hours of UV light, of shorter wavelength than 320 nm, is crucial to stimulate perithecia production, however, Gilbert and Tekauz (2000) stated that many isolates formed perithecia in culture without UV light treatment, when grown under optimum temperatures. In addition to temperature, moisture is a critical factor in ascospore discharge and dispersal from the perithecium. Paulitz (1996) reported that ascospores had a diurnal periodicity of release, with 1800 – 2300 hours as peak hours for ascospore release. Acospores were released at a temperature range of 11 – 30^0 C and relative humidity of 60 – 95 % (Paulitz, 1996). Tschanz et al. (1976), reported ascospore discharge is associated with a reduction of relative humidity after a moist period, and Paulitz (1996) describes a high correlation (R = 0.89) between time of ascospore release and increase in relative humidity. Both Tschanz et al. (1976) and Paulitz (1996) agree that a longer period of relative humidity or a rainfall of 5 mm or over would inhibit ascospore discharge. Gilbert and Tekauz (2000) have suggested that this conflicting phenomenon could be explained by assigning a threshold level for relative humidity at which ascospore release slows or stops. Optimum temperature for macroconidia formation on Coons agar ranges from 28 - 32^0 C (Anderson, 1948 Tschanz, 1976). Wet and warm weather is necessary for production of macroconidia in the field on host residue (Sutton, 1982).

Studies have shown that wind and rain both play a vital role in spore dispersal. Wind disperses ascospores and splashing rain disperses macroconidia of *G. zeae* and *F. graminearum*, respectively (Sutton, 1982). The perfect stage of *F. graminearum, G. zeae* produces ascospores, which contribute to the local inoculum but may also travel for longer distances so that airborne inoculum produced outside the field can initiate disease (Fernando et al., 1997). The latter quantified spore dispersal by examining ascospore and

macroconidial inoculum using dispersal gradients and came to the conclusion that disease results mainly from primary infection and is a monocyclic disease. In the same experiment FHB determinations were also made within plots to determine spread of the pathogen. In both inoculated plots it was found that prevailing winds were a significant factor in FHB spread. However, ascospores induced FHB over a greater area with a range of 5 to 22 meters. Therefore they also concluded that airborne ascospores are the primary inoculum. Excessive moisture may inhibit ascospore release but during this period splash dispersal of mycelia and macroconidia can become the primary inoculum (Paulitz, 1996). Inch et al. (2005) reported that under field conditions in Manitoba, ascospore discharge is correlated with a drop in air temperature and a rise in relative humidity. Spore deposition begins slowly at approximately 1500h, which corresponds to a drop in RH and rise in air temperature. Higher numbers of spores were trapped as RH increased, peaking at 2100 h and continuing until 0400h. Few ascospores were trapped between 0500 and 1400 h.

Del Ponte et al. (2002) studied the spatial pattern of FHB incidence in winter wheat fields in New York over a period of three years. They suggested that the primary deposition of spores came from diffused atmospheric populations of *G. zeae* and these airborne spores could have originated largely from inoculum sources external to the wheat fields tested. *Fusarium* spp. can also survive in diseased kernels left in the field after harvest as was demonstrated for *F. graminearum* by Inch and Gilbert (2003). Maldonado-Ramirez et al. (2005) investigated the long-distance transport of spores of *G. zeae* in the planetary boundary layer of the atmosphere, which extends from about 50 m to nearly 1 km above the surface of the earth. They collected abundant viable spores of

the pathogen which supported the case of long-distance transport of inoculum of *G. zeae* in regional epidemics of FHB.

The epidemiology and disease cycle of FHB has been studied by linking the relationship between wheat anthesis and the environment (Sutton, 1982; Abramson et al., 1987; McMullen et al., 1997). Anthesis is the most susceptible growth stage of cereals to *Fusarium* infection (Arthur 1891; Atanasoff 1920; Dickson et al., 1921; Pugh et al. 1933; Andersen 1948; Lacey et al., 1999). Wheat spikes are susceptible to *F. graminearum* infection for a short period of time (10 to 20 days) from anthesis through to the soft dough stage of kernel development (Gilbert et al., 1997). Optimum temperature for FHB infection ranges between 20 to 30^0 C; temperatures higher than 25^0 C and wet periods longer than 24 hours favour infection (Anderson 1948; Sutton, 1982). Anthers are considered the most important site of primary infection (Pearce et al., 1976), however, roles of other structures like stomata of the palea, lemma and glumes are regarded as important as well. Some studies suggest that waxy glumes serve as a barrier to infection and help to exclude moisture whereas tight glumes serve to restrict access of airborne inoculum to the flowering structures (Kang and Buchenauer, 2000a). The risk of an FHB epidemic is high when natural inoculum is abundant during warm and humid weather when plants are flowering. In natural circumstances, primary infection occurs when ascospores or macroconidia released from soil-borne debris and infected hosts are deposited on or inside flowering spikelets. From an infected floret, the fungus can move both up and down the spike from one spikelet to another on the same spike (Bushnell et al., 2003). This spread is an important component in the overall damage caused by the disease. The spores that land in a floret during anthesis by splashed rain or wind, germinate and developing mycelium penetrates the ovary and establishes itself in the

embryo and remains in the seed. When an infected seed germinates the mycelium starts growing again.

2.1.4 FHB inoculation techniques and disease evaluation

Genetic and molecular studies, including QTL mapping and genetic transformation all depend on phenotypic scoring of visual symptoms, i.e. how well we rate a test plant. Several studies have concluded that evaluation with measurement of spread of FHB within the wheat spike provides a reliable estimate of a cultivar's resistance at least under controlled conditions (Schroeder and Christensen 1963; Bai and Shaner 1996; Dill-Macky 2003). Based on project goals, the level of precision required, the number of lines under evaluation, and available resources, different inoculation techniques that introduce inoculum into or on the wheat spike at 50% anthesis have been implemented. The main inoculation methods are described in the following paragraphs.

1) Spray Inoculation: This method is generally used in field research to evaluate large numbers of lines but it is also employed in indoor experiments. Evaluation of Type I and Type II resistance in the field situation is done after natural infection or spraying the spikes with a macroconidial suspension of *F.graminearum*. In individual rows, the growth stage is recorded and plants are sprayed with a macroconidial suspension of 50,000 spores/ml when 50% of the plants in the row are at 50% anthesis and again two to three days later. After each macroconidial inoculation, plots are irrigated to maintain high humidity. Irrigation starts with the first day of inoculation, and ends within three weeks after the last inoculation, giving enough time to evaluate the entire material. Symptoms are rated 18 – 21 days after inoculation. When using spray inoculation however, Type I and Type II resistance cannot be easily separated on the same plant because of multiple infections rendered by spray inoculation. The extent of disease is quantified in terms of

the FHB index (FHBI), expressed as a product of percent incidence (% spikes with symptoms) and percent severity of infected spikes (% infected spikelets on infected spikes).

2) Single floret injection (SFI) or Point inoculation: Point inoculation is used to evaluate Type II resistance, usually under controlled conditions by inoculating a single central floret or two opposite florets. The inoculum is delivered using a hypodermic syringe, micropipette or a small piece of inoculum-soaked cotton batting or colonized millet kernel into the floret (s) of the central spikelet of the wheat spike at mid-anthesis (Bekele 1984; Stack 1989; van Ginkel et al., 1996). The volume of inoculum used ranges from 5 to 10 µl per floret of a macroconidial spore suspension, with a concentration of 50,000 macroconidia/ml (Stack and McMullen 1985; Bai and Shaner 1996; Rudd et al., 2001). After inoculation, wheat spikes are covered with glassine bags and plants are incubated in a misting chamber providing high humidity for 12 to 72 hours at a temperature of 22-25 ^{0}C (Wang and Miller 1988). Although most programs provide continuous misting, Bai and Shaner (1996) have carried out successful experiments supplying intermittent moisture over three successive nights. The point inoculation technique though generally performed in the greenhouse has also been used in the field (Mesterhazy, 1997; Cambell and Lipps, 1998; Wang and Miller, 1988). Mesterhazy (1997) reported that using glassine bags inverted over hand-misted spikes in the field was successful in maintaining 100% humidity of inoculated spikelets for 24 hrs, although this technique is best limited to situations where cool temperatures and/or low light intensities prevail (Dill-Macky, 2003). The cotton batting method was once used at CIMMYT's research plots in Toluca, Mexico without misting; however, consistently

high humidity in this location when plants were in anthesis avoided the need for any additional moisture above that retained by the cotton batting (Dill-Macky, 2003).

3) Corn kernel inoculum: Another method currently in use is corn kernel inoculum, although some programs (e.g. ECORC, Ottawa) also use barley/millet grains. This method is used in large FHB nurseries to achieve uniform disease development (Gilbert et al., 2008). This alternative inoculation protocol allows one to prepare corn inoculum in the off season. The inoculum is applied over a one - two day period, at the rate of $20g/m^2$, about two to three weeks prior to heading (booting stage). Irrigation is generally started soon after the kernels are spread on the ground to promote perithecia formation by the time of wheat flowering. Rudd et al. (2001) stated that this method could be the closest to natural epidemics.

The relative speed and ease of visual assessments provides the earliest possible data for selection among lines (Wong et al., 1992; Gilbert and Tekauz 1995; McMullen et al., 1997). Visual assessments are usually carried out on immature material, prior to full spike maturity, 18 to 21 days after inoculation. However, visual assessment of frozen material collected after 18 to 21 days of inoculation of the spike is not unusual.

Incidence is one component used to quantify disease under natural infections, or after artificial inoculation using spray or infected grain inoculum. Disease incidence is measured as the percentage of wheat spikes showing disease symptoms, and requires evaluation of multiple spikes (Groth et al., 1999). Different researchers evaluate ten or more spikes to determine FHB incidence (Wilcoxson et al., 1992; Campbell and Lipps, 1998).

Disease severity or symptom spread can be measured by counting the infected spikelets/florets and the total spikelets/florets of the wheat spike (Schroeder and

Christensen 1963; Bai et al., 1999). Wilcoxson et al. (1992) included necrotic spikelets as FHB-infected spikelets, while Bai et al. (1999) incorporated all or any disease symptoms such as dark brown, water soaked spots on the glumes to bleached spikelets and Buerstmayr et al. (2003) included the desiccated spikelets along with the bleached spikelets as FHB-infected spikelets. For resistance in the field, quantification of overall disease is calculated from disease incidence (% FHB infected spikes per row) and the disease severity (% infected spikelets/spike) (Gilbert and Tekauz 1995; McMullen et al., 1997; Shaner, 2003). A simple visual scoring system on a scale of 0 – 100%, where 0 = no disease and 100 = complete spike infection, on inoculated wheat spikes developed by Stack and McMullen (1985) has been used by several researchers (Bai et al., 1999; Waldron et al., 1999; Anderson et al., 2001). This measurement can be expressed in terms of an FHB index. Stack and McMullen (1995) have also made a pictorial scale with 10 categories: 0, 7, 14, 21, 33, 50, 66, 79, 90, and 100%, for the visual assessment of the percent infection of infected spikelets on a spike, with one infected spikelet equivalent to about 7%.

Because FHB has such a deleterious effect on grain quality, post harvest assessment of kernels is essential. Kernels are assayed to determine severity of the disease. Kernels with blight symptoms (bleached, shriveled) are counted as FDK and their frequency in a sample determines the grade of wheat and the price that will be paid for it (McMullen et al., 1997; Gilbert and Tekauz, 2000). Seed infection assessment may give much higher estimates of severity than would be indicated by the percentage of visually blighted spikelets (Fauzi and Paulitz, 1994; Fernando et al., 2000). Mycotoxin produced in grain is a serious consequence of FHB on grains. Thus these traits (FDK and DON) are also used to evaluate FHB resistance.

2.1.5 Mycotoxins in FHB-affected grain

Mycotoxins are naturally occurring toxic secondary metabolites produced by fungi infesting agricultural crops both during crop growth and storage. Mycotoxins are a major concern as grain affected by FHB is of primary importance when considering food and feed safety. *Fusarium* species produce several agriculturally important trichothecene mycotoxins which include deoxynivalenol, zearalenone and fumonisin (Miller and Young 1985; D'Mello et al., 1999; Pronk et al., 2002).

Fusarium culmorum and *F.graminearum* produce Type B trichothecenes, e.g. nivalenol (NIV), deoxynivalenol (DON) and its acetylated derivatives three-acetyl-DON (3-ADON), fifteen-acetyl-DON (15 A-DON) and fusarenon-X (4-ANIV) (Placinta et al., 1999). Deoxynivalenol is the predominant trichothecene found in contaminated grain in Europe and North America (Mirocha et al., 1989; Scott, 1997). Regional relationship studies show that *F. graminearum* isolates originating in North America produce 15-ADON whereas isolates originating in Europe have been found to produce 3-ADON (Mirocha et al., 1989). Some studies have shown that there is a relationship between the host and toxin production in *F. graminearum*. There is a tendency for isolates from wheat and corn to produce DON and its derivatives while the isolates from barley tend to produce NIV and its derivatives (Seo et al., 1996).

Recently, significant shifts from DON- to NIV-producing *F. graminearum* in Northern Europe (Waalwijk et al 2003) and from the original 15-ADON to 3-ADON chemotype in North America (Ward et al., 2002) have been observed. Analysis of FHB pathogen diversity in North America in 2008 revealed that a significant population structure associated with trichothecene chemotypes is present and that 3-ADON producing *F. graminearum* isolates are prevelant (Ward et al., 2008). In western Canada

it is reported that the 3-ADON chemotype frequency increased more than 14-fold from 1998 to 2004 (Ward et al., 2008). Guo et al. (2008) reported that 3-ADON and 15-ADON chemotypes ranged from 0 – 95.7 and 4.3 – 100%, respectively, from two wheat cultivars in 15 locations in Manitoba, Canada, from the samples collected in 2004 -2005.

Deoxynivalenol is also known as vomitoxin, because it is associated with emesis and feed refusal in non-ruminant animals such as swine (Forsyth et al., 1997). Chronic exposure of farm animals to DON is an important issue in several countries. Studies conducted by Placinta et al. (1999) with pigs indicated that DON is a potent feed intake and growth inhibitor, the levels of reduction typically being of the order of 20% and 13 %, respectively, for a dietary concentration of 4 mg DON/kg of feed. Feed intake and milk production in dairy cows were not affected, indicating ruminants were more tolerant of DON (Charmley et al., 1994). Zearalenone is an estrogenic metabolite which commonly occurs with DON in cereal crops. Its effect has been evident at levels as low as 1.5 – 3 mg/kg. In cows zearalenone induces infertility, interferes with conception, ovulation, fetal development, and reduces milk production (Placinta et al., 1999). Fumonisins are produced by *F. verticillioides* (Sacc.) Nirenberg (formerly *F. moniliforme* J Sheld.) and are associated with a variety of adverse health effects in livestock. The fumonisins (FB_1, FB_2 and FB_3) cause neurological disorders and pulmonary oedema syndrome in pigs (Prelusky et al., 1994). Studies show that while the effects of these toxins individually may be not strong, there may be synergistic interactions when two or more occur together (Harvey et al., 1996; Pronk et al., 2002). Harvey et al. (1996) demonstrated that DON and FB_1 caused marginal reduction in weight gain of pigs when provided individually, but a marked synergistic growth depression occurred when both mycotoxins were present. Pronk et al. (2002) studied

six important trichothecenes and reported that NIV is the one most often found in cereal grains and animal feed, often together with DON and zearalenone. NIV is also found in human food, although only in cereals and processed grains and not in food derived from animals given contaminated feed. Also occasionally, the occurrence of 3-ADON and 15-ADON in these commodities has been reported. 3-ADON affects the dividing thymus, spleen and intestinal cell and exerts immunotoxic activity.

2.1.6 Variation in pathogenicity/aggressiveness

High variation in aggressiveness has been found among *F. graminearum* isolates from different geographical regions (Bai and Shaner, 1996; Miedaner et al., 1996; Muthomi et al., 2000; Akinsanmi, 2004). A significant quantitative variation for aggressiveness was observed within individual field populations of *F. graminearum* from Germany and among isolates from a world collection (Miedaner et al., 2001). Differences in aggressiveness among *F. graminearum* isolates collected from North Carolina, USA, China, Canada and central Europe have also been reported (Walker et al., 2001, Gilbert et al., 2001; Toth et al., 2005; Wu et al., 2005).

Deoxynivalenol (DON) produced by *F. graminearum* during fungal infection has been proposed as a virulence factor (Proctor et al., 1995); an isolates aggressiveness also appears to depend on its DON-producing capacity (Mesterházy 2002; Miedaner et al. 2000). DON inhibits protein synthesis (Miller, 1989) and slows down wheat seedling growth (Bruins et al., 1993). Several studies have reported that FHB severity is positively correlated with toxin production (Atanassov et al., 1994; Proctor et al., 1995; Salas et al., 1999). Desjardins et al. (1996) reported a reduction in DON production and disease severity when the gene encoding trichodiene synthase (*Tri*5), a catalytic enzyme involved

in the DON biosynthetic pathway in *F. graminearum,* was disrupted. The resistance level of a cultivar influences DON production significantly. In the most resistant genotypes, toxin production was low (near zero), whereas the same isolates produced high toxin levels in susceptible cultivars (Mesterházy, 2002). Mesterházy (2002) concluded that the level of resistance is more important in governing DON accumulation in a given cultivar than is the aggressiveness of an isolate. In susceptible cultivars, the DON- producing ability is decisive, but in highly resistant cultivars, resistance is the major factor suppressing both disease development and DON accumulation (Mesterházy, 2002). Bai et al. (2001a) reported that the DON-nonproducing isolates could infect wheat spikes in both the greenhouse and field, but could not spread beyond the point of initial infection, suggesting that DON is an aggressiveness factor, rather than a pathogenicity factor. DON may not be necessary for primary infection by the fungus, but may enhance symptom development and spread of the pathogen within the spike (Bai and Shaner, 2004). This hypothesis suggests low DON content in an infected kernel or expression of a DON detoxifying gene from the fungus in wheat may improve disease resistance in wheat (Bai and Shaner 2004). Trichothecene 3-*O*-acetyltransferase (*Tri101*) encoding an enzyme that catalyzes the conversion of toxic *Fusarium* trichothecenes including DON to less-toxic products, has been proposed as a metabolic self-protection mechanism within *F. graminearum* (Kimura et al. 1998). Therefore, expression of *Tri101* may limit the accumulation of DON and enhance the level of resistance in wheat. A recent study (Gilbert et al., 2010) on relative aggressiveness and accumulation of 3-ADON and 15-ADON in spring wheat using two methods of inoculation reported that DON level was twice as high in grains from a susceptible wheat cultivar infected with 3-ADON isolates as 15-ADON isolates. There was a suggestion that with the increasing percentage of 3-

ADON producers in Canada there could be an increase in DON level in FHB- epidemic years. Also that aggressiveness of isolates was not dependent on whether isolates produced 3-ADON or 15-ADON.

2.1.7 Management strategies

No single control method is adequate for the control of FHB. Optimum management of FHB in wheat and barley is best achieved with multiple strategies; a single strategy generally fails during epidemic conditions (McMullen et al., 2008). Available methods and strategies, including cultural considerations and chemical methods, need to be practised in an integrated manner. Crop rotation together with cultivation of the infested crop residues and other hosts can be effective in controlling FHB in most years (Bai and Shaner., 1994; Shaner and Buechley, 2000). However, in epidemic years fungicide sprays at the appropriate time may help to minimize the damage (Mesterhazy, 2003; Paul et al., 2007). Environmental conditions (temperature, rainfall) required to decompose crop residue after tillage are also highly important for FHB development (Miller et al., 1987). Probable reasons for inadequate fungicide efficiency may be due to poor fungicide efficacy, incorrect timing of application, incorrect usage of fungicide dosage and environmental interactions (Mesterhazy, 2003).

Several studies have reported that crop rotations which follow a wheat-corn-barley rotation may promote FHB epidemics (Sutton, 1982; Parry et al., 1995). Wheat rotation with soybean may not be a viable option either as *F. graminearum* has been isolated in high frequency from soybean residues (Fernandez and Fernandes 1990; Baird et al., 1997). The FHB epidemic in 1993 may have been a consequence of more maize

production in the plains of the Red River Valley and reduced tillage in the 1980s combined with favourable weather conditions of the early 1990s (Shaner, 2003).

Currently, there is no fungicide that controls FHB completely or with high efficacy. Fungicide resistance among *Fusarium* pathogens has been recorded. D'Mello et al. (1999) reported that development of fungicide resistance was accompanied by a more persistent pattern of 3-ADON production in *F. culmorum*. Seed treatment does not eliminate head blight but it may reduce incidence and consequently DON concentration in grain (Teich and Hamilton, 1985), as seed treatment appears to be effective against seedling blight and for promoting stand establishment (Gilbert et al., 1997). Several fungicides (benomyl, flusilazole, mancozeb, propiconazole, tebuconazole, thiabendazole, thiophanate methyl, and triadimephen) are in use but have produced inconsistent results (Gilbert and Tekauz, 1995; Shaner, 2003). Shaner (2003) reported that the foliar fungicide tebuconazole was the best among all the fungicides tested and other studies confirmed this result in artificially inoculated trials (Henriksen et al., 2005; Spanic et al., 2008). Subsequently, Prosaro (a pre-mix of prothioconazole and tebuconazole) has been reported to be more effective than when used individually (McMullen et al., 2008).

Biological control may be an additional strategy in an integrated pest management system for FHB. Reduction of saprophytic survival and ascospore production of *F. graminearum* in cereal crop residues have been evaluated by applying fungal agents such as *Trichoderma harzianum* (Fernandez, 1992; Inch, 2009), or bacterial agents such as *Bacillus subtilis* strain AS 43.3 *Cryptococcus* sp. OH 71.4 and *C. nodaensis* OH 182.9 and yeast antagonists OH 71.4, OH 181.1, and OH 182.9 (D. A. Schisler et al., 2002 and 2004; Zhang et al., 2005).

In recent years, much of the research undertaken for the control of FHB has been concentrated on understanding and exploiting the genetic resistance of cereal plants to FHB-causing pathogens since the control of FHB spread by fungicides and crop rotation do not adequately protect the crop. Selecting cereal cultivars resistant to FHB is currently viewed as the most viable and sustainable option for reducing mycotoxin contamination of grain (D'Mello et al., 1999). Exploitation of genetic resistance to FHB in wheat has been successfully used to restrict kernel contamination with DON. Cultivars with good resistance, listed as MR (moderately resistant) in the Seed guides, to FHB including Canada western red spring wheat 5602HR, Waskada, Fieldstar and WR859 are a few good sources in the Canadian FHB resistance pool (Seed Manitoba 2011). Under high levels of disease all varieties will sustain damage from FHB. The use of susceptible cultivars has been blamed in part for the severe FHB outbreaks in North America (McMullen et al., 1997).

Maximum control of FHB can be achieved by integrated management using a number of cultural, biological and chemical strategies along with the exploitation of host plant resistance. McMullen et al. (2008) conducted studies at multiple locations across the United States using integrated management strategies including variety resistance, fungicides and crop rotation for FHB and DON management under natural field infections. They reported that the lowest field FHB severity, lowest DON and the highest yields and test weights were achieved using a combination of management strategies.

2.1.8 Sources of Resistance

Breeding for resistance and finding new sources of resistance appears to be the principal option for long lasting disease control. In general, there are four categories of resistant germplasm currently used in bread wheat breeding programs worldwide: (1) spring wheat from Asia including Chinese cultivars Sumai 3 and its derivatives (Del Blanco et al., 2003; Ittu et al., 2008), Ning 7840, Ning 894037 (Shen et al., 2003; Zhou et al., 2003), Wangshubai (Zhang et al., 2004; Mardi et al., 2005; Ma et al., 2006); Japanese cultivars Shinchunaga, Nobeokabouzu-Komugi, Saikai, Nyubai and the Korean cultivar Chokwang (Ban and Suenaga 2000; Yang et al., 2005). (2) Spring wheat from South America including Frontana and Encruzilhada from Brazil (Steiner et al., 2004). (3) Winter wheat from Europe including Kooperatorka, Praag 8 and Bizel (Snijders 1990c; Badea et al., 2008), Russian and Ukrainian winter wheat cultivars Hostianum 237, Odyessya 16 and their derivatives (Zappel et al.,2008); Swiss cultivars Arina (Paillard et al., 2004), German cultivars Cansas, Petrus and Dream (Schmolke et al., 2005; Klahr et al.,2007), French cultivars Renan and Apache (Grevais et al, 2003; Holzapfel et al., 2008), Dutch cultivar Romanus (Badea et al., 2008) Romanian cultivar Fundulea F201R (Ittu et al., 2002 and 2008) and (4) North American advanced breeding lines with improved FHB resistance (Snijders 1994; McCartney et al., 2007; McKendry, 2005; Fedak et al., 2009).

Additional sources of resistance to FHB, other than hexaploid wheat, have also been identified. Synthetic bread wheats (CIMMYT) seem to have a wide range of variability for FHB resistance, especially for Type I and Type II. Some accessions of *Aegilops tauschii* Coss have been be used as resistant sources in breeding programs (Gilchrist et al., 1997; Su et al., 2000; A. Breker et al., 2003). Fedak et al. (2000) have

worked at length with wheat-rye (*Secale cereale* L.), Wheat-*Thinopyrum* and wheat-*Dasypyrum* addition lines for FHB resistance but failed to generate highly resistant lines because FHB inheritance is complex and multigenic. Fedak (2000) has also investigated FHB resistance in *Lophopyrum ponticum* and *Elymus humidus*. Similarly, several other studies including those of Wan et al. (1997), Rudd et al. (2001) and Han and Fedak (2003), working with several genera and species in the family *Triticeae* have had little success as transfer of this resistance to adapted cultivars has proved difficult. Although these studies have indicated that resistance to FHB from wild relatives of wheat can be obtained, their usefulness in cultivar breeding programs has to date been deemed unsuitable or questionable. There are problems with the lack of chromosome pairing between wheat and its wild relatives, the quantitative nature of FHB resistance and the inferior agronomic characteristics in the hybrid progenies (Chen et al., 1997; Comeau et al., 2001; Buerstmayr et al., 2003; Oliver et al., 2007). Gilbert (1998) has screened several accessions of *Triticum turgidum spp. dicoccoides* (Koern. ex Aschers. et Graebn) Aarons and a few lines were found to have useful levels of resistance. However, this resistance has not been introgressed into elite durum germplasm. The most resistant accessions were from the species *Triticum timopheevii, T. karamyschevii,T militinae, T. dicoccum,T. monococcum* (Fedak et al., 2004).

The transfer of FHB resistance genes to wheat from alien genomes without homology to the wheat genome is more difficult compared to alien genomes that are homologous or closely related to wheat genomes (Cai et al., 2005). In addition, the resistance found in alien species is usually associated with undesirable traits which are difficult to breed out from the progeny (Bai and Shaner, 2004). Chromosome manipulation is needed to introgress FHB resistance genes into wheat from distantly

related alien species (Cai et al., 2005) and significant time and effort may be required for pre-breeding to remove these undesirable characters (Bai and Shaner, 2004).

The resistance of wheat to *F. graminearum* is as important as the aggressiveness of *F. graminearum* in terms of FHB development. Nonetheless, we still have no clear understanding of the genetic mechanisms of resistance to this fungus. Several factors seem to be responsible for different levels of resistance and susceptibility including morphological, physiological and active defense mechanisms (Snijders, 1990c; Mesterhazy, 1995; Miedaner, 1997; Rudd et al., 2001; Buerstmayr et al., 2002). Active resistance mechanisms as discussed by Mesterhazy (1995), include five different types: Type I – resistance to initial infection, Type II – resistance to pathogen spread within the wheat spike, Type III – resistance to kernel infection, Type IV – tolerance, Type V – resistance to toxins, of which Type I and II (earlier proposed by Schroeder and Christensen, 1963) and Type III (FDK), have become the basis for FHB disease resistance breeding. Although studies of the inheritance of FHB host resistance have produced conflicting results, all have agreed that the inheritance in spring wheat is quantitative, where the numbers of estimated resistance genes range from one to five major genes and many with smaller additive effects (Bai et al., 2000; Ban and Suenaga, 2000; Buerstmayr et al., 2002; Liu et al., 2005).

2.1.9 Genetics and Inheritance

To study the genetics of FHB resistance at least three approaches are used: quantitative genetics, cytogenetics (chromosome analysis), and molecular genetics.

Several studies on the inheritance of resistance in wheat to FHB have sought to develop optimal strategies in breeding programs using segregating populations including

F_2 progeny and backcrosses, recombinant inbred lines and doubled haploid lines. The Chinese cultivar Sumai 3 is primarily used in North American wheat breeding programs as a source of FHB Type II resistance (Gilbert and Tekauz, 2000; Stack, 2003). Sumai 3 is considered to be resistant or highly resistant and has been used in many breeding programs. Its resistance has high heritability and stability across environments (Buerstmayr et al., 1999; Ban and Suenaga, 2000). The genetics of this source of resistance have been studied in both Sumai 3 and its derivatives. Ban and Suenaga (2000) used three populations, two doubled haploid (DH) populations of Sumai 3 (resistant)/ Gamenya (susceptible), Sumai 3/Emblem (susceptible) and a population of recombinant inbred lines developed from Saikai 165 (Sumai 3 resistant derivative)/Emblem. Their results indicated that the resistance in Sumai 3 was conditioned by two major genes and that three resistance genes control the resistance in Saikai 165 with additive effects. Inheritance of FHB resistance studies conducted on crosses of Ning 7840, a Sumai 3 derivative, and Frontana, a Brazilian spring wheat cultivar, resistant to FHB, showed that two unique dominant genes, in both cultivars, controlled FHB resistance (van Ginkel et al., 1996). Bai and Shaner (1994) reported that increase in number of genes enhances the level of resistance to FHB in an additive manner: one gene may provide moderately resistant genotypes, two genes may provide resistant to highly resistant genotypes and three genes may confer high resistance. Bai et al. (2000) and Buerstmayr et al. (2000) have shown that additive effects are the main mode of gene action but dominance and epistasis were also observed in some crosses.

Estimates of heritability from segregating populations are useful in understanding the genetic consequences of hybridization and inbreeding. They can help the breeder in selecting and using superior individuals from a population. Heritability estimates for

FHB have been reported with a wide range of values depending on type of genetic materials studied and the method of estimation used. Snijders (1990) used F_2 populations with estimated broad sense heritability of 0.39 ranging from 0.05 – 0.89, and in the F_3 generation, he found heritability estimate of 0.23 ranging from 0 – 0.96. Miedaner et al. (2003) working in four environments, calculated heritabilities of 0.83 for head blight rating and 0.71 for DON content in one F3 population. Buerstmayr et al. (2000) reported broad sense heritability (H) of 0.75 and 0.77 in two winter wheat populations studied. It has been suggested that selection for FHB resistance can be effectively started in the F3 generation (Snijders, 1990c) and should include as many environments as possible (Virginia et al., 2006). Heritability of severity was approximately 0.30 in all populations and heritability of FDK ranged from 0.16 to 0.20. Singh et al. (1995) estimated heritability in the range of 0.66 -0.93 in F_6-derived populations of crosses of Frontana with Inia 66, Opata 85 and Pavon 76 using single floret inoculation. Waldron et al. (1999) measured heritability of resistance in recombinant inbred lines from a single cross of Sumai 3/Stoa and estimated it to be 0.78 indicating Sumai 3-derived FHB resistance is heritable.

The generation of different wheat cytogenetic collections including addition and substitution lines and monosomics, permits one to study the effect of a particular chromosome and determine if it carries genes controlling a trait of interest. Using monosomic analysis of Sumai 3, Yu (1982) determined that genes for FHB resistance were located on chromosomes 1B, 2A, 5A, 6D and 7D. A back-cross reciprocal monosomic analysis was performed by Buerstmayr et al. (1999) using the resistant Hungarian cultivar U-136.1 of pedigree Sagvari/Nobeokabouzu/Mini-Mano/Sumai 3. The likely locations of resistance genes controlling spread of FHB infection were

chromosomes 6B, 5A, 6D, 1B. Chromosomes 6D, 3B, 5A and 6B controlled seed weight after infection and homeologous groups 2 and 6 were involved in low DON content. Yao et al. (1997) generated a series of Chinese Spring/Sumai 3 substitution lines. Studies of Type II resistance suggested that the resistance genes in Sumai 3 are located on 2B, 3B and 6B and did not find resistance genes in the D genome. This is supported by Gilbert et al. (2000) who reported FHB resistance was probably not conferred by the D genome of Sumai 3. Results from cytogenetic analyses are rather inconsistent from one study to another. This could be due to the oligogenic inheritance of FHB resistance making the isolation of the effect of a single gene difficult and any discrepancies are further increased by the influence of environmental effects as some studies were conducted in field trials and others performed in the greenhouse. In tetraploid wheat (*Triticum durum*), the effect of chromosome 3A from *Triticum dicoccoides* has been more consistent (Ban and Watanabe, 2001; Stack et al., 2002).

Significant progress has been made in recent years with regards to molecular genetic studies on FHB resistance in wheat, firstly, identification of chromosomal location and effects of the major quantitative trait loci (QTLs), and secondly, identification of molecular markers associated with the QTLs (Kolb et al., 2001). Exploitation of molecular markers associated with FHB resistance genes has focused on Type II FHB resistance working with Sumai 3 as the source of resistance. Major QTLs, associated with FHB resistance, have been detected using various markers such as random amplified polymorphic DNA (RAPD) (Ban, 2000), restriction fragment length polymorphism (RFLP) markers (Waldron et al., 1999 Buerstmayr et al., 2002; Zhou et al., 2002; Gervais et al., 2003), amplified fragment length polymorphism (AFLP) markers (Bai et al., 1999; Buerstmayr et al., 2002; Zhou et al., 2002; Gervais et al., 2003)

and simple sequence repeat (SSR) markers (Anderson et al., 2001; Buerstmayr et al., 2002; Del Blanco et al., 2003; Gervais et al., 2003). Buerstmayr et al. (2009) have summarized the results from 52 studies dealing with detection of FHB resistance QTL in different wheat sources; 46 studies were carried out with hexaploid wheats, four with tetraploid wheats (secondary gene pool for common wheat) and two with wheat-related species (*Lophopyrum, Thinopyrum*) (tertiary gene pool for common wheat).

2.1.10 Stability of resistance

Resistance to *Fusarium* species is horizontal, non-species specific (van Eeuwijk et al., 1995; Bai and Shaner 1996). This indicates that resistant germplasm can be used globally. To date, it appears that Sumai 3 or Sumai 3-derived germplasm carries a stable resistance QTL on the short arm of chromosome 3B (3BS) (Waldron et al., 1999; Anderson et al., 2001; Liu and Anderson, 2003). Other studies have reported that chromosomes 1B, 2A, 2B, 2D, 3A, 5A and 6BS are also frequently associated with FHB resistance (Buerstmayr et al., 2009). The effect of the 3BS QTL has been confirmed across a wide range of genetic backgrounds and the results suggest that marker assisted selection should be effective (Zhou et al., 2003; Angerer et al., 2003; Cuthbert et al., 2006). Nevertheless, the resistance level conferred by this QTL alone is insufficient or its effectiveness in different genetic backgrounds is not predictable. There is a need to identify beneficial genes and alleles for all types of resistance and develop wheat cultivars that are highly resistant to FHB through introgression and the pyramiding of a full complement of FHB resistance genes.

2.2 Barley yellow dwarf

Barley yellow dwarf disease (BYD) is a viral disease of the Poaceae including wheat (*Triticum aestivum* L.), oats (*Avena sativa* L.), barley (*Hordeum vulgare* L.) (Oswald and Houston, 1951), rice (Oryza sativa L.) and maize (*Zea mays* L) (Watson and Mulligan, 1960). It is the most economically important and prevalent viral disease of cereal crops worldwide and may cause average yield losses of 30% when crops are infected (Oswald and Houston, 1953; Burnett, 1984; D'Arcy and Burnett, 1995; Lister and Ranieri, 1995; Haber, 1990). This disease is caused by barley yellow dwarf viruses (BYDVs) (Oswald and Houston, 1951; Slykhuis, 1956; Watson and Mulligan 1960; Slykhuis, 1962), members of the family *Luteoviridae*, genus *Luteovirus* (van Regenmortal, 2000) and vectored by several aphid species (A'Brook, 1981).

In North America, cereal disease characterized by yellowing, stunting and yield reduction was familiar long before the name BYD was recognized. Manns (1909) described the disease symptoms of oat blade as 'blade blight' which was probably partly due to BYD. It was thought that this disease, and a similar one in barley and other grasses, was caused by bacteria that were aphid transmissible (Manns, 1909; Carleton, 1916).

Barley yellow dwarf is prevalent year round as small grain cereals are grown throughout the continent from the Gulf of Mexico to the Northern Canadian Prairies. Cereals are grown in winter in the southern and coastal regions and barley, maize, oat and spring wheat varieties are seeded in the northern regions in the spring. This green-bridge enables a potential infection by BYDV every year. Hewings and Eastman (1995) state that BYD may have become more apparent because of recent successes in controlling rust diseases as well as breeding cereals that are tolerant to BYDVs. Breeding

tolerant crop varieties may have also increased BYDVs in the crop environment as tolerant cultivars can maintain a relatively high virus content during most of the growing season, but do not readily succumb to BYD disease. Lister and Ranier (1995), reported that BYD viruses are pathogens of grasses in general rather than primarily of cultivated cereals. However, the records of widespread disease worldwide show that BYDVs are ubiquitous in small grains.

In Canada, BYD was first reported in 1956 (Slykhuis, 1956) and *Rhopalosiphum padi* L. was determined to be one of the most efficient vectors of this virus (Slykhuis et al., 1959; Haber, 1990). Although BYDV is considered to cause more severe damage on oats and barley than on wheat, Gill (1975) assessed loss in seed weight to be 60 - 86 % as a result of natural severe epidemic infection of BYDV on spring wheat in Canada. Carrigan et al. (1980) reported BYDV significantly reduced grain yield, number of spikes, plant height, kernel weight, kernel number and above-ground plant mass in winter wheat trials.

In the United States, BYD, also known as red leaf of oat, was first formally identified and named by Oswald and Houston in 1951 as a result of a widespread epidemic in the California barley crop (Oswald and Houston, 1951). They determined that the yellowing and the stunting observed in barley were due to a virus transmitted only by aphids and were not mechanically transmissible. Significant global annual yield losses in barley, oats and wheat are attributable to BYDV infection (Pike, 1990; D'Arcy, 1995).

2.2.1 Disease symptoms

Symptoms of BYD infection in cereals can include all or some of the following: a yellowing or reddening of the leaves that usually begins at the leaf tips before spreading through the whole leaf in a basipetal direction; abnormal growth that may be expressed as reduced plant height, reduced tillering, or blasting of florets; abnormal morphology of the leaves which may have serrated edges, be very stiff, and have a dark blue-green colour (Burnett et al., 1995). Discolouration of leaves usually begins 7-20 days after infection and may be preceded by the development of water-soaked areas on the leaves. These changes in plant growth and chlorophyll content have physiological consequences that result in decreased yield. Symptoms vary with the host species and cultivar, the age and physiological condition of the host plant at the time of infection, the strain and dosage of virus, and the environmental conditions. High light intensity and relatively cool temperatures (15 – 18 C) usually favour expression of BYD symptoms (D'Arcy, 1995). BYD diagnostics based only on symptomatology may not be sufficient to assess the presence of disease as symptoms induced by BYDV are often difficult to distinguish from symptoms caused by other pathogens such as aster yellow mycoplasma, nutritional deficiencies like nitrogen or phosphorous, and moisture or weather stress (McKinney et al., 1952; D'Arcy, 1995). The timing of infection is very important for symptom expression because infection prior to tillering contributes to yield reduction while infection after heading has very little effect on plant growth and yield reduction (Forter et al., 1983).

According to Goulart et al. (1989), severe BYDV symptoms develop, and the largest differences between the resistant and susceptible lines are observed, when infection occurs at the three leaf stage. The symptoms of BYDV can be confounded with

nitrogen deficiencies, frost, drought, water logging, or other non-pathological causes that occur after stem elongation.

The earliest cytopathological effects of BYDV were described by Esau (1957) as "necrotic obliteration" of the sieve elements and nearby parenchyma and companion cells in the leaf vascular bundles. Disruption of the phloem reduces translocation so sugars are not transported from the leaf, leading to a reduction in the plant's dry weight. This in turn inhibits photosynthesis causing the amount of chlorophyll to decrease and the level of respiration to increase (Jensen, 1968). This observation was supported by Lamboy et al. (1991) who increased chlorosis and stunting in both healthy and infected plants by spraying the leaves with a 10% sucrose solution. In wheat, the grain from BYDV infected plants often has a higher percentage of protein but this is actually due to reduced starch production which in turn results in low 1000 kernel weight (Fitzgerald and Stoner, 1967; Jensen et al., 1971).

Barley yellow dwarf virus greatly influences the growth and metabolism of its host. Overall plant vigour can also be affected by the interaction of BYDV and other pathogens. Infection from diseases such as stem rust is more severe when the virus is present (Bissonnette et al., 1994). The losses due to leaf blotch, caused by *Septoria avenae* on BYDV–infected oats, are twice those of oats infected with *Septoria* alone (Comeau and Pelletier, 1976), BYDV causes a rise (4 - 8ºC in barley and 2 - 4ºC in wheat) in the critical threshold temperature at which 50% of the plants are killed in fall seeded winter crops. This is extremely important considering that a change of only 0.5ºC can significantly affect long-term field survival of crop plants (Paliwal and Andrews, 1989). Reports on effects of BYDV infections in oats and durum wheat on the development of the root disease caused by *Cochliobolus sativus* showed that plants

diseased by both pathogens were more severely affected than when plants were infected by each pathogen alone (Scott, 1968). A study conducted by Koch and Huth (1997) concluded that wheat plants were more susceptible to *F. culmorum* in BYDV epidemic years and Liu and Buchenauer (2005) reported that the DON content in grain of plants infected with BYDV and FHB was higher than that of plants infected only with FHB.

2.2.2 Barley yellow dwarf virus: vector and the host range

Barley yellow dwarf virus is transmitted exclusively by aphids (D'Arcy, 1995). Twenty-five aphid species (Homoptera: Aphididae) are listed as vectors of BYDV in North America, however, only four species, *Rhopalosiphum padi* (L.) (bird cherry-oat aphid), *Rhopalosiphum maidis* (Fitch), (corn leaf aphid), *Sitobium avenae* (F) (English grain aphid) and *Schizaphis graminum* (Rodani) (green bug), are recognized as major vectors of BYDV (Halbert and Voegtlin, 1995). *Rhopalosiphum padi,* is considered the most important aphid pest of cereals in North America and Europe and the principal vector for transmission of BYDV (Slykhuis et al., 1959; Watson and Mulligan 1960; Rochow et al., 1965; Froster and Rochow, 1983; Haber, 1995; Schotzko and Bosque-Perez, 2000). It damages its hosts in two ways – by direct feeding and by transmitting BYDV (Stern, 1967). *Rhopalosiphum padi* is able to transmit BYDV more efficiently than other aphid vectors and has the ability to alternate between cereal crops and grasses that can become BYDV reservoirs (Coon, 1959; Gildow, 1990).

Luteoviruses do not replicate in their aphid vectors, and thus have no direct influence on the performance of aphids. Therefore BYDV affects its aphid vectors primarily through induced changes in the physiology and biochemical aspect of the host plant (Montllor and Gildow, 1986; Mowry, 1994; Fiebig et al., 2003). In BYDV-infected wheat leaves the chlorophyll content and the rate of photosynthesis are reduced (Jensen

and Sambeck, 1972). It is reported that in infected plants, soluble carbohydrates and starch contents are considerably higher and respiration is stimulated, compared to healthy plants (Jensen, 1970; Fereres et al., 1990**).** In addition, concentration of amino acids is increased in BYDV-infected leaves (Ajayi, 1986). Virus-induced changes in host plant metabolism can influence insects such as phloem-feeding aphids as their development depends heavily on the quality of the phloem sap (Douglas, 1993). Rhodes et al. (1996) stated phloem-feeding aphids use carbohydrates as their main source of energy, and amino acids for their protein metabolism. Increased fecundity rates were observed in aphids, *R. padi* and *S. avenae,* feeding on BYDV-infected plants (Markkula and Laurema, 1964; Fereres et al., 1989; Mowry, 1994).

Most aphids that transmit viruses are highly mobile, and capable of rapidly transmitting viruses among plants. *Rhopalosiphum padi* occurs in high population densities, particularly in cereal crops such as wheat. During summer, aphids can reproduce asexually with winged or wingless individuals which contribute to a rapid population build-up depending on the crop, cropping methods and weather conditions (Easton, 1977). *Rhopalosiphum padi* nymphs pass through four molts and the adults are viviparous wingless females. The feeding method of *R. padi* ensures its success and importance as an insect pest in agriculture (Forbes, 1977). The mouth parts consist of two pairs of flexible stylets, a labrum and labium. Aphid feeding may occur by capillary action or by using a pharyngeal pump (Klingauf, 1987). When the infected *R. padi* moves from plant to plant, virus spread can take place. Aphid feeding is achieved by stylet-probing until the phloem vessels are reached, and if that plant happens to be infected, virions in the phloem are also ingested with the phloem sap (Forbes, 1977). The behavioral ecology of *R. padi* is strongly influenced by olfactory stimuli of various

types (Petterson, 1994). The behavior of both apterae and alate *R. padi* is affected by volatile cues from the host plant (Quiroz and Niemeyer, 1998). Jimenez-Martinez et al. (2004) studied the response of nonviruliferous apterae *R. padi* to BYDV-infected wheat plants of both virus-resistant transgenic and nontransformed virus-susceptible genotypes. They reported that volatile cues from virus-susceptible plants infected with BYDV were more attractive and/or arrestant to aphids than volatile cues from virus-infected transgenic and/or noninfected plants of either genotype. Medina-Ortega et al. (2009) suggested that responses of *R. padi* to BYDV-infected plants are caused by attraction rather than arrestment.

The host range of BYDV includes more than 150 species in the *Poaceae* family (D'Arcy, 1995). No report of BYDV isolates infecting dicotyledonous plants has been presented to date (Miller and Rasochova, 1997). However, *R. padi*, the BYDV vector over-winters principally on *Prunus virginiana*, L. and *Prunus padus* (Halbert and Voegtlin, 1995). Over-wintered eggs hatch in spring giving rise to individuals called stem mothers or fundactrices. Usually one or more wingless (apterous) generations are produced on the shrubs. Winged (alate) spring migrant aphids develop from the overwintered eggs on the woody host and move to colonize summer hosts such as cereal crops and grasses. In the cooler season (autumn), the colonies produce a generation of winged males and females (gynoparae) that migrate back to the over-wintering hosts and produce wingless egg-laying females (oviparae), which mate with the males prior to laying over-wintering eggs (Halbert and Voegtlin, 1995).

Barley yellow dwarf virus is present in small grains in North Central US and the Canadian Prairie regions every year but with varying severity and extent. Barley yellow dwarf virus aphid vectors do not overwinter in the northern regions of the cereal growing

area in North America. The inoculum for a virus epidemic may be brought in from distant sources by migrant aphids (Hewigs and Eastman, 1995). In the western Canadian setting these cereal aphid species arrive in early summer on winds from the south (S. Haber personal comm.).

2.2.3 Barley yellow dwarf virus

Barley yellow dwarf viruses are members of the genus *Luteovirus* and family *Luteoviridae*. *Luteoviruses* that cause BYDV exhibit a range of biological, serological and chemical properties. Six different serotypes currently have been identified as belonging to two groups based on serological relatedness and cytopathological effects (Cheng et al., 1996). As the viral RNAs of BYDVs and other *luteoviruses* have been sequenced, these two sub-groups apply to the whole group on the basis of genome organization (Miller, 1994). The serotypes of BYDV in sub-group I are PAV (*Rhopalosiphum padi avenae* virus*)*, MAV (*Macrosiphum avenae* virus) and SGV (*Schizaphis graminum* virus), those in sub-group II are RPV (*Rhopalosiphum padi* virus*)*, RMV (*Rhopalosiphum maidis* virus*)* and GPV (*S. graminum* and *R. padi* virus). The BYDVs of sub-group 2 are more similar to other sub-group 2 *luteoviruses* such as beet western yellow virus, than to sub-group I *luteoviruses* (Martin and D'Arcy, 1995). Currently, only BYDV-PAV and BYDV-MAV are designated as barley yellow dwarf viruses (van Regenmortel, 2000). The International Committee on the Taxonomy of Viruses (ICTV) working group on *luteoviruses* has renamed the former BYDV-RPV as cereal yellow dwarf virus (CYDV), and assigned it to the genus *Polerovirus* (van Regenmortel, 2000).

The genomes of all the *luteoviruses* consist of a single positive sense strand of RNA that code for 5-6 genes, 5.5 to 6 kb in size (Miller and Rasochova, 1997). The BYDV virion is an isometric-icosahedral (+) ssRNA virus. The virus particle or the virion is small, spherical and about 24-25nm in diameter and the genome includes up to six open reading frames (ORFs) (Miller et al., 1987). Microscopic examination of tissue infected with *luteoviruses* shows that virus replication is restricted to the phloem tissue, almost exclusively to the sieve elements and companion cells, but occasionally also in phloem parenchyma (Waterhouse et al., 1988; Mayo and Ziegler-Graff, 1996; Miller et al., 2002). Since BYDV is phloem limited, it accumulates to only low titres (Domier, 1995).

2.2.4 Vector-virus interaction

Pathogens and vectors colonizing the same plant often compete for the same nutritional resources from the host. Barley yellow dwarf virus disease involves the interaction between plant, virus and aphid vectors, resulting in a complex and varied disease cycle. BYDV is exclusively aphid-borne, thus epidemiology of the virus largely depends on the dispersal pattern of the vector population (Fiebig et al., 2003). During transmission, luteoviruses circulate throughout their aphid vector's body, requiring virus recognition, penetration, and transport through aphid cells (Gildow, 1999). Luteoviruses do not infect and replicate in the vector aphids. This type of virus transmission is classified as circulative-non-propagative transmission (Gray and Banerjee, 1999). Persistent viruses have several important diagnostic characteristics. The virus is retained for the life time of the aphid. There is a close interaction between the virus and the aphid

vector. There is a latent period of the virus between acquisition and inoculativity in the aphid that lasts from 24 to 48 hours (Sylvester, 1987).

In all aphid species that transmit circulative viruses, the mechanism of acquiring and transmitting the virus is similar. The aphid acquires the virus when it is feeding on the sap of the infected plant. It takes 15-60 minutes of feeding to achieve greater than 50% chance of acquiring the virus depending on the virus-vector combination (Gray et al., 1991; Cheour et al., 1993: Power et al., 1991). The probability of virus transmission increases with longer acquisition, inoculation and latency periods. Increased access periods will increase transmission efficiency up to an optimal time of about 24 – 48 hours. The latent period refers to the period between virus ingestion and the time at which the aphid can successfully transmit the virus. This time includes acquisition into the hemocoel, movement to the accessory salivary gland, and transport into the salivary duct. In general, a minimal latent period for efficient transmission is at least 24 h, and may extend to as much as 3–4 days. During this time the aphid does not transmit virus even though it has fed on an infected plant and acquired the virus. The length of the latent period is influenced by many genetic and environmental factors, including the inherent transmission efficiency of each aphid population for a specific virus isolate, virus concentration in the plant tissue, and environmental factors such as temperature (Power, A.G. and Gray, S.M. 1995; van der Broek, L.J., Gill, C.C. 1980). The nymphal stages of some aphid species, such as *S. graminum*, are more efficient at transmitting the virus than the adult (Halstead and Gill, 1971).

Luteovirus transmission can be divided into four distinct processes: a) virus ingestion from the host plant into the lumen of the aphid's alimentary canal, b) acquisition of the virus through the aphid's gut, c) retention in the tissues and hemocoel,

and d) transmission through the salivary gland and into the phloem tissue of a host plant (Gray and Gildow, 2003). Transmission of the *luteoviruses* occurs after the virus passes through the aphid salivary gland and into the common salivary duct that extends the length of the stylets. During tissue penetration and feeding, salivary enzymes and other excretions are pumped into the plant through a small salivary duct located laterally in the stylets and the viruses suspended in the salivary secretary material are thereby inoculated to susceptible host cells (Gray and Gildow, 2003). The virus enters the alimentary canal when an aphid feeds on an infected plant and moves through the fore-gut and mid-gut into the hind-gut. There is receptor- mediated uptake of the virus by the hind-gut epithelial cells into the hemocoel. This is the primary transmission barrier (Power and Gray, 1995; Jimenez et al., 2004). The virus particle must then migrate into the accessory salivary gland through the basal lamina that acts as a selective filter. This selective migration through the basal lamina appears to be a critical step in transmission and involves the interaction of the coat protein with the aphid and contributes to the vector specificity.

2.2.5 Vector specificity

The BYDV epidemics in 1951 and 1959 led to a beginning of research on the vector specificity of what are now classified as *luteoviruses*. Oswald & Houston (1953) first described BYDV in California as a pathogen of small grains which could be transmitted by several common cereal grain aphid species. It was possible that the predominant isolate of BYDV in California was similar to BYDV-PAV and a mixture of several other viruses (Gildow, 1990), which would have been transmitted by several common aphid species in the area. For these reasons, the vector-specific nature of BYD

luteoviruses was not immediately obvious until Bruehl and Toko (1957) discovered the differential transmission of two strains of BYDV by two different aphid species, *Rhopalosiphum padi* and *Sitobion avenae*. Following an exchange of BYDV isolates and aphid vectors between Washington and New York, Rochow (1958) and Bruehl (1958) reported that observed differences in vector-specificity were determined by the virus isolates and not by differences in populations of an aphid species (Gray and Gildow, 2003). Toko and Bruehl (1957) predicted that BYDV would probably be found to be a complex of related viruses with biological differences. Since then several studies have been conducted on BYD-virus complexes and their circulative transmission pathway. The transmission barriers have been identified, and the genetic (both aphid and virus) factors regulating the transmission process are beginning to be understood. Environmental or abiotic factors also play a role in determining virus vector interactions, but in general, these factors seem to influence the efficiency of the interaction rather than determine the ability of the interaction to take place (Gray and Gildow, 2003). The specificity of viruses with their vectors is associated with the biochemical properties of some of the proteins that are present in the particles (Mayo and Ziegler-Graff, 1996; Medina-Ortega et al., 2009).

2.2.6 Resistance to BYDV in barley and wheat

Barley yellow dwarf can be controlled by applications of insecticide to seeds, reducing aphids in infested wheat crops by spraying with insecticides, avoidance of early planting, and use of tolerant or resistant cultivars (Burnett et al., 1995; McKirdy and Jones, 1996; Francki et al., 2001; Royer et al., 2005; Flanders et al., 2006). Incorporating genetic resistance/tolerance to pathogens and pests is often the most desirable control

method to growers as it is effective when successfully transferred, and economical (Henry et al., 2002). BYDV is controlled mainly by use of varieties that are tolerant or resistant to certain BYDV isolates (Miller and Rasochova, 1997).

There are no known sources of adapted germplasm available in North America that provides complete resistance to BYDV without compromising yield and grain quality. A single first BYDV resistance gene was identified in the barley cultivar 'Rojo' (Suneson, 1955). This gene, *Yd1*, is recessive and only confers an intermediate level of resistance and is therefore not widely used. A second gene, *Yd2*, confers incomplete resistance to BYDV (Rasmusson and Schaller, 1959) in specific cultivars depending upon the plant's genetic background and the infecting BYDV serotype. *Yd2* is effective against PAV and MAV but not against RPV. *Yd2* is located on chromosome 3 of barley (Scallar et al., 1964). Wheat disomic addition lines with *Yd2* have been field-tested and express the BYDV field resistance (tolerance) to some level (McGuire, 1984). *Yd2* is considered a resistance gene since a decrease in virus titre has been shown with some BYDV serotypes (Larkin et al., 1991).

A partially effective dominant tolerance gene, *Bdv1*, has been identified in wheat varieties such as Condor and nine other wheats (Singh et al., 1993). Tolerance to BYDV based on *Bdv1* might originate from the Brazilian spring wheat variety Frontana. In field studies this gene located on the short arm of chromosome 7D was found to be linked to genes *Lr34* and *Yr18* conferring adult plant resistance to leaf and yellow rust, respectively (Singh et al., 1993). However, Ayala et al. (2002) reported only a minor QTL explaining 7% of the phenotypic variation attributable to the 7D chromosome region. The large number of QTLs having mostly small effects found in this study and

the continuous distribution of all evaluated traits is indicative of the polygenic nature and complexity of BYDV tolerance in wheat.

Qualset et al. (1973) suggested that combining different sources of tolerance in wheat may confer higher levels of resistance than those achieved with *Yd2* in barley. Selection for breeding for host tolerance/resistance to BYDV is practised on all the continents where cereals are grown (Burnett, 1990). In wheat, attempts have been made to introgress resistance from wheat grass *Thinopyrum intermedium* by substituting a group 7 chromosome. The BYDV resistance gene on 7Ai1 (7X) coming from *T. intermedium* was first identified in the disomic addition line L1 and was located on the long arm (Brettell et al., 1988; Francki et al., 2001). This BYDV resistance gene was designated *Bdv2*. Using L1 as the resistance source, a series of wheat-*T. intermedium* 7D-7Ai1translocation lines carrying the *Bdv2* gene (called TC lines) was developed (Banks et al., 1995; Sharma et al., 1995). Zhang et al. (2004) developed SCAR (sequence-characterized amplified region) markers linked to the *Bdv2* resistance gene. Introgression of *Bdv2* into elite wheat varieties has met with practical difficulties, especially when validation of resistance relies on bioassays. In the field, natural infection is rarely uniform. If viruliferous aphids have to be used in the field, skill and infrastructure are required to rear and release adequate aphid populations. The field bioassay may be done once a year. Laboratory –based bioassays can be conducted throughout the year, but they too depend on maintaining viruliferous aphids and performing large numbers of ELISA assays. The expression of the *Bdv2* resistance gene in an elite bread wheat background was studied, but was not found promising (Ayala et al., 2001; Zang et al., 2004; Veskrna et al., 2009).

Ayala et al. (2007) developed 2 EST-PCR markers for the *Bdv2* region. However, high-density EST-derived markers are necessary for dissecting the mutant populations around *Bdv2* and for the eventual cloning of the *Bdv2* gene. Gao et al. (2009) developed 8 novel EST-PCR markers which are different from other reported markers, enriching the selective markers and lays a foundation for the high-resolution mapping of the region harbouring *Bdv2*. Furthermore, they also tried to analyze the relative genetic distances of the 8 novel markers with the gene *Bdv2* by an F_2 population of Yw642/Zhong 8601. However, the results showed that all the EST-PCR markers co-segregated with the *Bdv2* region as the alien *Bdv2* region segment could not recombine with the homoeologous wheat chromosome segment in the F_2 population, suggesting that the F_2 population of Yw642/Zhong 8601 could not be used to construct a *Bdv2* genetic map. Therefore, in order to make a genetic map of *Bdv2,* it is necessary to develop an ideal mapping population, in which recombinants can occur. Gao et al. (2009) suggested that some genes in the *Bdv2* region are potentially involved in the BYDV defense response mediated by the *Bdv2* gene. The sequencing of the specific bands for the *Bdv2* region indicated the sequences were highly homologous with the original wheat EST sequences that were used to design the primers. Researchers expect that the eventual cloning of the *Bdv2* gene will assist in understanding the defense mechanism of BYDV-resistance conferred by the *Bdv2* gene in wheat (Gao et al., 2009).

2.2.7 Alternative sources of resistance

It has proved difficult to achieve desired levels of resistance by conventional breeding using available sources of resistance. Genetic transformation using relevant alien genes derived from intermediate wheatgrass, *Thinopyron intermedium,* has the

potential of providing resistant germplasm of wheat while retaining desired agronomic characteristics (Ayala-Navarrete et al., 2009). Coat protein mediated resistance to viruses has been reported in several crops including wheat (McCarthy et al., 1996; Megrath et al., 1997; Hansen et al., 1998; Berger et al., 1999). McGrath et al. (1997), while working with transgenic genotypes of oat and barley expressing the coat protein of BYDV, reported low ELISA values indicating a reduced virus titre on some infected plants. Working with transgenic lines expressing the coat protein gene of isolates of BYDV-PAV and BYDV-GPV, Li et al. (2001), reported a reduction in virus accumulation observed after infection and a delay in symptom development compared to untransformed plants. Wang et al. (2000) transformed barley with hpRNA of the 3' half of the viral genome. As a consequence of the transformation, low virus titre and enhanced resistance were found in the transformants. They proposed that the double-stranded RNA which is formed by the stem of hpRNA expressed in the transformants activated cellular enzymes which recognize and cleave not only dsRNA but also ssRNA of the same sequence (3'half of the viral genome). Therefore, the transformant exhibited a low virus titre and enhanced resistance to BYDV.

The first such coat-protein mediated resistance was identified in the tobacco mosaic virus–tobacco system (Powell et al., 1986). Coat-protein mediated resistance confers broad-or-narrow based protection but the reasons for these differences are not understood. Replicase-mediated resistance often confers complete immunity to the virus but it is usually effective against only closely related strains (Golemboski et al., 1990). Movement protein mediated resistance is not a likely mechanism for BYDV resistance since there is no evidence for movement proteins being involved in the spread of the virus (Lapidot et al., 1993). Antisense RNA mediated resistance is sometimes effective

against strains similar to the template strain (Hammond and Kamo, 1995). Oats and barley plants more resistant than the untransformed controls have been generated but the transgene has not been stably inherited (McGrath et al., 1997).

Since BYDV infects many members of the Poaceae it may be possible to use genetic information from other plants to either transgenically produce resistance in wheat or identify resistance genes in the wheat genome. A high density microsatellite consensus map for bread wheat has been drawn (Somers et al., 2004) and could be studied with the comparative consensus grass map (Gale and Devos, 1998). The potential genetic co-linearity between the wheat genome and that of rice may also provide a useful tool for mapping and breeding (Bennetzen et al., 1998). Within the Triticeae there is a very high level of conservation in marker order which will facilitate such mapping (van Deynze et al., 1995). This will allow marker data from well-characterized small genomes such as rice to be used in more complex species like wheat. The molecular characterization and cloning have been initiated describing the phenomenon involved in defense mechanism of BYDV resistance in wheat (Zhang et al., 2004; Ayala et al., 2007; Gao et al., 2009; Ayala-Navarrete et al., 2009). So far transformation has not produced cereals with virus tolerance, so conventional breeding efforts must continue.

CHAPTER 3

INHERITANCE OF FUSARIUM HEAD BLIGHT RESISTANCE IN SPRING WHEAT LINES WUHAN AND MARINGA

3.0 Inheritance of Fusarium head blight resistance in spring wheat lines Wuhan and Maringa

3.1 Abstract

Fusarium head blight (FHB), a fungal disease caused principally in North America by *Fusarium graminearum* Schwabe, is a serious, worldwide economic threat to all classes of common wheat (*Triticum aestivum* L.). Host-plant resistance offers the best approach for reducing the devastating effects of FHB, but sources of resistance are limited. The wheat accession 'Sumai 3' and its derivatives are widely used for FHB resistance in wheat breeding programs. The Chinese wheat line Wuhan, may provide additional resistance genes for FHB supplementing the resistance conferred by Sumai 3. The Brazilian wheat line, Maringa with useful BYDV tolerance is moderately susceptible to Type II FHB resistance (resistance to spread within the spike). However, the genetics of resistance have not been studied in either of these lines. The objectives of this study were to estimate the genetic control, gene number and heritability of FHB resistance traits in Wuhan and Maringa. F_1 seeds were produced from reciprocal crosses of Wuhan and Maringa with Roblin, a Canada western red spring (CWRS) wheat, which is highly susceptible to FHB. Corn pollen mediated doubled haploid (DH) populations were developed from the F_1 hybrids to investigate the genetic constitution of resistance to FHB in Wuhan and Maringa. The parents, F_1s, and F_1-derived DH lines (150 and 199 DH lines of Wuhan/Roblin and Maringa/Roblin, respectively) from reciprocal crosses were point inoculated with *F. graminearum* in greenhouse experiments to evaluate disease severity (DS). Macroconidial spray inoculations and spread of corn inoculum were used in field

environments conducted in a single year at three locations in Manitoba and one location in Ottawa to evaluate disease incidence, disease severity, FHB Index (% incidence x % severity/100) and *Fusarium*-damaged kernels (FDK). There is no maternal inheritance in Wuhan and Maringa FHB resistance. The generation mean analysis indicated that the Type II resistance in both Wuhan and Maringa are conditioned by at least two or more genes with additive genetic effects. The broad sense heritability was high in all crosses, ranging from 0.71 to 0.94 and 0.73 to 0.93 for the Roblin/Wuhan and Roblin/Maringa populations, respectively. Correlations between greenhouse and field screening environments were positive, statistically significant, but low (0.43 and 0.19). Transgressive segregation for resistance was observed in both populations suggesting multiple gene resistance. This study provides insight into the genetic control of FHB resistance in Wuhan which complements the available sources for FHB resistance in spring wheat and points to the possibility of pyramiding genes.

3.2 Introduction

The fungal disease fusarium head blight (FHB), also known as scab or Fusarium ear blight, is caused principally by *Fusarium graminearum* Schwabe [Teleomorph: *Gibberella zeae* Schwein. (Petch) in North America. It is a serious, worldwide economic threat to all classes of wheat (*Triticum aestivum* L and *Triticum turgidum* L var durum) and other small grain cereals (Schroeder and Christensen, 1963; Parry et al., 1995). The disease is prevalent in warm, humid or semi-humid areas of the world and may cause severe losses in grain yield and quality (Parry et al., 1995, McMullen et al., 1997; Miedaner, 1997; Gilbert and Tekauz, 2000; Dardis and Walsh, 2002; Stack, 2003). The FHB epidemic in 1993 incurred the greatest loss in a single year (over $1 billion) in the northern wheat growing areas of the United States and Manitoba, Canada (Gilbert et al., 1994; McMullen et al., 1997) and since then, losses to the Manitoba wheat industry have been estimated at over $150 million in epidemic years (Humphreys et al., 2001). Clear and Nowicki (2009) suggest greater than a billion dollar loss over 30 years, in Canada, because of reduction in yield and quality due to FHB.

The symptoms of FHB on a wheat spike are characterized by the premature bleaching of single or multiple spikelets after anthesis when the rest of the crop is still green. Bleached spikelets may be seen at the tip, base, or throughout the spike (Shaner, 2003). In wet and warm weather, pink sporodochia form along the edge of the glume or at the point of attachment of the spikelet to the rachis. In severe cases, the entire spike becomes bleached and the rachis turns black. Infected spikelets are often sterile and infection of the rachis may result in grain above the point of infection not filling; grain from blighted spikes is often shriveled (Bailey et al., 2003). Symptoms of FHB are

restricted to the point of inoculation or infection in the most resistant genotypes, but spread to adjacent spikelets causing the entire spike to become bleached in susceptible genotypes (Wang and Miller, 1988).

FHB epidemics are sporadic and highly dependent upon disease-conducive environmental conditions (Mesterhazy, 2003). However, in some regions air-borne inoculum due to extensive corn acreage and reduced or no tillage practices increase the potential for FHB epidemics in wheat fields (Schaafsma et al., 2005). Incorporating genetic resistance into wheat cultivar(s) is an economically sound and environmentally effective means of controlling this disease as chemical and agronomic controls are not entirely successful (Bai and Shaner, 1994; McMullen et al., 1997; Gilbert and Tekauz, 2000). McMullen (2008) has shown that through integrated FHB management strategies, including a combination of two or more control strategies such as crop rotation and use of fungicides, the effects of FHB can be mitigated to some extent, especially in moderately resistant varieties.

Several factors combine to make selection for FHB resistance complicated. First, there is no accession identified that is immune to FHB. The existing sources provide only partial resistance (Mesterhazy, 1997; Ban and Suenaga, 2000; Gilbert and Tekauz, 2000). A few cultivars are found to have adequate levels of resistance to the disease but none is completely resistant to the pathogen (Wilcoxson et al., 1992; Buerstmayr et al., 2002; Liu et al., 2005; McCartney, 2007). Secondly, screening for resistance to FHB requires mature plants and reactions are conditioned by both physiological and morphological factors sometimes resulting in inconsistent disease severity values (Snijders, 1990c; Rudd et al., 2001; Buerstmayr et al., 2002). At least five active resistance mechanisms have been discussed that include Type I – resistance to initial infection, Type II –

resistance to pathogen spread within the wheat spike, Type III – resistance to kernel infection, Type IV – tolerance, Type V – resistance to toxins (Schroeder and Christensen, 1963; Mesterhazy, 1995). This categorization has become the basis for FHB disease evaluation in resistance breeding. Thirdly, FHB disease severity and the FHB disease index (product of percent disease severity and % incidence divided by 100) show a continuous distribution in segregating populations due to the interaction between different loci and the environment (Snijders 1990c; Singh et al., 1996; van Ginkel et al., 1996).

It is vital to continue the search for diverse sources of new and improved FHB resistance to avoid annual losses. A small number of resistant germplasm lines have been identified in a few accessions from different parts of the world such as spring wheat from Asia including the Chinese cultivar Sumai 3 and its Ning derivatives, and Wangshuibai (Del Blanco et al., 2003; Shen et al., 2003a; Zhou et al., 2003; Ma et al., 2006; Ittu et al., 2008); Shinchunaga, Nobeokabouzu-Komugi and Nyubai from Japan (Ban and Suenaga 2000) and the Korean cultivar Chokwang (Yang et al., 2005); spring wheat from south America including Frontana, and Encruzilhada from Brazil (Steiner et al., 2004); winter wheat from Europe including Praag 8, Bizel, Cansas, Petrus, Renan, Apache and Romanus (Snijders 1990; Sip & Stuchlikova 1997; Schmolke et al., 2005; Klahr et al.,2007; Badea et al., 2008; Gervais et al, 2003; Holzapfel et al., 2008), Russian and Ukrainian winter wheat cultivars Hostianum 237, Odesskaya 16 and their derivatives (Zappel et al.,2008); and North American advanced breeding lines with improved FHB resistance (McKendry, 2005; McCartney et al., 2007).

The Chinese cultivar Sumai 3 is widely used in wheat breeding programs in North America and other parts of the world as a source of FHB Type II resistance

(Gilbert and Tekauz, 2000; Stack, 2003). The genetics of this source of resistance have been studied in both Sumai 3 and its derivatives using segregating recombinant inbred, or doubled haploid populations (Ban and Suenaga 2000; van Ginkel et al., 1996). Although studies of inheritance of FHB host resistance have produced conflicting results, all have agreed that the inheritance is quantitative, with the numbers of estimated resistance genes ranging from one to five with smaller additive effects depending on the type of population used (van Ginkel et al., 1996; Bai et al., 2000; Ban and Suenaga, 2000; Buerstmayr et al., 2002; Liu et al., 2005).

The exploitation of other sources of FHB resistance is needed to diversify the genetic base of resistance in elite wheat germplasm and to enable a strategy for pyramiding independent genes in order to obtain an increased level of enduring resistance. Recently identified newer sources include the winter wheat Ernie, an adapted cultivar in the USA (McKendry et al., 1995) and the European winter wheat Arina (Ruckenbauer, 2001). Fedak et al. (2001) reported that Wuhan 1 has a similar level of FHB resistance to Sumai 3 but may have different resistance genes. Maringa, a derivative of Frontana which is moderately resistant to FHB (Steiner et al, 2004), is reported to be moderately susceptible to FHB.

Diallel analysis has revealed significant general combining ability and specific combining ability for FHB resistance (Snijder 1990b; Buerstmayr et al., 1999). In some crosses the F_1 was superior to the most resistant parent. This suggests more resistant homozygous progeny can be selected from the population derived from such crosses due to transgressive segregation.

It is possible that Wuhan and Maringa, singly as well as in combination can be used as a complementary source of FHB resistance to Sumai 3. Sumai 3 with the highest

level of FHB resistance is the progeny of two moderately susceptible parents, Italian wheat Funo and the landrace Taiwan Xiaomai wheat. However the genetics of resistance in Wuhan and Maringa are not known. The objectives of this study were (1) to investigate the nature of inheritance of resistance to FHB in Wuhan and Maringa under greenhouse and field environments and (2) to estimate the heritability of FHB resistance in Wuhan/Roblin and Maringa/Roblin progeny.

3.3 Materials and methods

3.3.1 Population development

Wuhan 1 (referred to as Wuhan henceforth), a Chinese wheat line resistant to FHB, Maringa, a Brazilian wheat moderately susceptible to FHB and Roblin, Canadian western red spring (CWRS), highly susceptible to FHB (Table 3.3.1), were obtained from the Cereal Research Centre in Winnipeg. F_1 seeds were obtained from reciprocal crosses of Wuhan and Roblin, and Maringa and Roblin. The pedigree of Wuhan is unknown. Several lines of Wuhan (Wuhan 1, 2, 3…) are reported to exist (Person. commun. G. Fedak).

Table 3.3.1 Origin, pedigree, some physical characteristics and reactions to barley yellow dwarf (BYD) and fusarium head blight (FHB) of the parental lines used, Wuhan 1 and Maringa.

Genotype	Origin	Pedigree	Awn/ Non-awn	Height	Reaction to FHB
Roblin	Canada	BW15/BW38//RL4359/RL4353	Non-Awned	Short	Highly susceptible
Maringa	Brazil	Frontana/Kenya 58//Ponta Grossa1	Awned	Tall	Intermediate
Wuhan	China	Unknown	Awned	Medium	Resistant

3.3.2 Generation of doubled haploid (DH) lines from F_1 seeds

Doubled haploid (DH) lines are homozygous and thus homogeneous and ideal for genetic analysis because they do not express dominance variation and segregation within lines. The corn pollen (*Zea mays* L.) -mediated DH technology (Figure 3.3.1) used in this research refers to the *in vitro* culturing of haploid embryos. The F_1 hybrids were used for the development of DH populations. Pollen from a maize cultivar, [bred from the four-way cross, *Zea mays* L. cvs: Seneca 60/Golden Bantam//Manitoba Sweet/Indian Flint corn, for this particular technology for wheat and oats (Aung, T., 1998)] was used to stimulate embryo development in wheat caryopses. When the plants were at the two to three-tiller stage, they were treated with colchicine for chromosome doubling according to the protocol adapted from the Cereal Research Centre, Winnipeg (Appendix 1).

Regenerated doubled haploid plants were grown for one generation to produce a sufficient amount of uniform seed for the experiments. Increases were carried out between September 2002 and May 2003. A total of 412 DH lines, from two reciprocal crosses were generated during March 2001 – April 2002 from F_{1s}. Some of the DH lines (14 lines from Roblin/Wuhan, 19 lines from Wuhan/Roblin, 13 lines from Roblin/Maringa and 17 lines from Maringa/Roblin) perished during a cabinet breakdown so 349 DH lines were available for assessment.

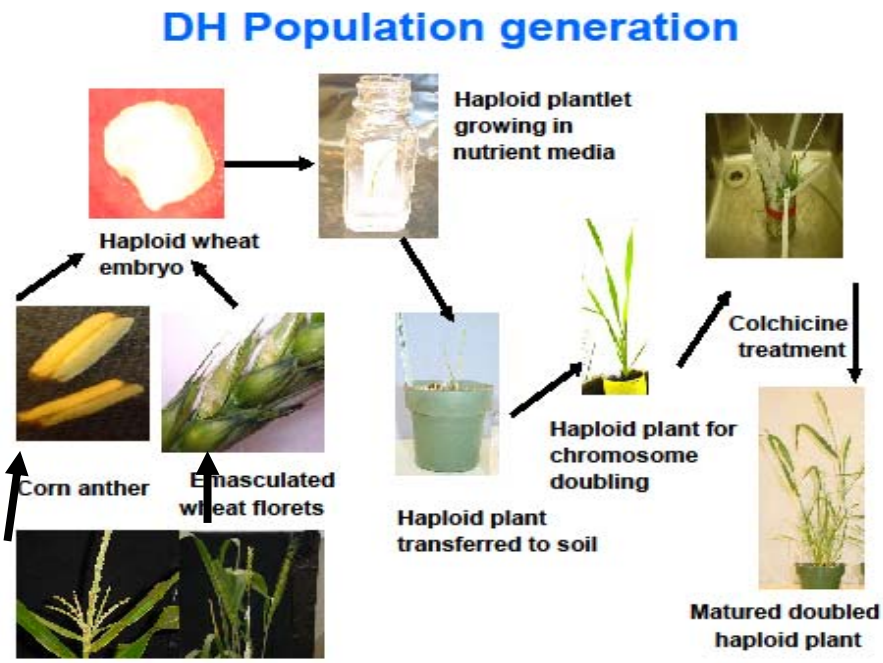


Figure 3.3.1 Process involved in corn-mediated doubled haploid technology in wheat.

1. Maize pollen was used to develop haploid wheat embryo
2. Embryo was rescued and were transferred to nutrient medium
3. Haploid plantlet growing in nutrient media were transferred to soil medium
4. Chromosomes were doubled via colchicine treatment generating a doubled haploid plant
5. Doubled haploid plants produce fertile seeds

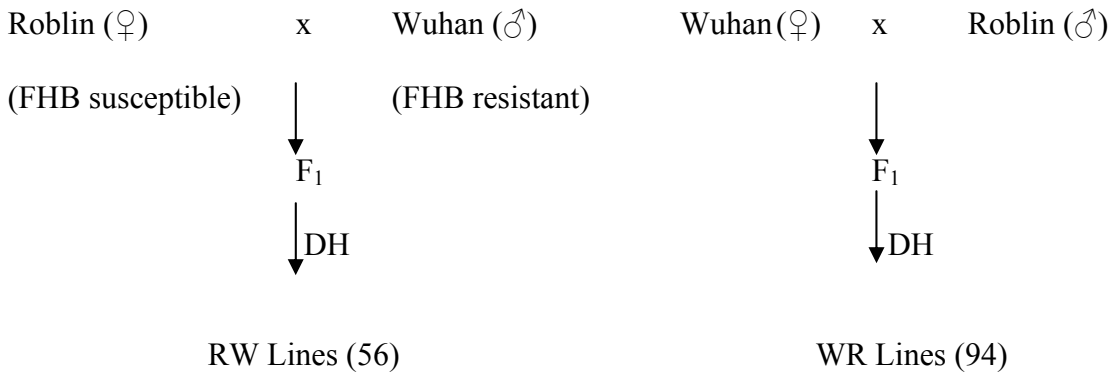


Figure 3.3.2a Generation of Roblin/Maringa and Maringa/Roblin populations via corn pollen mediated doubled haploid technology.

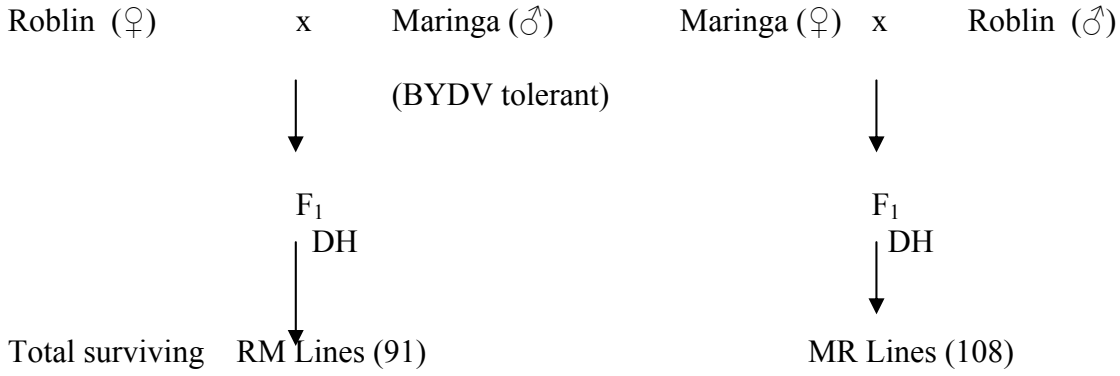


Figure 3.3.2b Generation of Roblin/Wuhan and Wuhan/Roblin populations via corn pollen mediated doubled haploid technology.

3.3.3 Pathogen and inoculum production

Currently there is no evidence of strain or isolate specificity in FHB resistance and inoculum for all experiments was prepared from a mixture of three *F. graminearum* isolates (JM #6, EEI #20/6 and EEI #23/6) that were tested earlier for aggressiveness (Dr. J. Gilbert, Cereal Research Centre, Agriculture and Agri-Food Canada, Winnipeg). The mixture of isolates was propagated in carboxymethyl cellulose medium (CMC, 1.0 g NH_4NO_3, 1.0 g KH_2PO_4, 0.5 g $MgSO_4$. $7H_2O$, 1.0 g yeast extract, 15 g CMC, L H_2O, 0.2 g streptomycin sulphate [Sigma-AldrichCo., St.Louis, Mo]). Inoculum was diluted with sterile water and standardized to 5 x 10^4 macroconidia mL^{-1} using a hemacytometer. A drop of Tween 20 was added per 100 mL inoculum to reduce surface tension. New CMC cultures were prepared every two weeks.

3.3.4 FHB disease evaluation in the greenhouse

Parents, F_1s, and F_1-derived DH lines were evaluated (2002 –2003) for FHB resistance under controlled environmental conditions. Ten seeds per parental line, ten seeds per F_1 and a total of 349 DH lines (Wuhan/Roblin – 94; Roblin/Wuhan – 56; Roblin/Maringa – 91 and Maringa/Roblin – 108, four seeds per line) were taken for the experiment. Plants were grown in 4 cm x 20 cm cones in growth cabinets or a greenhouse with 16/8 h light/dark at 22/18^{o} C, respectively. The experiment was conducted using hydroponics, growing two seeds per cone for each line in a completely randomized block design with two replications. The experiment was repeated once. The plants were grown in growth cabinets until 50% anthesis and transferred to the greenhouse.

When plants were at 50% anthesis, they were screened for FHB Type II resistance (resistance to disease spread within the spike) with *F. graminearum.* Two central and opposite florets of the primary spike on each plant were inoculated using a micropipette to inoculate florets with a macro-conidial suspension of a mixture of *F. graminearum* isolates (10µl of a suspension with 50,000 macroconidia spores/mL). The plants were then exposed to 100% RH for 24 hours at 22 - 24^{o} C in a humidity chamber to promote disease development after which they were then returned to the greenhouse bench.

In the greenhouse, level of disease infection on the wheat spikes was scored at 7, 14, and 21 days after inoculation. Disease severity (DS) ratings were assessed by counting number of infected spikelets and total number of spikelets, and calculating the percent infection (infected spikelet(s)/total spikelets x 100) 21 days after inoculation. It is noted here that all the florets on the spike were taken into consideration although some of the spikelets above the inoculation point could have gone through early senescence as a result of the restriction of water flow due to FHB infection.

3.3.5 FHB disease evaluation in the field

The DH populations were evaluated for FHB in replicated field trials at three FHB nurseries in Manitoba (Carman, Glenlea and Portage La Prairie) and one location at the Eastern Cereals and Oilseeds Research Centre (ECORC), Ottawa, Ontario, in the summer of 2003. Two populations Roblin/Wuhan (RW) and Roblin/Maringa (RM) were not included in Ottawa trials due to unavailability of DH seeds. All locations completed seeding between May 22 and June 4, 2003. The experiment was conducted as a randomized complete block design with two replications. The test materials were seeded

at a rate of 60-65 seeds/row in 1 meter row length with 17 cm row spacing in Carman, and 1.5 meter row length with 30 cm row spacing in other locations. The parents, Roblin, Maringa, and Wuhan were included as checks and were seeded after every 20 rows of DH lines.

In Carman and Glenlea, the plants in the rows were identified and marked to denote 50% anthesis and date of first inoculation. The spikes of the entire row were spray-inoculated with a macro-conidial suspension of a mixture of three *Fusarium graminearum* isolates (50,000 macroconidia spores/ml) when the plants in the row were at 50% anthesis. Inoculation was repeated two to three days later to cover the late emerging wheat spikes. In the Portage la Prairie FHB nursery, the plants were inoculated by spreading *F. graminearum*-infested corn kernels at a rate of 20g/m^2. The corn kernel inoculum was applied in the field two-three weeks before anthesis. In Ottawa, the plants were inoculated using corn kernel and barley seed inoculum. The FHB nurseries in Portage la Prairie and Ottawa were equipped with an overhead irrigation system which was activated for half an hour every day after plant inoculation for three weeks. The Glenlea nursery was irrigated with a sprinkler system for 30 minutes following each inoculation. At the Carman nursery, plots were mist-irrigated for five minutes every hour for ten hours after inoculation for two weeks.

Spike emergence dates as well as 50% anthesis for each DH and parental line were recorded. For the field experiment two methods of FHB assessment were used: Fusarium head blight index (FHBI) and *Fusarium*-damaged kernels (FDK). Disease incidence (DI - infected wheat spikes per row) and disease severity (DS - infected spikelets per spike) for each row were recorded at 18 – 21 days post-inoculation as the percentage of diseased spikes in plots and disease severity according to a 0 – 100% scale

for visually infected spikelets on a whole plot basis. The FHBI was calculated as the product of disease incidence x disease severity/100.

For assessment of FDK, wheat spikes from Carman, Glenlea and Ottawa field trials were harvested at the end of the season when plants were mature. The Glenlea field nursery was harvested using offset double thresher. The Carman nursery was hand-harvested and threshed using a single head thresher at the Cereal Research Centre, Winnipeg. Seeds were cleaned using an air blast seed cleaner. While cleaning, care was taken to ensure FDK were retained in the harvested sample. The Ottawa field trials were maintained and harvested by Drs. G. Fedak and W. Cao, ECORC. Harvested seed samples were placed in paper bags. Percent FDK was expressed by counting the visually damaged kernels and total number of kernels from two 50g field-harvested grain samples from each DH line and parents (number of damaged kernels/total number of kernels x 100). *Fusarium*-damaged kernels were identified as shriveled, light weight and chalky-white kernels with occasional pink colouration. These kernels were distinguishable from visually healthy plump kernels within the sample.

3.3.6 Statistical analysis

Statistical analyses were performed using SAS® 9.2 (SAS version 9.2, SAS Institute Inc., Cary, N.C.). All data were tested for normality using the PROC UNIVARIATE. The F_1 means, population means and least square means (LSM) of the DH lines were evaluated. Least square means of the 25 % top ranking DH lines were compared to check the consistency in their ranking over all locations. T-tests were performed on the least square means to test if the data could be pooled over crosses, experiments and locations. Analyses of variance (ANOVA) for DI, DS, FHBI and FDK

for each site and a combined analysis of the locations were performed using PROC MIXED with location and genotype as fixed factors and DH line (genotype) and rep (location) treated as random in the model. All effects in the model were considered random for estimating heritability. Broad-sense heritability for each population was estimated from ANOVA using the formulae $h^2 = \sigma_G^2 \Big/ [\sigma_G^2 + (\sigma_e^2 / r)]$ for single location and greenhouse data, $h^2 = \sigma_G^2 \Big/ [\sigma_G^2 + (\sigma_{GL}^2 / l) + (\sigma_e^2 / rl)]$ for combined locations where σ_G^2 is the genotypic variance, σ_{GL}^2 is genotype x location variance, σ_e^2 is residual variance, *r* is the number of replications (blocks), *l* is the number of locations.

In the PROC MIXED model a contrast statement was added to estimate the genetic effects (maternal, non-additive and additive effects) for all four disease traits (DI, DS, FHBI and FDK) for both populations using least square means of genotypes. The model presented here is for the Roblin/Wuhan population, where genotypes equal WR $= \mu + ½\ g_W + ½\ g_R + H_{WR} + M_W$; RW $= \mu + ½\ g_W + ½\ g_R + H_{WR} + M_R$; R $= \mu + g_R + M_R$; W $= \mu + g_W + M_W$, where μ = population mean, g = genetic effect, M = maternal effect and H = heterosis

The contrasts: (1) Maternal effect: WR –RW = MW - M_R;

(2) Non-additive or heterosis: [(WR + RW) – (R + W)]/2 = [($\mu + ½\ g_W + ½\ g_R + H_{WR} + M_W + \mu + ½\ g_W + ½\ g_R + H_{WR} + M_R$) – ($\mu + g_R + M_R + \mu + g_W + M_W$)]/2 = [($2\mu + g_W + g_R + 2\ H_{WR} + M_W + M_R$) – ($2\mu + g_W + g_R + M_W + M_R$)]/2 = H_{WR};

(3) Direct additive genetic: (W-R) – (WR – RW) = ($\mu + g_W + M_W - \mu + g_R + M_R$) – ($\mu + ½\ g_W + ½\ g_R + H_{WR} + M_W - \mu + ½\ g_W + ½\ g_R + H_{WR} + M_R$) = ($g_W - g_R$) + ($M_W - M_R$) – ($M_W - M_R$). The coefficients used for these variables were expressed as fractions based

on the expected proportion of genes contributed by each genotype for maternal, non-additive and additive effects and are summarized in the following table format.

Genotype	Contrast 1	Contrast 2	Contrast 3
RW	-1	0.5	1
Roblin	0	-0.5	-1
WR	1	0.5	-1
Wuhan	0	-0.5	1

Pearson correlation coefficients between any two traits were computed using PROC CORR at $\alpha = 0.05$, to determine the degree of association among the traits of interest.

3.4 Results

Generation of doubled haploid (DH) populations through corn pollen fertilization was successful; some degree of genotypic variation was observed among the reciprocal crosses (Table A1.1). The reciprocal cross Maringa x Roblin (MR and RM) F_1s developed more caryopses (85 and 80%, respectively, from the pollinated florets) than Roblin x Wuhan (RW and WR) F_1s (65 and 66% respectively). More fertile DH plants (50% for MR and RM F_1s and 30% for RW and WR F_1s) from the rescued embryos were obtained for MR F_1s resulting in a larger DH population for these lines. In both crosses, many caryopses contained only watery sacs without embryos indicating embryo abortion at an early stage.

Difference in days to heading of the three parent cultivars in the greenhouse was 8 -12 days and in the field it was 10 – 15 days. Roblin had early spike emergence and

Wuhan was the late line. Heading days for the DH lines spanned 20 days in the green house and 39 days between the earliest and the latest spike emergence in the field. Some Roblin\Wuhan reciprocal DH lines exhibited a grassy phenotype and did not produce spikes (Table 3.4.1).

Table 3.4.1 Spike emergence in four doubled haploid (DH) populations generated from reciprocal crosses between wheat cultivars Maringa/Roblin (MR) and Roblin/Wuhan (RW) expressed as a percentage relative to parents. Total number of lines: RW (56), WR (94), MR (108) and RM (91) tested in both greenhouse and field.

DH Line	≤ both parents Roblin,Wuhan or Maringa (%)	6 days later than the late parent Wuhan (%)	Grassy phenotype No spike emergence
WR	57	40	3
RW	54	39	7
MR	95	5	0
RM	84	16	0

Fusarium graminearum spray inoculation at Carman and Glenlea started on July 14 and finished August 13, 2003, because of the differences in heading/anthesis dates for different DH lines disease ratings were conducted 21 days after inoculation. In Portage La Prairie and Ottawa experiments, inoculum depended on ascospores produced by corn and barley grain spawn.

The residuals of the dependent variables from the univariate analysis exhibited a (near) normal distribution. Arcsine transformation of data did not change the distribution; therefore the untransformed data were used in all analyses.

Results of the t-tests assessing whether the means of the FHB disease severity obtained from the greenhouse experiments of two reciprocal populations from Roblin/Wuhan and Roblin/Maringa, respectively, are presented in Table 3.4.2. The t-value for the Roblin/Wuhan reciprocal crosses at 95% cl (confidence level) is significant (pooled t-value = 0.038) indicating there is a difference between the two populations. This result could in part be due to the fact that the population size for Roblin/Wuhan (56 DH Lines) is smaller than Wuhan/Roblin (94 DH lines). For Roblin/Maringa populations, the means are not significantly different (pooled t-value = 0.051). Despite the statistical significance the two populations were pooled to perform the analysis of variance considering the small differences in the mean values of the F_1s and the DH populations.

Table 3.4.2 The t-Test for FHB disease severity (DS) for Roblin/Wuhan and Maringa/Roblin doubled haploid populations artificially inoculated with *Fusarium graminearum* in the greenhouse.

Cross	N	Method	Mean	Variance	Df	Std Err	t-Value	Pr>t
RW	224		45.80			1.34		
WR	376		49.19			0.98		
Diff (1-2)			-3.39			1.63		
Diff (1-2)		Pooled	-3.39	equal	598		-2.08	0.0381
Diff (1-2)		Satterthwaite	-3.39	unequal	449.14		-2.05	0.0409
MR	432		64.84			0.90		
RM	364		62.32			0.92		
Diff (1-2)			2.52			1.29		
Diff (1-2)		Pooled	2.52	equal	794		1.95	0.0514
Diff (1-2)		Satterthwaite	2.52	unequal	785.4		1.96	0.0501

Alpha level =0.5

3.4.1 Analysis of variance of the phenotypic data

The analyses of variance for the FHB disease severity of the two combined populations, Roblin/Wuhan (reciprocal crosses) and Maringa/Roblin (reciprocal crosses) from the greenhouse are presented in Tables 3.4.3 and 3.4.4. In both populations the DH line and the cross are highly significant sources of variation. Differences between DH lines were highly significant (F-value = 40.07 for Roblin/Wuhan and 5.86 for Maringa/Roblin) for disease severity in both populations.

Table 3.4.3 Analysis of variance for FHB disease severity (DS) for the reciprocal crosses of Roblin/Wuhan doubled haploid populations artificially inoculated with *Fusarium graminearum* from the greenhouse experiment.

Dependent variable: Disease Severity (DS)

Source	df	SS	MS	F Value	Pr>F
Expt	1	206.489	206.489	5.84	0.0163
Cross	1	1616.886	1616.886	45.71	<.0001
Expt*Cross	1	89.847	89.847	2.54	0.1120
DH line (Cross)	148	209768	1417.354	40.07	<.0001
Expt *DH line(Cross)	148	3169.104	21.412	0.61	0.9997
Error	300	10612	35.373	.	.

Table 3.4.4 Analysis of variance for FHB disease severity (DS) for the reciprocal crosses of Maringa/Roblin doubled haploid population artificially inoculated with *Fusarium graminearum* from the greenhouse experiment.

Dependent variable: Disease Severity (DS)

Source	df	SS	MS	F Value	Pr>F
Expt	1	34.356	34.356	0.22	0.63
Cross	1	1256.544	1256.544	8.13	0.0046
Expt*Cross	1	8.603	8.603	0.06	0.8136
DH line (Cross)	197	178526	906.223	5.86	<.0001
Expt *DH line	197	21968	111.513	0.72	0.9951
Error	398	61518	154.567	.	.

For the field experiments, the top ranking 25 % of the DH lines from all three locations were consistent, although there were some occurrences of small changes in rank within the group. The analyses of variance were performed separately by location and genotype; genotype at each location and combined location and genotype for each reciprocal population. However, only the location and genotype combined analysis for each population is presented in Tables 3.4.5 and 3.4.6.

Analyses of variance for DI, DS, FHBI and FDK for combined field locations were significant for DH line (Genotype) and Loc * DH line (Genotype) for the Roblin/Wuhan population indicating the high of variation in the DH lines. Genotype was also a significant source of variation in the case of FDK for this population at $\alpha = 0.05$ (Table 3.4.5). For the Roblin/Maringa population all sources of variation were significant. For FDK, none of the sources of variation were significant for this population (Table 3.4.6).

Table 3.4.5 Analysis of variance for FHB disease incidence (DI), severity (DS), index (FHBI) and *Fusarium*- damaged kernels (FDK) for Wuhan /Roblin doubled haploid lines artificially inoculated with *Fusarium graminearum* from three, Carman, Glenlea and Portage la Prairie, field locations.

Dependent variable: Disease Incidence (DI)

Source	df	SS	MS	F Value	Pr>F
Genotype	3	222480	74160	3.53	0.0169
Loc	2	5672.09	2836.05	1.14	0.3239
Loc*Genotype	6	5581.95	930.33	0.30	0.9355
Rep (Loc)	3	24.32	8.11	0.07	0.9740
DH line (Genotype)	141	278542	1975.49	5.26	<.0001
Loc* DH line (Genotype)	282	105906	375.55	3.42	<.0001
Residual	635	69651	109.69	.	.

Dependent variable: Disease Severity (DS)

Source	df	SS	MS	F Value	Pr>F
Genotype	3	199189	66396	4.40	0.0056
Loc	2	189.86	94.93	0.03	0.9731
Loc*Genotype	6	4828.96	804.83	o.20	0.9761
Rep (Loc)	3	786.82	262.27	2.66	0.0471
DH line (Genotype)	141	202239	1434.32	3.21	<.0001
Loc* DH line (Genotype)	282	126007	446.83	4.54	<.0001
Residual	635	62510	98.44	.	.

Dependent variable: FHB index (FHBI)

Source	df	SS	MS	F Value	Pr>F
Genotype	3	246885	82295	4.39	0.0056
Loc	2	1011.02	505.51	0.16	0.8548
Loc*Genotype	6	6036.51	1006.08	0.26	0.9551
Rep (Loc)	3	166.11	55.37	0.72	0.5430
DH line (Genotype)	141	245863	1743.71	4.18	<.0001
Loc* DH line (Genotype)	282	117602	417.03	5.39	<.0001
Residual	635	49142	77.39	.	.

Dependent variable: *Fusarium*-damaged kernels (FDK)

Source	df	SS	MS	F Value	Pr>F
Genotype	1	13175	13175	8.68	0.0038
Loc	1	2593.79	2593.79	3.47	0.0709
Loc*Genotype	1	1865.84	1865.84	2.60	0.1088
Rep (Loc)	2	335.63	167.82	1.25	0.2880
DH line (Genotype)	141	214806	1523.48	2.25	<.0001
Loc* DH line (Genotype)	141	101311	718.52	5.35	<.0001
Residual	283	37981	134.21	.	.

Table 3.4.6 Analysis of variance for FHB disease incidence (DI), severity (DS), index (FHBI) and *Fusarium*- damaged kernels (FDK) for Maringa/Roblin doubled haploid lines artificially inoculated with *Fusarium graminearum* from four (Carman, Glenlea, Portage la Prairie and Ottawa) and field locations.

Dependent variable: Disease Incidence (DI)

Source	df	SS	MS	F Value	Pr>F
Genotype	3	267760	89253	6.24	0.0005
Loc	3	22456	7485.43	3.24	0.0272
Loc*Genotype	6	12394	2065.68	0.72	0.6323
Rep (Loc)	4	2459.91	614.98	4.56	0.0012
DH line (Genotype)	191	295907	1549.25	4.16	<.0001
Loc* DH line (Genotype)	483	180286	373.26	2.77	<.0001
Residual	886	119571	134.96	.	.

Dependent variable: Disease Severity (DS)

Source	df	SS	MS	F Value	Pr>F
Genotype	3	207346	69115	6.26	0.0005
Loc	3	85586	28529	13.16	<.0001
Loc*Genotype	6	23372	3895.32	1.32	0.2483
Rep (Loc)	4	1481.89	370.47	2.72	0.0286
DH line (Genotype)	191	231209	1216.89	3.19	<.0001
Loc* DH line (Genotype)	483	184388	381.76	2.80	<.0001
Residual	886	120703	136.23	.	.

Dependent variable: FHB index (FHBI)

Source	df	SS	MS	F Value	Pr>F
Genotype	3	240529	80175	4.66	0.0037
Loc	3	76073	25358	7.53	0.0001
Loc*Genotype	6	20546	3424.30	0.76	0.5986
Rep (Loc)	4	2413.52	603.38	4.91	0.0006
DH line (Genotype)	190	345524	1818.55	3.61	<.0001
Loc* DH line (Genotype)	483	243354	503.84	4.10	<.0001
Residual	886	108866	122.87	.	.

Dependent variable: *Fusarium*-damaged kernels (K) (FDK)

Source	df	SS	MS	F Value	Pr>F
Genotype	1	17089	17089	0.72	0.3973
Loc	2	87093	43546	1.20	0.3860
Loc*Genotype	1	42128	42128	1.42	0.2346
Rep (Loc)	3	100594	33531	1.27	0.2842
DH line (Genotype)	191	4232383	22159	0.75	0.9857
Loc* DH line (Genotype)	293	8701510	29698	1.12	0.1287
Residual	485	12811177	26415	.	.

3.4.2 Disease severity generation means

In the greenhouse, darkening or discolouration at the point of inoculation with *F. graminearum* was visible by 7 days after inoculation. Disease progression was limited to a few glumes near the point of inoculation at day 14 for the susceptible parent and DH lines, but progressed quickly between 14 and 21 days after inoculation. The disease ratings were converted to percent severity.

The three parents differed significantly and consistently in FHB disease severity in all experiments. The resistant parent Wuhan was significantly better than Maringa and Roblin, and Maringa was significantly better than the susceptible parent Roblin (Table 3.4.7). The mean for Roblin was 86.42 and 82.80, for Maringa, 50.63 and 48.35 and for Wuhan 18.20 and 22.56 for greenhouse and field respectively. The means of the F_1s and reciprocal F_1s (RW F_1 = 31.1; WR F_1= 33.1; RM F_1 = 52.77 and MR F_1 = 49.21) when evaluated in the greenhouse, were not significantly different which suggested that resistance was not maternally inherited. The FHB disease severity ranged from 9.0 – 100 in the Roblin/Wuhan population, 11.76 – 100 in the Wuhan/Roblin population, 13.33 - 100 in the Maringa/Roblin population and 15.79 – 100 in the Roblin/Maringa population in the greenhouse experiments.

Table 3.4.7 Means and standard error of fusarium head blight disease severity for parent cultivars and doubled haploid lines inoculated with *Fusarium graminearum* in two greenhouse experiments and four field locations.

Cultivar	Mean Severity (field)	Std error	Mean Severity (greenhouse)	Std error
Roblin	82.80[a](127)	0.832	86.42 [a] (40)	1.348
Maringa	48.35[b](127)	1.012	50.63 [b] (40)	0.636
Wuhan	22.56[c](127)	0.647	18.20 [c] (40)	0.429
DH lines: Wuhan x Roblin*	48.48 (150)	0.701	48.51 (150)	1.632
DH lines: Maringa x Roblin*	57.23 (199)	0.616	63.66 (199)	1.072

Means with the same letter are not significantly different

LSD 1.77 $\alpha = 0.05$

Numbers in parenthesis are the number of plants (parents) or DH lines (reciprocal crosses) assessed

* Doubled haploid lines from reciprocal crosses combined

The means, mid parent values and the ranges for the field experiments are presented in Tables 3.4.8 and 3.4.9. All four disease traits of the DH lines across environments ranged from low to high, DI: 5 – 100; DS: 5 – 100; FHBI: 0.25 – 100 and FDK: 1.4 – 100. The F_1 mean values for disease severity were lower than the mid parent values in all cases, however, the DH line means were close to mid parent values in most cases for all traits (DI, DS, FHBI and FDK) in both Roblin/Wuhan and Roblin/Maringa populations indicating the additive genetic effect for inheritance.

Table 3.4.8 Means and ranges of Wuhan, Roblin, and DH populations from Wuhan/Roblin combined reciprocal crosses for fusarium head blight disease traits (incidence, severity, index and *Fusarium*-damaged kernels) artificially inoculated with *Fusarium graminearum* from four field experiments.

Trait	Location	Mean			Mid-parent value	DH line Range
		Wuhan	Roblin	DH line		
Disease	Carman	22.8	80.6	60.2	51.7	7.5 - 100
Incidence	Glenlea	23.0	82.3	55.4	52.7	10 - 100
	Ottawa	29.3	87.5	62.4	64.4	20 - 92.5
	Portage	19. 6	83.4	53.0	51.5	15 - 90
	Mean	23.7	83.5	54.9	55.1	13.1 - 95.6
Disease	Carman	25.0	81.6	49.7	53.3	7.5 - 100
Severity	Glenlea	22.5	87.3	48.1	54.7	5 - 100
	Ottawa	21.3	77.5	33.4	49.4	7.5 - 70
	Portage	20.6	82.5	43.6	51.5	20 - 85
	Mean	22.3	82.2	43.7	52.2	10. - 88.75
FHB	Carman	5.6	65.6	33.3	35.6	1 - 90.5
	Glenlea	4.9	71.2	29.1	38.1	0.25 - 95
Index	Ottawa	8.9	67.8	23.9	38.4	1.75 - 62.88
	Portage	4.8	67.4	29.9	36.1	3.5 – 68
	Mean	6.1	68.0	29.1	37.1	1.63 - 79.1
Fusarium-	Carman	18.6	75.3	50.3	46.9	4.28 – 95
damaged	Glenlea	11.9	70.6	43.7	41.2	1.4 – 95
Kernels	Ottawa	21.0	80.0	50.6	50.5	15.5 – 88
	Mean	17.2	75.3	48.2	46.2	7.06 - 92.67

Table 3.4.9 Means and ranges of Maringa, Roblin and DH populations from Maringa/Roblin combined reciprocal crosses for fusarium head blight disease traits (incidence, severity, FHBI and *Fusarium*-damaged kernels) artificially inoculated with *Fusarium graminearum* from four field experiments.

Trait	Location	Mean			Midparent	DH line Range
		Maringa	Roblin	DH line	Value	
Disease	Carman	45.3	92.3	72.5	68.7	20.0 - 100
Incidence	Glenlea	38.5	86.5	74.9	62.6	25 - 100
	Ottawa	38.5	90.0	68.1	64.2	35 - 92.5
	Portage	36.4	88.5	58.9	62.4	15 - 100
	Mean	39.7	89.3	68.6	64.5	23.75 - 98.1
Disease	Carman	51.2	77.1	64.8	64.2	20 - 100
Severity	Glenlea	51.7	95.6	67.1	73.6	20 - 100
	Ottawa	26.9	76.8	42.2	51.8	15 - 74.25
	Portage	53.3	76.3	53.7	64.7	15 - 95
	Mean	41.7	81.5	56.9	61.5	17.5 - 92.3
FHB	Carman	23.1	67.8	51.5	45.5	5 - 100
	Glenlea	22.1	84.5	51.7	53.3	6 - 100
Index	Ottawa	10.3	69.3	33.9	39.7	6 - 74.25
	Portage	16.1	64.5	32.0	40.3	2 - 85.5
	Mean	17.9	74.0	42.28	45.9	4.75 - 89.9
Fusarium-	Carman	48.2	83.8	63.5	66.0	13.78 - 95
damaged	Glenlea	30.7	87.3	64.5	59.0	17.1 - 95
Kernels	Ottawa	28.4	87.5	51.8	57.9	17.5 - 100
	Mean	35.8	86.2	59.9	61.0	16.13 - 96.7

3.4.3 Frequency distribution

The frequency distributions for FHB disease severities for Wuhan/Roblin and Maringa/Roblin DH populations from greenhouse and field experiments showed a continuous distribution (Figure 3.4.1: A and B; and Figure 3.4.2: A and B). The DH lines segregated for disease severity with values that covered the entire parental range without clearcut demarcations for bimodal or trimodal peaks. Transgressive segregation beyond the resistant and susceptible parents among the DH lines was observed at all locations indicating the quantitative nature of inheritance; however the majority of the segregants were inferior (highly susceptible). The distribution skewed towards Roblin, the susceptible parent, for all traits in all doubled haploid populations.

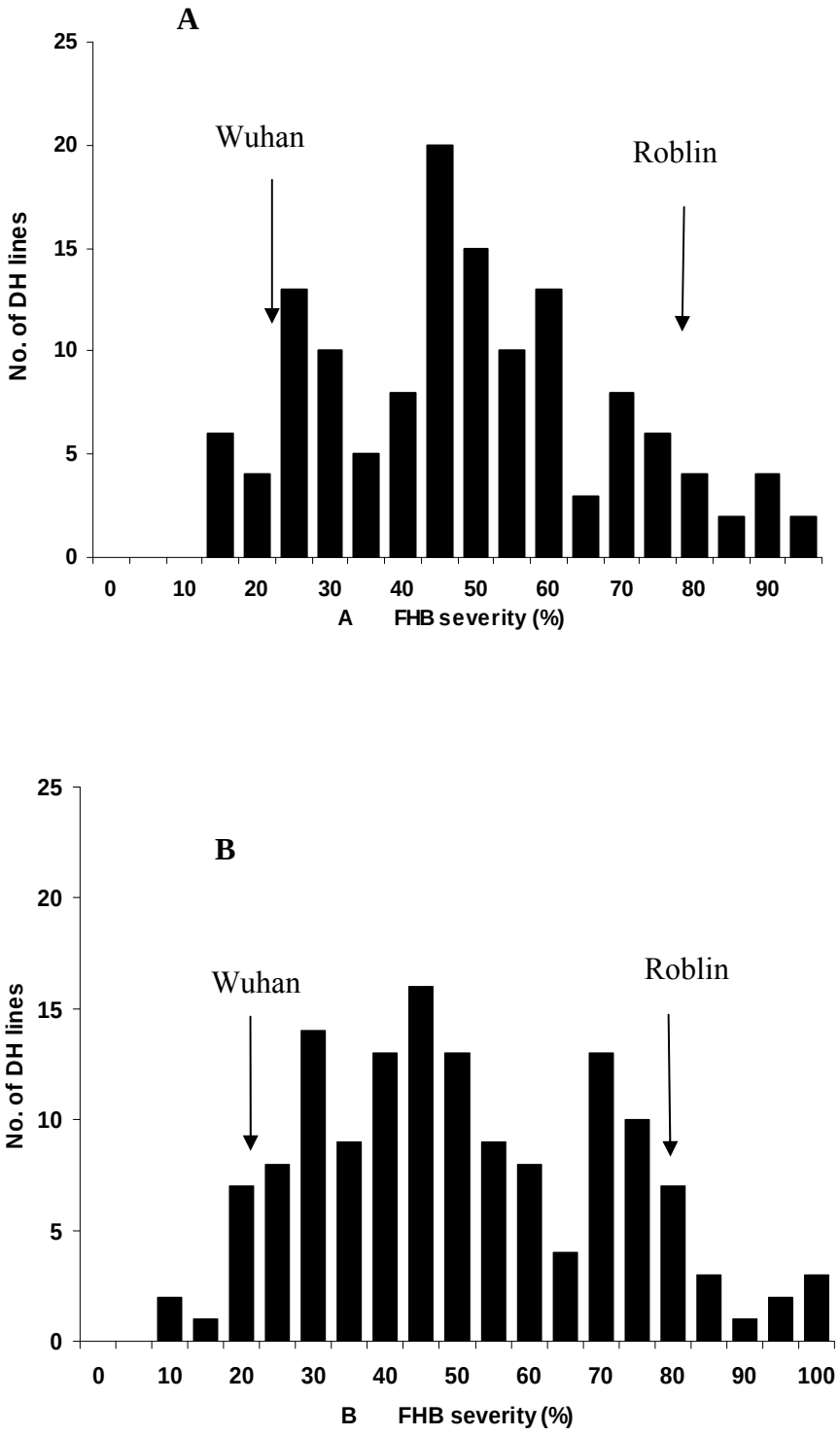


Figure 3.4.3 A and B. Phenotypic distribution of fusarium head blight severity (FHB) (with Type II resistance to FHB), in a doubled haploid population developed from the cross of Wuhan/Roblin in A) greenhouse and B) field experiments.

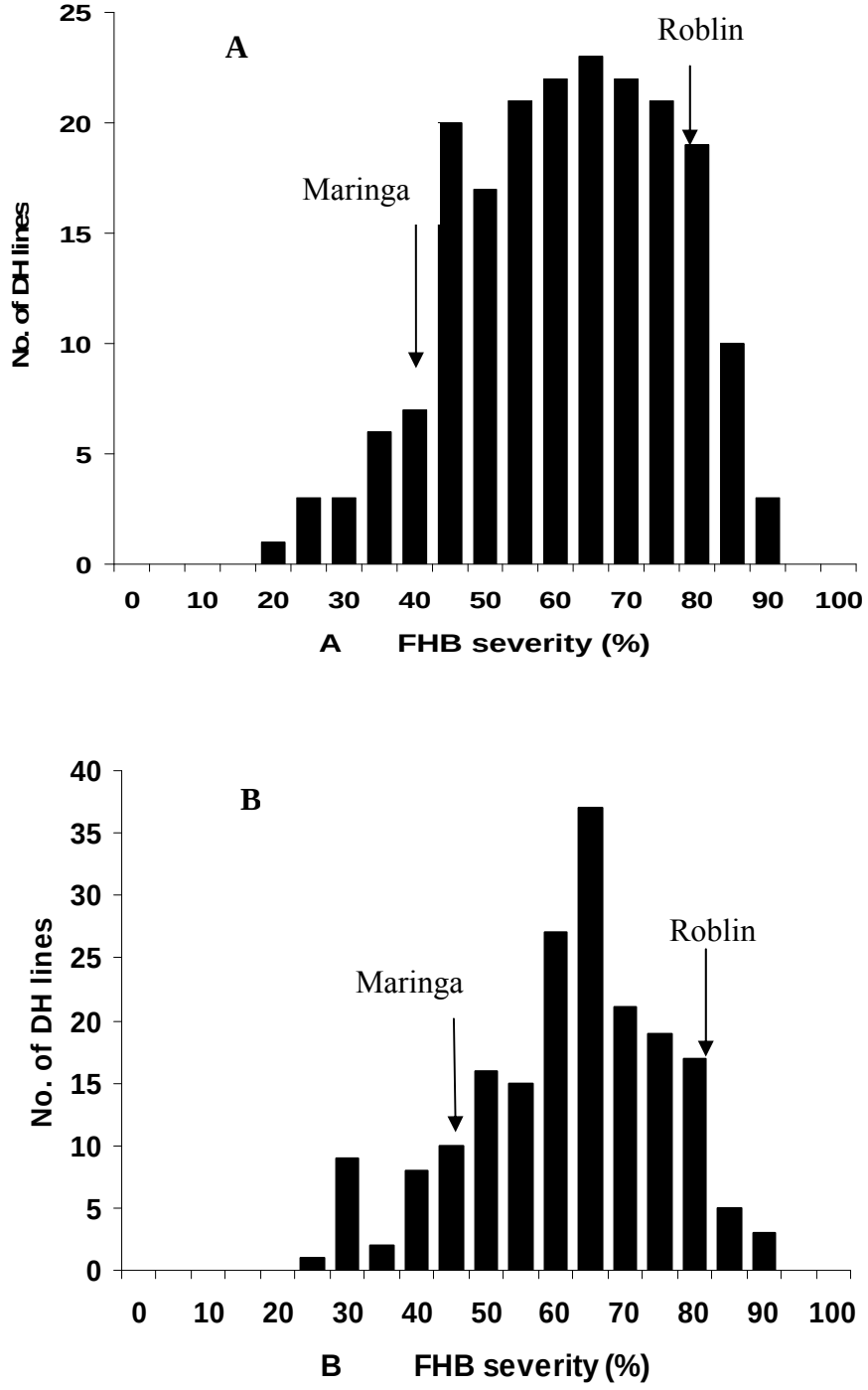


Figure 3.4.4 A and B. Phenotypic distribution of fusarium head blight severity (FHB) (tested for Type II resistance to FHB), in a doubled haploid population developed from the cross of Maringa/Roblin in A) greenhouse and B) field experiments.

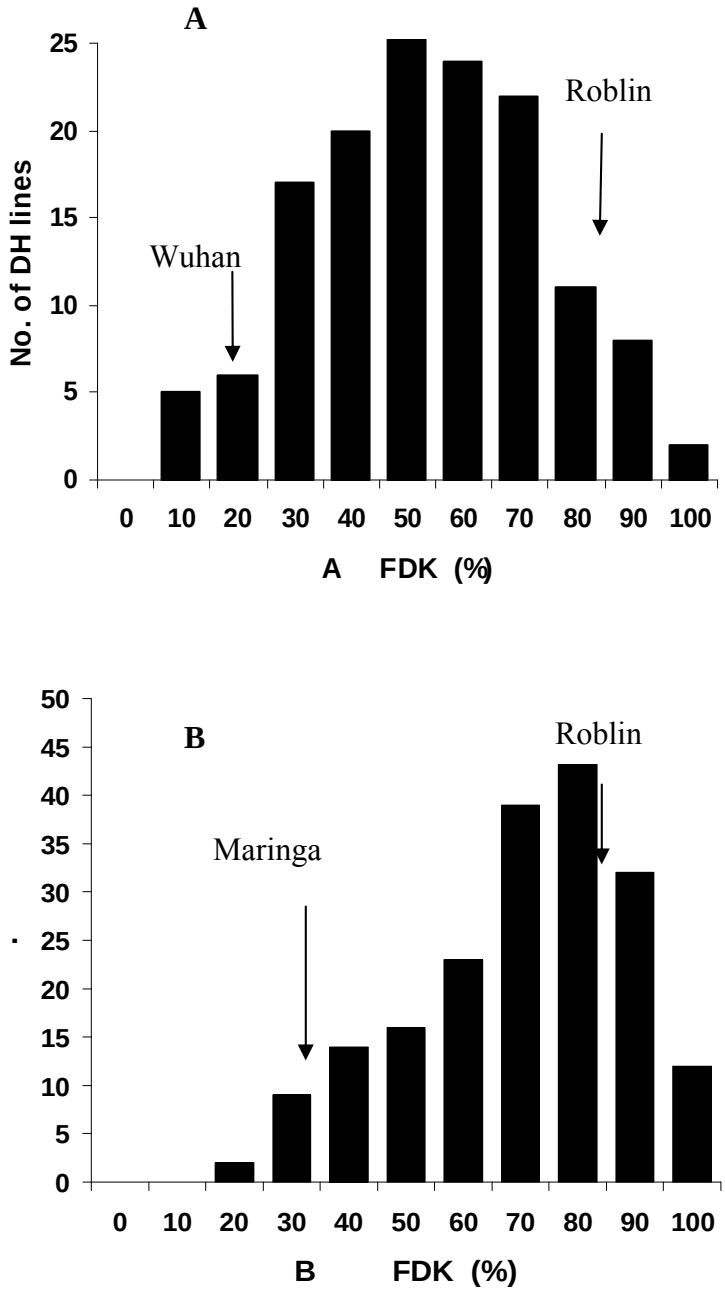


Figure 3.4.5 A and B. Frequency distribution of *fusarium*-damaged kernels (FDK), in a doubled haploid population developed from the cross A) Wuahn/Roblin and B) Maringa/Roblin. Data collected from three field experiments.

The frequency distributions of the DH lines for *Fusarium*-damaged kernels assessed from three field locations were continuous in both populations (Fig 3.4.5 A and B). In the Roblin/Maringa population the distribution is skewed towards the susceptible parent.

3.4.4. The estimation of maternal, non –additive and direct additive genetic effects

The interlationship of genetic and maternal contributions in both the populations, derived from crossing Roblin/Wuhan and Roblin/Martinga, estimated for all disease traits DI, DS, FHBI and FDK are presented in Tables 3.4.10 A and 3.4.10 B. Analysis of variance showed that additive genetic deviation was a significant source of variation for all traits for both populations. The maternal and non-additive genetic effects associated with crossing Roblin, Wuhan and Maringa were not important for any disease trait tested.

Table 3.4.10 Estimates of deviations and tests of significance of genetic and maternal contributions in reciprocal (A) Wuhan/Roblin (B) Maringa/Roblin populations for fusarium head blight (FHB) disease traits.

A) Wuhan/Roblin

		Trait			
Label	DF	DI	DS	FHBI	FDK
Maternal	141	5.47	0.31	2.49	-
Non-additive	141	1.02	3.24	-8,15	-4.99
Direct-additive	141	-68.71 *	-60.78*	-68.66*	-9.98

B) Maringa/Roblin

		Trait			
Label	DF	DI	DS	FHBI	FDK
Maternal	190	18.39	16.67	21.78	-
Non-additive	190	26.86	26.01	28.39	-2.63
Direct-additive	190	-69.05 *	-59.09*	-70.18*	-5.27

DI = disease incidence; DS = disease severity; FHBI = fusarium head blight index,
FDK = *Fusarium*-damged kernels * = significant at 0.05

3.4.5 Correlation analysis

All phenotypic correlations between disease measurements were positive and significant at $P < 0.05$. Correlation coefficients ranged from 0.82 to 0.95 among all traits (DI, DS, FHBI and FDK) associated with FHB resistance in the field experiment for the Wuhan/Roblin population (Table 3.4.11). The correlation between the mean greenhouse disease severity and field severity was lower (0.43) but statistically significant at $\alpha = 0.05$. In the field trials, the correlation between severity and FDK was strong, at $r = 0.89$.

For the Maringa/Roblin population correlation coefficients for the field measurements ranged from 0.72 to 0.94 among all traits (DI, DS, FHBI and FDK) associated with FHB resistance (Table 3.4.12). The correlations between the field traits were highly significant ($\alpha = 0.05$) and positive. The correlation between the mean greenhouse and field disease severity was not as high.

Table 3.4.11 Correlation coefficients (N = 143) among traits associated with fusarium head blight resistance in doubled haploid lines of reciprocal crosses between Wuhan and Roblin spring wheat. Correlation coefficients (r) were calculated using mean values for two greenhouse experiments and field data measurements from four field locations Carman, Glenlea, Ottawa and Portage (2003).

Traits	**DS (F)**	**FHBI (F)**	**FDK(F)**	**DS (GH)**
DI (F)	0.818**	0.917**	0.834**	0.456**
DS (F)		0.947**	0.886**	0.432**
FHBI (F)			0.886**	0.433**
FDK (F)				0.464**

** Indicates significance at $P < 0.01$

DI (F) = disease incidence (field)

DS (F) = disease severity (field)

FHBI (F) = fusarium head blight index (field)

FDK (F) = *Fusarium*-damaged kernels (field)

DS (GH) = disease severity (greenhouse)

Table 3.4.12 Correlations coefficients (N = 192) among traits associated with fusarium head blight (FHB) resistance in doubled haploid lines of reciprocal crosses between Maringa and Roblin spring wheat. Correlation coefficients (r) were calculated using mean values for two greenhouse experiments and field data measurements from four field locations (Carman, Glenlea, Ottawa and Portage la Prairie (2003)

Traits	**DS (F)**	**FHBI (F)**	**FDK(F)**	**DS (GH)**
DI (F)	0.715**	0.901**	0.828**	0.091 ns
DS (F)		0.915**	0.885**	0.191**
FHBI (F)			0.940**	0.154*
FDK (F)				0.193**

*,** indicate significance at $P < 0.05$ and $P < 0.01$, respectively

ns indicates no significant correlation

DI (F) = disease incidence (field)

DS (F) = disease severity (field)

FHBI (F) = fusarium head blight index (field)

FDK (F) = *Fusarium*-damaged kernels (field)

DS (GH) = greenhouse disease severity

3.4.6 Field heritability

Disease severity is an important component in the overall damage caused by the infection. The broad- sense heritability estimate was calculated using the FHBI as it includes both DI and DS from the analyses conducted separately by location and genotype. The environmental variance was small and the genotypic variance was large for FHBI at all four locations (Carman, Glenlea, Portage and Ottawa) and accordingly heritability estimates were high, ranging from 0.78 to 0.96 for Wuhan/Roblin population and 0.73 to 0.91 for Maringa/Roblin population, respectively, when analyzed for

individual populations at different environments (Table 3.4.13). Further, the estimates of broad-sense heritability from both DH populations of each reciprocal cross combined at each location, and location and cross combined is listed in Table 3.4.14. The estimate of heritability went down in percentage from single cross - single location when crosses were combined at each location and when crosses and locations were combined.

Table 3.4.13 Estimates of fusarium head blight index (FHBI) variance components and heritability of the 349 doubled haploid lines derived from Roblin/Wuhan and Roblin/Maringa reciprocal crosses from field experiments.

	Roblin/Wuhan			Wuhan/Roblin		
Location	σ^2_E	σ^2_G	h^2	σ^2_E	σ^2_G	h^2
Carman	39.87	615.82	0.94	54.86	402.74	0.88
Glenlea	65.85	340.53	0.84	41.05	416.61	0.91
Ottawa	NA	NA	NA	57.19	201.83	0.78
Portage	18.94	452.97	0.96	24.15	212.58	0.90
	Roblin/Maringa			Maringa/Roblin		
Carman	20.88	576.33	0.88	67.09	648.50	0.91
Glenlea	98.47	392.96	0.80	51.71	507.02	0.91
Ottawa	NA	NA	NA	73.84	209.15	0.74
Portage	37.38	99.80	0.73	48.15	189.80	0.80

NA = Roblin/Maringa population was not assessed at Eastern Cereals and Oil Seeds Research Centre (ECORC)

Table 3.4.14 Estimates of fusarium head blight index (FHBI) variance components and heritability of the doubled haploid lines derived from Wuhan/Roblin and Maringa/Roblin reciprocal crosses from field experiments, reciprocal crosses combined and location and crosses combined.

	Wuhan/Roblin	Maringa/Roblin
Carman (C)	0.92	0.92
Glenlea (G)	0.90	0.85
Portage la Prairie (PLP)	0.90	0.76
C+G+PLP combined	0.76	0.69

3.5 Discussion

Laurie and Reymondie (1991) reported that haploid production from corn pollen-mediated wheat embryo development was less genotype-dependent than haploid production through the *Hordeum bulbosum* technique or anther culture in wheat. However, varietal differences in the efficiency of embryo formation in wheat x maize crosses and some degree of genotypic variation were observed among the reciprocal crosses (Suenaga and Nakajima, 1993). The reciprocal Maringa/Roblin (MR and RM) F_1s produced more embryos (85 and 80%, respectively, from the pollinated florets) than Roblin/Wuhan (RW and WR) F_1s (65 and 66% respectively). More fertile DH plants (50% for MR and RM F_1s and 30% for RW and WR F_1s) from the rescued embryos were obtained for MR F_1s resulting in a larger DH population for these lines. In both crosses,

many caryopses contained only watery sacs without embryos indicating embryo abortion at an early stage.

Some of the DH lines grew as healthy as the other lines but remained grassy with no tiller production. The term grassy phenotype (Table 3.4.1) was used to represent those DH lines which did not produce wheat spikes when the rest of the population generated fertile spikes. This may be due to several reasons including the parental wheat line Wuhan has an unknown pedigree. It is possible that Wuhan has a winter wheat background which was expressed after the process of doubled haploid technology. The requirement of a low temperature exposure for flowering, a condition known as vernalization, separates spring wheats ***(****Triticum aestivum* L.) from winter wheats. Research into genetic control of vernalization has shown that several genes are involved. Pugsley (1971 and 1972) demonstrated that at least four genes were involved in control of the vernalization requirement *(Vrn1, Vrn2, Vrn3* and *Vrn4).* The genes were found to be dominant for the spring habit. A mutation in any one of the *Vrn* alleles could have expressed a winter habit in the genotype.

Wuhan, from China, is reported to have a moderate to high level of FHB resistance, and Maringa, from Brazil, possesses an intermediate level of FHB resistance. Phenotypic characterization of FHB resistance can be problematic whether it is screened indoors in a greenhouse or outside in field trials. Confounding environmental effects and inheritance of multiple traits have significant effects on selection for FHB resistance (Anderson, 1948; Miedaner et al 2001). An accurate assessment of resistance requires multiple tests over locations and years (Parry et al., 1995). In this study we tried to decrease some of the variables by using doubled haploid technology which enables the generation of homozygous populations in a relatively short time, phenotyping in

replicated greenhouse experiments and conducting replicated trials at multiple field locations.

This study focused mainly on breeding for Type II resistance from Wuhan and Maringa, but components of Type I and Type III (FDK) resistance were also evaluated. Using the disease severity phenotypic distribution of the DH lines it may be possible to predict the presence of a tri-modal distribution for Wuhan/Roblin populations, however presence of transgressive segregation indicates involvement of two genes or more in inheritance of FHB resistance. For the Maringa/Roblin populations the frequency distribution appears to be more continuous suggesting a quantitative inheritance (Figures 3.4.3 and 3.4.4). The frequency distributions for individual population and individual location are presented as appendix 2 figures 1- 6. The FHBI (chart not shown) which includes both disease severity and incidence, and FDK chart (Figure 3.4.5 A and B) for DH populations had continuous frequency distributions and wide phenotypic variations. These findings are congruent with other studies, indicating that FHB resistance is controlled by multiple genes and quantitatively inherited (Waldron et al, 1999; Ban and Suenaga, 2000; Buerstmayr et al., 2002; Jiang et al., 2006; Bonin and Kolb, 2009). The environmental conditions play an important role in determining the severity of FHB infection and FHB resistance (Parry et al., 1995). Analysis of variance (Tables 3. 4.5 and 3.4.6) showed that the DH lines varied significantly for all field traits. The experimental design and data collection methods were effective in removing sources of variation from entry effects. The significant level of variation for all field traits may be due to varying disease pressure because of different methods of inoculation (macroconidial spray, corn grain spawn inoculum), differences in pathogen isolates and differences in the irrigation systems used in different locations. Statistically, the most significant factors affecting

FHB ratings in the population were location and genotype. The interaction between DH lines*location for DI, DS, VRI and FDK for all locations was the second largest source of variation. Disease severity was measured both in the greenhouse using point inoculation and in the field using spray inoculation to ensure consistent infections. The overall rating results were not different and were moderately correlated. Lower correlation between disease severity in the greenhouse and the field experiment may be due to the fact that disease severity in the greenhouse is a reflection of Type II resistance while it is most possibly the result of both Type I and Type II resistance in the field. Field symptoms, based on average DH line FHBI values were consistent among Carman and Glenlea spray-inoculated field experiments and means were higher than the average for Ottawa and Portage grain-spawn inoculated sites. However, FDK mean values were consistent among all experimental locations.

The inheritance studies of FHB reactions on Wuhan have not been previously reported, however, some QTL work has been conducted on FHB resistance associated with plant height with Wuhan–1/Nyubai derivatives (Somers et al., 2003; McCartney et al., 2007). The data presented in this paper indicate that the smaller number of genes controlling FHB reaction caused by *F. graminearum* in Wuhan can be used as a source of resistance to FHB. The present results are consistent with previous findings of quantitative inheritance of FHB resistance in other resistant sources such as Sumai 3 (Ban and Suenaga 2000), however reports of the number of genes involved have differed according to the source of resistance (Bai et al., 2000, Hall and Sanford, 2003; Hozapfel et al., 2008).

The continuous distribution of disease severity ratings from all populations for all traits as well as the presence of transgressive segregants indicated that resistance in

both Wuhan and Maringa is a quantitative trait. Transgressive segregants may be valuable sources of resistance. Sumai 3 was derived as a transgressive segregant from two moderately susceptible parents. The fact that the population means of the greenhouse experiment and the field trials were not different may indicate both environments were conducive to the development of disease symptoms. The DH lines on average were similar to the mean of the two parents indicating genetic additive effects. Population means and the mid parent values for FHBI and FDK were similar in both crosses indicating multiple gene involvement in inheritance of these traits. However, for disease severity and FHBI, the midparent values at two locations (Portage La prairie and Ottawa) were not similar to the population mean indicating that the inheritance of at least one gene could be dominant. This study showed that FHB resistance in Wuhan and Maringa both are conditioned by additive genetic effects for all FHB disease traits tested and hence both or either, disease severity or FHBI can be used for selection in breeding for FHB resistance in Wuhan and Maringa.

The heritabilty estimates for FHBI were high, similar to many other studies reported earlier (Yang et al., 2005; Liu et al., 2007). The high heritability estimate for FHBI indicates that a large portion of the variance is due to genetic effects. The high heritability estimates may be due to the large difference in resistant and susceptible parental reaction to FHB. The high heritability for disease severity and FHBI suggests the possibility to improve FHB resistance through recurrent selection of the DH population.

3.6 Conclusion

Buerstmayr et al. (2000) stated that the development of FHB resistant cultivars should be possible by phenotypic selection under epidemic conditions, and should be largely independent of plant height, flowering date, awnedness and genotype of the maternal parent within the cross. Both disease severity and FHBI have been considered reliable traits to use as selection criteria for FHB reaction as presented in earlier studies (Miedaner et al., 2006).

The cross between Maringa, intermediate reaction to FHB resistance, good spike length, long flag leaf, awned and tall straw, producing good vigorous plants, and Roblin, highly susceptible to FHB, short strawed adapted Canadian cultivar with early heading produced about 30% of the progenies with a superior reaction to FHB than the parents. This may be in line with the recommendations of Simon et al (2004) to use varieties with intermediate resistance in crosses. Sumai 3 with the highest level of FHB resistance was also derived from two moderately susceptible parents, Italian wheat Funo and landrace line Taiwan Xiaomai wheat. Accordingly any other moderately susceptible wheat lines might offer an alternative source of resistance to FHB in addition to Sumai 3. McCartney et al. (2004) conducted a haplotype study using microsatellite markers linked to FHB resistance QTLs in a diverse collection of FHB resistant germplasm including Sumai 3 and Wuhan 1. They included six FHB resistance QTLs in five chromosomes and the study reported that the haplotype of Sumai 3 and Wuhan 1 are different at the 2DL and 4B loci. A further study to evaluate FHB resistance QTLs performed by McCartney et al. (2007) stated that the 3BSc allele was the most effective in Sumai 3 where as 2D allele was effective in Wuhan 1.

Improved breeding lines, which may eventually be developed into cultivars of hard red spring wheat, are necessary to respond to changing disease pressures and to provide growers with high-yielding varieties that will be acceptable in the marketplace. Wheat varieties with improved disease resistance should reduce the use of fungicides, thus resulting in potential environmental benefits. The generated population can be used to study marker-assisted selection and help in finding FHB resistance QTLs. This study provides insight into the genetic control of FHB resistance in Wuhan which may complement the available sources for FHB resistance in spring wheat. As additive genetic effects are the major component conditioning FHB resistance in Wuhan and Maringa, a higher level of resistance may be obtained by selecting transgressive segregants in breeding programs.

CHAPTER 4

INHERITANCE OF BARLEY YELLOW DWARF VIRUS (BYDV) TOLERANCE IN SPRING WHEAT CULTIVAR MARINGA

4.0 Inheritance of Barley yellow dwarf virus tolerance in spring wheat line Maringa

4.1 Abstract

Barley yellow dwarf (BYD) caused by a group of related single-stranded RNA viruses, is the most widely distributed and economically important virus disease of the cereal grains. Tolerance to BYD virus was studied in a doubled haploid (DH) population derived from a cross between a Brazilian wheat cultivar Maringa, which is highly tolerant to BYDV and Roblin, a Canada western spring wheat susceptible to BYDV. The experiments were conducted in the greenhouse and in one field location for one growing season in 2003. One hundred and ninety nine F_1 – derived DH lines were generated and used for the assessment. The plants were inoculated with ten to fifteen BYDV-viruliferous aphids, *Rhopalosiphum padi* BYDV-PAV isolate 9301PAV, at the one to two leaf stage to obtain the greatest range of symptoms. Aphids were applied to the base of each test plant. The greenhouse experiment consisted of inoculated and non-inoculated plants and the field experiment consisted only of inoculated plants. The BYDV tolerance was measured by visual assessment of disease symptoms, plant height and spike mass measurements. In the greenhouse, other BYDV symptom indicators were also used including heading delay and flag leaf and peduncle lengths. The phenotypic distribution for all parameters exhibited transgressive segregation in both greenhouse and field trials indicating a multiple genes are involved in inheritance of BYDV tolerance in Maringa.

4.2 Introduction

Barley yellow dwarf (BYD) is the most widely distributed and economically important virus disease of cereal grains (D'Arcy, 1995; Lister and Ranieri, 1995). This disease is caused by a group of related single-stranded RNA viruses (Miller et al; 1987) called barley yellow dwarf viruses (BYDVs) species BYDV-PAV (*Rhopalosiphum padi avenae* virus*)*, BYDV-MAV (*Macrosiphum avenae* virus) (Oswald and Houston, 1951; Slykhuis, 1956; Watson and Mulligan 1960), in the genus *Luteoviruses,* family *Luteoviridae,* and cereal yellow dwarf viruses, (CYDVs) species SGV(*Schizaphis graminum* virus), RMV (*Rhopalosiphum maidis* virus*)* and GPV (*S. graminum* and *R. padi* virus) in the genus *Polerovirus*, family *Luteoviridae* (van Regenmortal, 2000; D'Arcy and Domier 2005). This virus group is distributed throughout the wheat growing areas of the world and can cause significant yield losses (Carrigan et al., 1980; El and Hill, 1990; Haber, 1990; McKirdy and Jones, 1996; Riedell et al., 1999; Veskrna et al., 2009). The disease can affect several other species in the family *Poaceae*, including barley, oats, rye, triticale, rice and many wild grass species (D'Arcy, 1995).

Symptoms of BYD vary with crop and variety. In wheat the most common symptoms include reduction of root growth and stunting of plants resulting in reduced plant height and yellowing of leaves, especially leaf tips along the vascular bundle. This colour change is associated with a reduction in chlorophyll and photosynthesis, which leads to a reduction in grain yield. The virus may also cause phloem degradation and collapse of the sieve elements, weakening plants and making them more susceptible to fungal infections (D'Arcy, 1995)

Wild annual and perennial grasses and cereal volunteers play an important role in the epidemiology of BYD, serving as host reservoirs of this virus complex. Their wide host range within the grass family is attributed to numerous aphid species that vector BYDVs (A'Brook, 1981). Each BYDV strain is preferentially spread by a narrow range of aphid species; the BYDV-PAV is spread by the bird cherry-oat aphid, *Rhopalosiphum padi* L, the English grain aphid (*Macrosiphum* (*Sitobion*) *avenae*) and occasionally by the green bug (*Schizaphis graminum*).

The bird cherry-oat aphid, *R.padi* is an important insect pest of wheat in North America and the principal vector for transmission of BYDV (Haber, 1990). Consequently, isolates that share the serotype of BYDV-PAV are the most prevalent in Canada and the USA (Gildow 1990; Haber, 1990). BYDV-MAV is also commonly found in North America, but when there is a double infection PAV is a stronger competitor than MAV within the host and is generally transmitted more efficiently which contributes to its predominance under field conditions (Power, 1996). *Luteoviruses* primarily infect plant phloem tissue and are transmitted only by a limited number of aphid species showing a high level of vector-specificity (Miller et al., 2002). During transmission, *Luteoviruses* circulate throughout their aphid vector's body, requiring virus recognition, penetration, and transport through aphid cells, although they do not infect or replicate in the vector aphids (Gildow, 1999). This type of virus transmission is classified as circulative –nonpropagative transmission (Gray and Banerjee, 1999).

In North America BYD is prevalent year round as small grain cereals are grown throughout the continent from the Gulf of Mexico to the Northern Canadian Prairies. Cereals are grown in winter in the southern and coastal regions and barley, maize, oat and spring wheat varieties are spring seeded in the north. Because of the continuous

cycle of seeding and harvest throughout the continent there is no time when crops or grass reservoirs are absent and BYDV vectors inactive. This green bridge enables a potential infection by BYDV every year.

The disease may be restricted by controlling any of the components of the pathogen-vector-host-environment complex; studies have led to the consensus that the most effective and sustainable strategy is the development and use of genetic resistance or tolerance to the virus complex (Henry et al., 2002). Incorporating resistance to pathogens and pests is the most desirable control method to growers as it is effective and inexpensive. Currently there is no highly effective resistance to BYDV in the primary or secondary gene pools of wheat (Fedak et al., 2001; Veskrna et al., 2009). BYDV is controlled mainly by use of varieties that are tolerant or resistant to certain BYDV isolates of serotypes or subgroups (Miller and Rasochova, 1997).

Several studies have been conducted to transfer BYDV resistance into winter wheat from its wild relatives (Chen et al., 1997 and 1998; Francki et al., 2001; Henry et al., 2002), however, transfer of this resistance from a winter wheat background to a spring wheat background has yet to be achieved (Comeau and Haber 2002; Kosova et al., 2008). Tolerance has been identified in several wheat lines since the 1950s (Burnett et al., 1995). A positive response of a host plant to virus invasion can therefore be either tolerance or resistance. However, these two mechanisms are not the same (Comeau and Haber, 2002). The definitions of tolerance and resistance put forward by Cooper and Jones (1983) state that when there is resistance, replication of the virus in the infected plant is inhibited. By contrast, in a tolerant response there are reduced symptoms while virus replication is unaffected. Comeau and Haber (2002), qualify the definition of Cooper and Jones (1993) by stating that true resistance may limit virus replication, but

not necessarily reduce damage to individual plants; tolerance, on the other hand, reduces damage, but this may or may not be correlated with a reduction of virus titre based on ELISA values.

Currently the major BYDV tolerance gene identified in bread wheat is *Bdv1*, which originates from the Brazilian wheat cultivar Frontana developed in 1940; it was introduced into the North American bread wheat cultivar, Anza, by Singh et al. (1993). A second gene *Bdv2* is derived from intermediate wheatgrass, a hexaploid species containing two copies of the E genome and one copy of the Ti genome. However, carriers of *Bdv1* and *Bdv2* fail to show the expected level of tolerance or resistance after infection by BYDV-PAV. The resistance conferred by *Bdv2* reduces virus titre and infection rate, but also appears to elevate sensitivity to BYDV-PAV (van Ginkel and Henry, 2002). Therefore, it is highly desirable to explore other spring wheat lines for higher levels of tolerance (Ayala et al., 2001; Chrpova et al., 2006).

The Brazilian wheat cultivar Maringa, derived from Frontana, has been found to possess a high level of tolerance to BYDV –PAV in greenhouse and field experiments (Pers. comm. A. Comeau and S. Haber). It is possible that the tolerance in Maringa was derived from its parent Frontana, and can be used as a source of BYDV tolerance in spring wheat.

The objectives of this study were 1), to develop a doubled haploid population from F_1 hybrids obtained from the cross Maringa (BYDV – tolerant)/Roblin (BYDV – susceptible, 2), to investigate the nature of inheritance of tolerance to BYDV, and 3), estimate heritability in Maringa/Roblin populations.

4.3 Materials and methods

4.3.1 Population development

Maringa, a Brazilian wheat cultivar highly tolerant to BYDV and Roblin, a Canada western hard red spring (CWRS) wheat cultivar susceptible to BYDV, were obtained from the Cereal Research Centre in Winnipeg, and crossed to produce F_1 seeds. Maringa is a cross between Frontana/Kenya 58//PGI. It is a tall, awned spring wheat variety. Roblin is a derivative of BW15/BW38//RL4359/RL4353. It is an awn-less, short-statured early maturing variety.

4.3.2 Generation of doubled haploid (DH) lines from F_1 seeds

Doubled haploid (DH) lines are homozygous and homogeneous and as such are ideal for genetic analysis because they do not express dominance variation and segregation within lines. The F_1 hybrids were used for the development of BYDV-tolerant germplasm. Corn pollen was used to stimulate embryo development in wheat caryopses. When the plants were at the two to three-tiller stage, they were treated with colchicine for chromosome doubling. The DH population development protocol is explained in Apendix 1 of this dissertation.

Regenerated doubled haploid plants were grown for one generation to produce a sufficient amount of uniform seed for the experiments. Increases were carried out between September 2002 and May 2003. Some of the DH lines (30 lines from Roblin/Maringa cross) perished during a cabinet breakdown so 199 DH (Figure 4.3.1) lines were available for assessment.

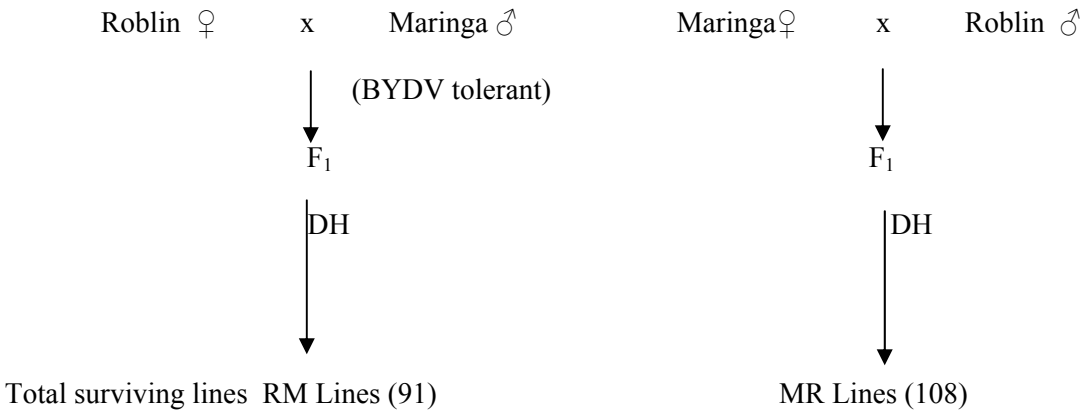


Figure 4.3.1 Generation of Roblin/Maringa (RM) and Maringa/Roblin (MR) doubled haploid populations via corn-mediated doubled haploid technology

4.3.3 Aphid rearing and inoculation

Before an aphid is capable of inoculating a healthy plant, it must acquire the virus by feeding on infected plants for a period of 12 to 30 hours. Once the aphid acquires the virus, it is capable of transmitting virus for the rest of its life. It generally requires 4 hours or more of feeding on a plant for the virus to be transmitted.

Viruliferous aphids were reared for greenhouse and field experiments. *Rhopalosiphum padi* insects infected with BYDV-PAV (isolate 9301PAV nonspecific) were reared on the oat cultivar Riel in growth chambers at 18^0 C. The aphids were transferred onto fresh, healthy oat seedlings every two weeks to increase and maintain the aphid population. Aphids for inoculation were gently dislodged from the oat

seedlings and suspended in talcum powder (to prevent balling of the aphids). Ten to fifteen aphids per plant were distributed. The exact number of aphids is not critical because two to ten aphids per plant give a similar level of infection (Sakira et al., 1985).

4.3.4 Barley yellow dwarf disease evaluation in the growth cabinets/greenhouse

Parent cultivars Roblin and Maringa, F_1s, and F_1-derived DH lines (RM 91, MR 108) were evaluated for BYDV tolerance under controlled conditions. Four plants from each DH line were grown in a 17 cm (3RR-66) fibre nursery. Each line had two pots of four plants (Figure 4.3.2). The experiments were conducted in a completely randomized design in the growth cabinet/greenhouse. The plants were started in growth cabinets with a 16 h light: 8 h day cycle at 20: 16 ºC temperature. The soil mixture used for the experiments was composed of 2:1:1 (v:v:v) soil, sand and peat. The plants were fertilized biweekly with 20:20:20 (N:P:K) (Peters Professional, Scotts – Siera Horticultural Products Co. Marysville, OH) until heading, and with 15:28:15 (N:P:K) after heading until maturity.

The test plants were inoculated with ten to fifteen BYDV-viruliferous aphids at the 1-2 leaf stage when they are most susceptible and have the greatest potential to subsequently express the full range of symptoms (Figure 4.3.2). Aphids were applied to the base of each test plant. The control plants were kept in a separate growth cabinet until the inoculated plants were sprayed with thiodan (1.5 gm/l) 5 days after infestation to remove the aphids. The plants were kept in the same conditions until heading. Wheat spike emergence dates were recorded. The plants were transferred to a greenhouse after heading and maintained until harvest. The plants were scored for symptoms after anthesis

using a visual scale 0 – 9, where 0 indicates no symptoms and 9 indicates stunting with complete yellowing (Table 4.3.1) according to Schaller and Qualset (1980).

In most of the susceptible species of Poaceae, BYDV symptoms are expressed through changes in many parameters, which must also be considered in evaluations of tolerance (Comeau and Haber, 2002). Tolerance has little to do with enzyme-linked immunosorbent assay (ELISA) titres or quantities of infectious virus. Instead it is the effect on parameters of host vigor, such as grain yield, delay in days to heading and height measurements that is important. A quantitative indoor assay (QI assay) as a tool to identify, select and develop BYDV tolerant cereal germplasm as described by Haber and Comeau (1998) is a useful tool to evaluate plant viral infection and was used in this experiment. The QI-assay is a yield prediction model for spring bread wheat following BYDV infection (Haber et al., 1997; Haber and Comeau, 1998). BYDV-infected plots with appropriate healthy controls are not always available due to several reasons including mobile vectors. This model uses criteria such as symptomatology, heading delay, flag leaf length, spike mass, biomass, harvest index, and plant height, or a combination of more than one factor. In this research we have used leaf symptoms (visual), plant height (from soil surface to the tip of the plant primary spike) (cm), flag leaf length (cm), head emergence or heading delay (days), peduncle length (cm) and spike mass for the greenhouse and leaf symptoms, plant height and spike mass from the field data to assess tolerant or susceptible wheat lines.

Figure 4.3.2 Inoculation of DH lines with bird cherry-oat aphids, infested with BYDV isolate 9301PAV at the one to two-leaf stage in the growth cabinet.

Table 4.3.1 Barley yellow dwarf virus (BYDV) visual symptom scoring system for wheat (Schaller and Qualset, 1980).

Score	Description
0	no visible symptoms
1	trace amounts of yellowing at the tips of a few leaves; vigorous plant appearance
2	restricted yellowing of leaves; larger proportion of yellowed areas; compared to class 1, more leaves discoloured
3	moderate to low amount of yellowing; no sign of dwarfing or reduction in tillering
4	moderate to somewhat extensive yellowing; no dwarfing; moderate to good plant vigor
5	more extensive yellowing; moderate to poor plant vigor; some dwarfing
6	high level of yellowing; poor plant vigor; apparent dwarfing
7	severe yellowing; small spikes; moderate dwarfing; poor plant appearance
8	Nearly complete yellowing of all leaves; dwarfing; tillering reduced (rosette appearance); reduced spikes with some sterility
9	Marked dwarfing; complete yellowing; few or no spikes; considerable sterility; forced maturity or drying of plant before normal maturity is reached

4.3.5 Barley yellow dwarf disease evaluation in the field

The F_1-derived DH lines (RM 91, MR 108) and parent cultivars Roblin and Maringa were evaluated for BYDV tolerance at the Agriculture and AgriFood Canada Research Station, Glenlea, Manitoba.

Seeding was completed on June 6, 2003. The experiment was conducted as a randomized complete block design with two replications. The test materials were seeded

at a rate of 40-45 seeds/row in 1 meter long rows with 30 cm row spacing. The parents, Roblin, and Maringa, were included as checks and were seeded every 20 rows. The BYDV nursery was inoculated on June 14, 2003 with 15 to 20 viruliferous bird cherry-oat aphids per plant at the two leaf stage. Aphids were applied to the base of the plants in a row. Plants were sprayed with Malathion® (diethyl (dimethoxy thiophosphororylthio succinate)) 5 days after infestation to remove the aphids. The plants in the field plots were regularly examined for any surviving or naturally infesting aphids. Visual symptoms were scored at heading six weeks after inoculation. Plants were rated for symptom severity August 6, 7, and 8, 2003. Plant height was recorded for each DH line. For assessment of the spike mass, ten spikes from each row of parents and DH lines were randomly selected and harvested at the end of the season when plants were mature. Harvested spike were placed in paper bags and stored until spike mass was recorded. The field experiment consisted of only the virus-inoculated plants.

4.3.6 Statistical Analysis

Analyses of variance (ANOVA) for disease tolerance were performed using PROC MIXED (SAS version 9.1, SAS Institute Inc., Cary, NC). All effects (DH line, block and replicate) in the model were considered random. Broad -sense heritability (H^2) for BYDV tolerance was estimated from the variance components and determined as the ratio of the genotypic variance to the sum of the genotypic and environmental variance, $H^2 = \sigma^2_G / (\sigma^2_G + \sigma^2_{E/r})$, where σ^2_G and σ^2_E are the genotypic variance and environmental variance, respectively, r is the number of replications. Comparisons were made between disease parameters from virus infected and the control plants. Percent reduction was

calculated using test and control plants. The percent reduction of the DH lines on each parameter was compared to the tolerant parent Maringa.

Pearson correlation coefficients between any two traits were computed using PROC CORR at $\alpha = 0.05$, to determine the degree of association among the traits of interest.

4. 4 Results

4.4.1 Disease symptoms severity means

All genotypes (Maringa, Roblin and doubled haploid lines) inoculated with BYDV showed symptoms of the disease to varying extents. The 199 doubled haploid lines from the reciprocal crosses in the 2003 greenhouse trials had symptom scores ranging from 2.2 to 8.1 and the disease symptom rating score for the field experiments ranged from 3.5 to 9.0. The difference in days to heading between two parent cultivars in the field were 10 days and the DH lines spanned 20 days. Sixty two DH lines were as early as Roblin.

The mean symptom scores for the parental controls in the greenhouse were 3.4 for the tolerant parent Maringa and 6.4 for the susceptible parent Roblin. For the field experiment the mean disease symptom rating scores were 4.3 and 7.5, for Maringa and Roblin, respectively, and for the doubled haploid population 4.5 and 6.5 for the greenhouse and for the field experiments respectively (Table 4.4.1). The combined effects of disease in the field were much more severe than in the greenhouse. The F_{1s} were tolerant with a mean rating score of 3.5, consistent with the tolerant parent.

Table 4.4.1 Means (standard error) for parent cultivars Roblin and Maringa, and doubled haploid lines from reciprocal crosses for barley yellow dwarf virus symptom scores from greenhouse and field trials.

Cultivar	Mean Symptoms (greenhouse)	Std error	Mean Symptoms (field)	Std error
Roblin	6.4 [a] (40)	0.848	7.5[a] (40)	0.832
Maringa	3.4 [b] (40)	0.636	4.3[b] (40)	0.912
Maringa x Roblin*	4.5 (199)	1.02	6.5 (199)	0.616

Means in columns with the same letter are not significantly different LSD 1.71 $\alpha = 0.05$

Numbers in the parentheses are the number of plants (parents) or DH Lines (reciprocal crosses) assessed

*doubled haploid lines from reciprocal crosses

4.4.2 Analysis of variance

The analyses of variance for plant height, disease symptoms and spike mass for the reciprocal DH population of Roblin/Maringa from the field trial showed that genotype was the largest source of variation for all parameters assessed indicating high diversity among the DH lines (Table 4.4.2). The effect of spike mass was not significant as spikes of the majority of the DH lines were light (contained only chaff) and did not have a significant mass showing a greater severity of disease effects in the field. The effect of height on genotype is significant at the 5% level for reciprocal crosses and the combined population. The broad -sense heritability for disease symptom score in the greenhouse and the field trials was 0.80 and 0.66, respectively.

Table 4.4.2 Analysis of variance for plant height, disease symptom score and spike mass for Maringa/Roblin reciprocal doubled haploid population artificially inoculated with barley yellow dwarf virus in one field location (Glenlea, MB) in 2003.

Variable: Height					
MR population					
Source	df	SS	MS	F	Pr > F
Rep	1	1.042	1.042	2.69	0.104
Genotype	107	60.273	0.553	1.45	0.0271
Residual	107	41.458	0.387	.	.
RM population					
Source	df	SS	MS	F	Pr > F
Rep	1	0.137	0.137	0.35	0.5558
Genotype	90	66.879	0.743	1.89	0.0014
Residual	90	35.362	0.392	.	.
Combined Population					
Source	df	SS	MS	F	Pr > F
Rep	1	0.251	0.251	0.64	0.4247
Genotype	198	127.487	0.644	1.64	0.0003
Residual	198	77.748	0.393	.	.
Variable: Symptom score					
MR population					
Source	df	SS	MS	F	Pr > F
Rep	1	7.223	7.223	11.18	0.0011
Genotype	107	195.651	1.825	2.83	<.0001
Residual	107	69.151	0.646	.	.
RM population					
Source	df	SS	MS	F	Pr > F
Rep	1	0.549	0.549	0.85	0.3591
Genotype	90	184.662	2.051	3.17	<.0001
Residual	90	58.201	0.647	.	.
Combined Population					
Source	df	SS	MS	F	Pr > F
Rep	1	6.156	6.156	9.45	0.0024
Genotype	198	380.438	1.921	2.95	<.0001
Residual	198	128.968	0.651	.	.
Variable: Spike mass					
MR population					
Source	df	SS	MS	F	Pr > F
Rep	1	0.006	0.006	3.68	0.0576
Genotype	107	0.199	0.001	1.11	0.283
Residual	107	0.179	0.001	.	.
RM population					
Source	df	SS	MS	F	Pr > F
Rep	1	0	0	0.05	0.8178
Genotype	90	0.156	0.001	1.58	0.0161
Residual	90	0.099	0.001	.	.
Combined Population					
Source	df	SS	MS	F	Pr > F
Rep	1	0.003	0.003	1.95	0.1642
Genotype	198	0.356	0.001	1.26	0.0508
Residual	198	0.282	0.001	.	.

4.4.3 Frequency distribution

The visual rating symptom scores (using Schaller and Qualset 1980 symptom scoring system) from the greenhouse and field experiments for the second generation F_1-derived doubled haploid lines did not show apparent discrete modes but gave a continuous distribution (Figure 4.4.3 A and B) suggesting quantitative inheritance. Transgressive segregation was observed on both ends of the distribution.

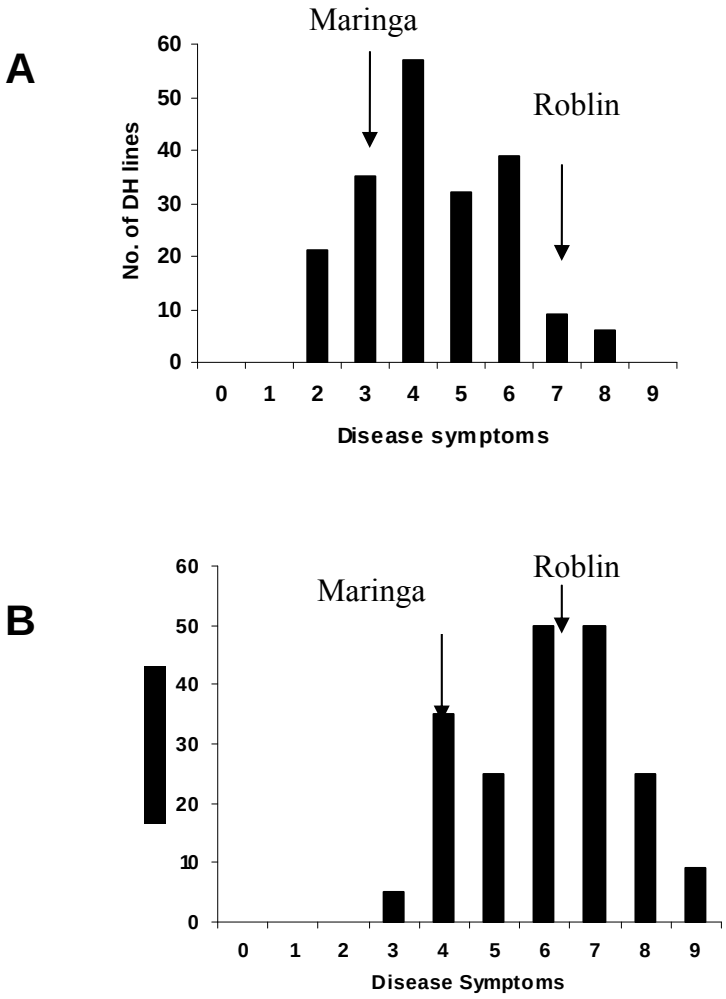


Figure 4.4.3 Frequency distribution of mean visual symptom scores for barley yellow dwarf virus infection in a doubled haploid spring wheat population derived from the cross Maringa/Roblin. A) greenhouse B) field trial

Note: BYDV symptom expression was scored on a scale ranging from 0 (no symptoms) to 9 (severe symptoms, no head emergence or plants are dead).

4.4.4 Association between the different parameters used in the quantitative indoor (QI) assay to evaluate BYDV

Of all the selection indices, measurement of yield is the most practical and relevant indicator for tolerance. A positive relationship was obtained amongst the different parameters using the QI assay for BYDV assessment (Table 4.4.4 and Figure 4.4.4 A-C) when the parameters were plotted against spike mass. Spike mass increased with increase in flag leaf length and peduncle elongation, although the correlation was not constant between height and mass.

The QI assay rating scale (0 – 9) (Haber and Comeau, 1998) is similar to that of Schaller and Qualset (1980). The QI assay was used to determine disease infection in the population by determining the percent reduction of all traits compared to the tolerant parent Maringa, and categorizing the DH lines in groups on a 0 – 9 scale. A relative score that was associated with tolerance or susceptibility was as follows: 0 immune; 1 - 4 tolerant; 5 – 6 intermediate and 7 – 8 susceptible. A score of 9 indicated the plants were senesced.

There was a low but statistically significant correlation between the greenhouse and the field experiments for BYDV disease symptoms *(r = 0.42, P = 0.0017)*. Correlation between the different parameters was also positive and significant (Table 4.4.5).

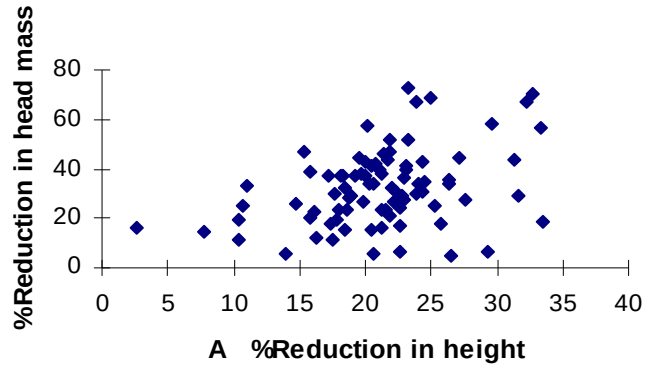
In the greenhouse symptom expression was well developed on the inoculated plants in all genotypes. The DH lines were reduced in plant height ranging from -7 to 24 %, in spike mass ranging from -5 to 60 %, in peduncle length ranging from -19 to 70%, and the flag leaf length from -3 to 47% compared to the control plants.

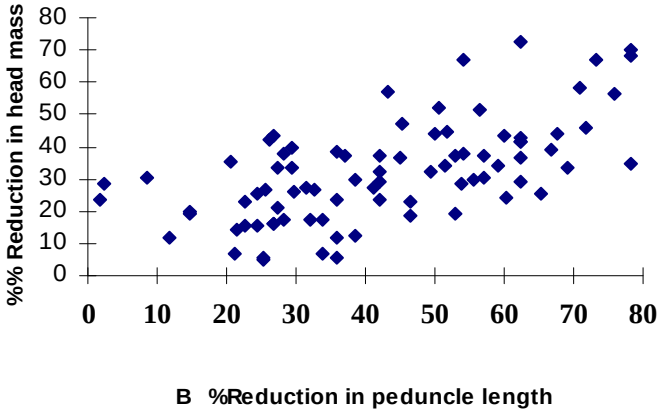
Table 4.4.4 Analysis of covariance for effect of parameters on spike mass calculated on Maringa/Roblin population (N= 84). Data collected form greenhouse experiments.

Source	Pr>F
Height/ Flag leaf	ns
Flag leaf/Height	*
Flag leaf/Peduncle	*
Peduncle /Flag leaf	ns
Peduncle/Height	ns
Height/ Peduncle	ns

Table 4.4.5 Correlation among parameters (plant height, spike mass and flag leaf length) associated with barley yellow dwarf virus tolerance in doubled haploid lines of spring wheat Roblin/Maringa

	Height	Spike mass	Flag leaf length
Peduncle length	0.35^{**}	0.61^{**}	0.52^{**}
Height	-	0.38^{**}	0.15^{ns}
Spike mass	-	-	0.53^{**}





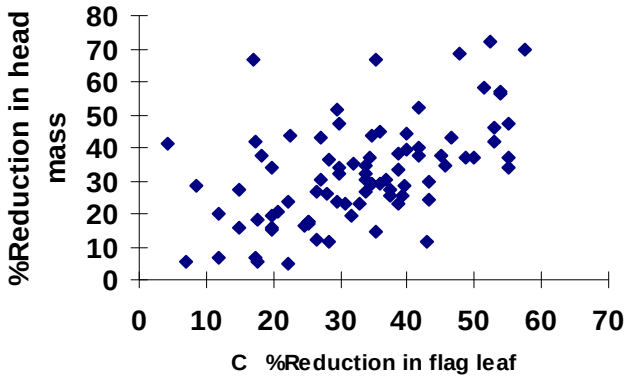


Figure 4.4.4 Charts A – C Relationship of different parameters with spike mass to assess the scores for Maringa/Roblin DH lines from green house experiments.

4. 5 Discussion

In this study the field selection of reciprocal DH lines from Roblin/Maringa was based on an index calculated using data from virus-inoculated plants. Parental checks, Roblin and Maringa, were grown at regular intervals within the experiments. Enzyme-linked immunosorbent assay (ELISA) values were not sought in this study as tolerance reduces damage, but may or may not be correlated to a reduction of virus content (Comeau and Haber, 2002).

The tolerant parent, Maringa had a visual symptom rating of 3. 4 (greenhouse) and 4.3 (field) which is consistent with results reported by Veskrna et al. (2009). They reported the Brazilian variety Maringa showed the best resistance/tolerance to BYDV-PAV infections. This study confirmed the enhanced tolerance of this variety compared to Roblin. The visual symptoms of BYDV attack are related especially to yellowing of leaves and the reduction in plant height and tillering in infected plants. DH lines that developed higher infection, in general displayed greater loss in spike mass, although some lines showing a lower infection level had a lower spike mass. An experiment conducted on winter and spring wheat by Veskrna et al. (2009) reported a higher susceptibility on average in spring wheat compared to winter wheat. They suggested the cause may be the more pronounced negative effect of stresses such as drought and higher temperature for spring crops than for winter crops. Maringa is a later-maturing variety than most of the adapted Canadian varieties; however some of the DH lines (16/62) showed an early maturity with lower percent reduction in spike mass ($\leq 20\%$) and symptom rating (≤ 4.5). The early spike emergence characteristic may have been inherited from Roblin which is an early maturing Canadian spring variety.

The high environmental variance observed in the field experiment may be due to extremes of temperature and light which unlike indoor conditions can not be controlled. Yield losses are greater in field experiments than in the greenhouse as a result of interactions between the damage in the host plant caused by the virus and environmental stresses (Riedell et al., 1999). Similarly, in this study the BYDV symptom expression in the field was more severe than the greenhouse. Plants were severely stunted; the tolerant parent Maringa which is tall in stature had a reduction of 25 percent in height. Spike weight in durum wheat is considered a good indicator of BYDV tolerance (Chéour et al., 1993).Yield, in terms of spike mass, from the artificially virus-inoculated field trial was severely reduced compared to the greenhouse spike mass as the wheat spikes were more robust in the greenhouse than in field. Several lines in the field were also infected by other diseases including leaf spots, glume blotch, leaf rust and environmental stress spots. Several lines showed no elongation of peduncle and displayed pronounced spike sterility. The flag leaves were very short and the wheat spikes small in most of the lines. These characteristics contributed to the DH line frequency skewing towards the susceptible parent.

Heritability of a trait expresses the relative importance of heredity in determining phenotypic values and is the proportion of total variation in the population that can be attributed to variation in genetic factors (Falconer and Mackay, 1996). Broad sense heritability, calculated by the ratio between genetic variance and phenotypic variance, is of more theoretical interest than practical importance in self-pollinated crops, because it expresses the extent to which individual phenotypes are determined by genotype. However, heritability in the narrow sense, calculated as the ratio between additive variance and dominance variance, expresses the extent to which phenotypes are

determined by the genes transmitted from each parent. It determines the degree of resemblance between relatives, and has the highest importance in breeding programs. However, in this experiment, it was not possible to calculate heritability in the narrow sense as the population was DH in which dominance variance is not detected. Hence, the values for broad sense heritability are assumed to be close to heritability in the narrow sense.

Barley yellow dwarf virus causes both physiological and biological changes in host plants (Jensen et al., 1971). The negative results of this study may be partially explained by the complexities of the BYDV pathosystem. It is reported that BYDV alters plant biology as it influences growth and metabolism. Studies have shown that depending on the BYDV strain and its virulence, infection may contribute to winter kill in cold, temperate regions, induce plant stunting, inhibit root growth, reduce or prevent flower production, or increase host susceptibility to opportunistic pathogens, drought and other stresses (Burnett, 1984; Irwin and Thresh, 1990; Fiebig et al., 2003). The negative values obtained in this experiment can be either because of the host pathogen interaction or the experimental error. Growing plants in the greenhouse under controlled conditions should have removed some of the environmental errors so it is not unlikely that some portion of the negative values can be explained by plant-virus-aphid interaction.

4.6 Conclusion

Several studies suggest that the nature of BYDV resistance/tolerance in spring wheat is polygenic (Barbieri et al., 2000; Ayala et al., 2002; Veskrna et al., 2009). The visual rating system used in this study also indicates that BYDV tolerance in Maringa is

quantitative in nature. As the environmental effects on the trait can mask tolerant genotypes a marker assisted approach would be beneficial after the quantitative indoor assay (QI-assay) is performed in the greenhouse experiments.

CHAPTER 5

COMBINING FUSARIUM HEAD BLIGHT (FHB) RESISTANCE AND BARLEY YELLOW DWARF (BYDV) TOLERANCE IN SPRING WHEAT

5.0 Combining fusarium head blight (FHB) resistance and barley yellow dwarf (BYDV) tolerance in spring wheat

5.1 Abstract

Fusarium head blight (FHB), a fungal disease caused principally by *Fusarium graminearum* Schwabe [teleomorph: *Gibberella zeae* (Schwein.) Petch] in North America, and barley yellow dwarf (BYD) caused by BYD luteovirus are two serious economic threats to small grain cereals. In addition to head blight, *F. graminearum* produces the mycotoxin deoxynivalenol (DON) in the grain which affects quality and milling properties. The objective of this research was to develop spring wheat germplasm that combines FHB resistance with tolerance to BYDV infection. Genes for FHB resistance distinct from those in Sumai 3, have been identified in the Chinese wheat cultivar, Wuhan, while good BYDV tolerance has been identified in the Brazilian cultivar, Maringa. One hundred and fifty doubled haploid (DH) lines were derived from F_1 seeds that had been generated from reciprocal crosses involving Maringa and Wuhan using corn-mediated DH technology. The DH lines and the two parents were evaluated for disease symptoms, reduction in height and spike mass for BYD in one greenhouse and one field environment in Manitoba and for disease incidence (DI), disease severity (DS) and *Fusarium*-damaged kernels (FDK) for FHB in one greenhouse and four field environments, three in Manitoba and one in Ottawa. A randomly selected subset sample of DH lines was evaluated in 2004 field nurseries for both BYD and FHB. Parents, F_1s and DH lines were point inoculated with *F. graminearum* in greenhouse experiments to evaluate FHB disease severity. Macroconidial spray inoculations and spread of corn inoculum were used in field environments. BYDV inoculations were performed by

placing ten to fifteen viruliferous aphids, *Rhopalosiphum padi* L. with BYDV-PAV isolate 9301PAV, at the one to two leaf stage for both greenhouse and field trials. Both resistance to FHB and tolerance to BYDV infection were measured by visual assessment of disease symptoms on separate plants. The phenotypic distribution for all parameters showed transgressive segregation. The broad sense heritability was high (0.90 to 0.97) for all traits evaluated. Fourteen DH lines were found more resistant than, or at least equal to, the resistant parent, Wuhan for FHB resistance and to the tolerant parent Maringa for BYDV. These 14 lines were subjected to successive inoculation of BYDV and FHB on the same plant to study the interaction and validate information obtained from previous experiments. Six lines from the 14 selected doubled haploids demonstrated high resistance to FHB and tolerance to BYDV infections when plants were inoculated with both *F. graminearum* and BYDV. Promising lines from this study included - MW B-27, MW B-55, WM B-29, WM B-39, WM E-41; WM B-21. These DH lines have combined both BYDV tolerance and FHB resistance and can be used in wheat breeding programs.

5.2 Introduction

Breeding for combined resistance to diseases has become an important and effective strategy in agriculture. This approach can reduce environmental pollution and production costs resulting from lower inputs such as use of pesticides in crops, especially cereal crops which are grown as monocultures. Breeding for resistance is even more urgent where effective fungicides are not available. Fusarium head blight (FHB), a fungal disease caused principally by *Fusarium graminearum* Schwabe and barley yellow dwarf (BYD) caused by BYD luteoviruses (BYDVs) are two serious, worldwide economic threats to small grain cereals. Fusarium head blight is difficult to control with fungicides (Schroeder and Christensen, 1963; Parry et al., 1995) and BYD can not be controlled with fungicides (D'Arcy, 1995; Lister and Ranieri, 1995). It would be of great benefit to the field of disease resistance breeding if BYDV tolerance and FHB resistance were combined in spring wheat lines (*Triticum aestivum* L.). The higher the diversity of the genetic system governing resistance, by pyramiding major and minor resistance genes, the lower the vulnerability of resistance, or the potential loss of resistance due to changes in virulence of the pathogen (Sip et al., 2005).

Fusarium head blight is prevalent in warm, humid or semi-humid areas of the world and can cause quantitative and qualitative losses in grain (Parry et al., 1995, McMullen et al., 1997; Miedaner, 1997; Gilbert and Tekauz, 2000; Dardis and Walsh, 2002; Stack, 2003; Waalwijk et al., 2003). The shrivelled *Fusarium*-damaged kernels (FDK) contribute to its reduced yield and low test weight and are a reservoir for mycotoxins.

The overall loss due to FHB is amplified by the presence of fungal toxins such as the trichothecene - deoxynivalenol (DON) and the estrogenic toxin zearalenone (ZEA) (Sutton, 1982; Gilbert et al., 2001; Mesterhazy, 2002). The accumulation of mycotoxins in the infected grain causes a variety of detrimental effects on humans and livestock if ingested (Snijders, 1990; McMullen et al., 1997; Whitlow and Hagler, 2005). Tricothecenes produced by *Fusarium* species are secondary metabolites which are not essential for growth or survival of the fungus, but they may be a virulence factor involved in pathogenesis (Desjardins and Hohn, 1997). Li et al. (2001) reported that the FHB - resistant wheat cultivar Sumai 3 accumulates higher levels of pathogenesis-related proteins (chitinases and glucanases) than its susceptible mutants. A comparative study on changes in gene expression, initiated by infection in the resistant cultivar Ning 7840 and susceptible cultivar Clark, demonstrated an enhanced expression of defence-related genes in Ning 7840 compared to Clark during the early stages of fungal growth (Bernado et al., 2007).

Resistance to FHB is a complex phenomenon; screening requires adult plants and reactions are conditioned by both physiological and morphological factors sometimes resulting in inconsistent disease severity values (Snijders, 1990c; Rudd et al., 2001; Buerstmayr et al., 2002). At least five active resistance mechanisms have been discussed that include Type I – resistance to initial infection, Type II – resistance to pathogen spread within the wheat spike (Schroeder and Christensen, 1963), Type III – resistance to kernel infection, Type IV – tolerance, Type V – resistance to toxins (Mesterhazy, 1995). This categorization has become the basis for FHB disease evaluation in resistance breeding. Thirdly, FHB disease severity and the FHB disease index (a product of percent disease severity and incidence) show a continuous distribution in segregating populations

due to the interaction between different loci and environment (Snijders 1990c; Singh et al., 1996; Ginkel et al., 1996).

Barley yellow dwarf (BYD) is a virus disease of plants of the family *Poaceae* and poses a serious threat, especially, to wheat (*Triticum aestivum* L.) and other small grain cereals (D'Arcy, 1995; Lister and Ranieri, 1995; Comeau and Haber, 2002). The disease is caused by a group of related single-stranded RNA viruses in the family *Luteoviridae* (Miller et al, 1987) called the barley yellow dwarf viruses (BYDVs). BYDV strains and their principal vector associated are *Rhopalosiphum padi* virus (RPV) and *Macrosiphum avenae* virus (MAV) (Oswald and Houston, 1951; Slykhuis, 1956; Watson and Mulligan 1960), genus *Luteoviruses,* and cereal yellow dwarf viruses, (CYDVs) species *Schizaphis graminum* virus (SGV), *Rhopalosiphum maidis* virus (RMV) and *S. graminum* and *R. padi* virus (GPV), genus *Polerovirus*, (Van Regenmortal et al., 2000, D'Arcy and Domier 2005). The virus group is distributed throughout the wheat growing areas globally and can cause significant yield losses (Carrigan et al., 1980; El Yamani, 1990; Haber, 1990; McKirdy and Jones, 1997; Riedell et al., 1999; Veskrna et al., 2009).

Symptoms of BYD vary with age and physiological condition of crop and variety. In wheat, the most common symptoms of BYD include reduced root growth, stunted growth of plant due to reduced internode elongation and yellowing of leaves, especially leaf tips along the vascular bundles. Leaves of infected plants may appear stiff and more erect than the healthy ones. Discoloration is common among older infected leaves (Oswald and Houston 1953). The discolored area enlarges with time from the tip to the base of the infected leaf and may finally cover the whole leaf. This colour change is associated with a reduction in chlorophyll content and photosynthesis, which leads to a reduction in grain yield. BYD also affects yield by reducing the number of

tillers, suppressing heading, causing sterility and reducing number of kernels per spike (D'Arcy 1995). The virus may also cause phloem degradation and collapse of the sieve elements, weakening plants and making them susceptible to other diseases (D'Arcy, 1995).

Annual and perennial grasses, and volunteer cereals, and even neighbouring grain crops play an important role in the epidemiology of BYD, serving as host reservoirs of the BYDV virus complex. Their wide host range within the grass family is attributed to numerous aphid species that vector BYDVs (A'Brook, 1981). BYDVs are phloem limited within the host and they are transmitted in a persistent manner by aphids. Each BYDV strain is preferentially spread by a narrow range of aphid species. The BYDV-PAV is spread primarily by the bird cherry-oat aphid, *Rhopalosiphum padi* L. (Slykhuis et al., 1959; Watson and Mulligan 1960; Rochow et al., 1965; Haber, 1990) and is the most prevalent serotype in Canada and the USA (Gildow, 1990; Haber, 1990). BYDV-MAV is also commonly found in North America, but when there is an infection with both MAV and PAV, PAV is a stronger competitor than MAV within the host and is generally transmitted more efficiently which contributes to its predominance under field conditions (Power, 1996).

Incorporating genetic resistance to pathogens and pests is the most desirable control method to growers as it is effective, and inexpensive (Henry et al., 2002). BYDV is controlled mainly by use of varieties that are tolerant to certain BYDV isolates of a serotype or subgroup to varying degrees (Miller and Rasochova, 1997; Fedak et al., 2001; Veskrna et al., 2009). Tolerance to BYDV based on *Bdv1* has been reported to have originated from the Brazilian spring wheat cultivar Frontana, parent of the cultivar Maringa. This gene was also found to be linked to genes *Lr34* and *Yr18* conferring adult

plant resistance to leaf and yellow rust respectively in field environments (Singh et al., 1993). Sip et al. (2004) reported that damage to cereal crops is more severe under conditions of double stresses, (BYDV and other stresses such as deep frosts during winter or drought during the growing season). Therefore, resistance to BYDV is considered to be the most effective way of controlling damage caused to cereal crops by biotic and abiotic stress.

Both BYDV and FHB are economically important to cereal crops but they differ considerably in their nature of parasitism. BYDV is a systemic disease, restricted to the host's phloem cells, and can infect the plant from the seedling to adult stages bringing morphological and physiological changes. On the other hand, FHB causes more local infections restricted primarily to the wheat spikes and peduncle. From ultrastructural investigations it is reported that FHB changes from biotrophic (initial infection) to necrotrophic stages (Kang and Buchenauer, 2000b). The benefits of combining FHB resistance and BYDV tolerance are not merely additive as damage from BYD predisposes to greater losses from FHB (Liu and Buchenauer, 2005).

The objectives of this study were to combine barley yellow dwarf virsuses tolerance and FHB resistance in a spring wheat doubled haploid population and to identify genotypes that combine tolerance to barley yellow dwarf virus and resistance to FHB.

5.3 Materials and Methods

5.3.1 Population development

Procedures for population development, generation of DH populations and details on the pedigrees of Wuhan and Maringa (Table 3.3.1) have been described in Chapters 3 of this thesis. A total of 150 DH lines, from two reciprocal crosses were generated during March 2001 – April 2002 (Figure 5.3.1).

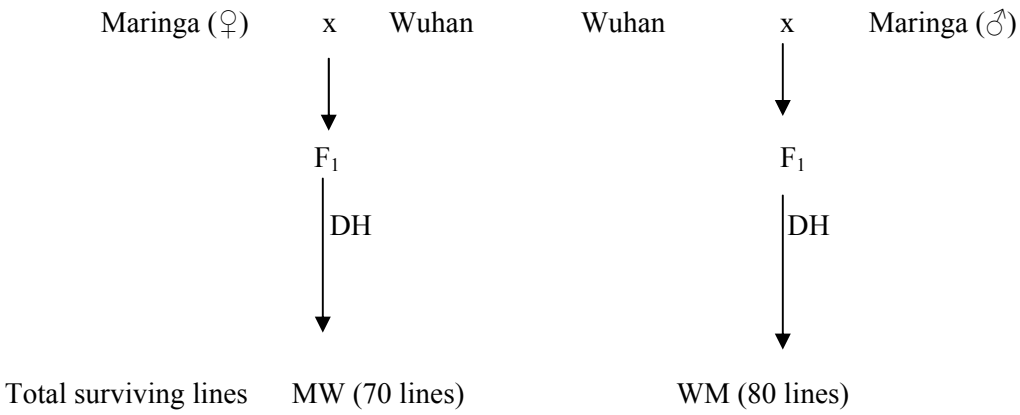


Figure 5.3.1 Generation of Maringa/Wuhan and Wuhan/Maringa doubled haploid populations via corn-mediated doubled haploid technology.

5.3.2 Fusarium head blight phenotyping

Methods and procedures of FHB and BYDV evaluation in growth cabinets/greenhouse experiments are detailed in Materials and Methods sections of Chapter 3.

The parents (Maringa and Wuhan), F_1s, and F_1-derived DH lines were evaluated for FHB resistance in a controlled environment. Ten seeds per parental line, ten seeds per F_1 and two seeds per DH lines (MW – 70 lines; WM – 80 lines) were screened.

In 2003 the DH populations were evaluated for FHB in replicated field trials at three FHB Nurseries in Manitoba (Carman, Glenlea and Portage La Prairie) and one location at the Eastern Cereals and Oilseeds Research Center (ECORC), Ottawa, Ontario. In 2004, the twenty lines (14 lines that performed as well as, or better than, the resistant parent Wuhan and Maringa, three intermediate and three susceptible lines) were selected based on their 2003 performance and evaluated again in all four locations. The parents, Maringa, Roblin and Wuhan were included as checks and were seeded after every five rows in the 2004 field trials.

5.3.3 Barley yellow dwarf phenotyping

Methods and procedures for BYDV evaluation in growth cabinets/greenhouse experiments are detailed in Materials and Methods sections of Chapter 4.

Parent cultivars Maringa and Wuhan, F_1s, and F_1-derived DH lines (MW 70, WM 80) were evaluated for BYDV tolerance under controlled conditions. Four plants from each DH line were grown in 17cm fibre nursery pots (3RR-66) with two replications.

The field trials included the 150 F_1-derived DH lines (MW 70 line, WM 80 lines) which were evaluated for BYDV tolerance at Agriculture and AgriFood Canada

Research Station, Glenlea, Manitoba in 2003. In 2004, twenty doubled haploid lines (14 lines that performed as well as, or better than, the tolerant parent Maringa, three intermediate and three susceptible lines) that performed as well as or better than the resistant parent Maringa, were selected for evaluation based on the 2003 selection procedures. Wheat cultivars Maringa, Roblin and Wuhan, were included as checks and were seeded every 20 rows of DH lines in the 2003 growing season and after every five rows in the 2004 field trials.

5.3.4 Evaluation of DH lines with successive inoculation of FHB and BYDV

Twenty of the 150 Wuhan/Maringa DH lines with improved BYDV tolerance and FHB resistance (compared to the parent cultivars) which were identified in separate greenhouse and field experiments in 2003 and 2004 were assessed BYDV and FHB reaction with successive inoculations. Riel oat and Manley barley were included as checks in addition to the three parent cultivars Wuhan, Maringa, and Roblin. Inoculum was applied for both diseases on the same plant at the 2-3- leaf stage for BYD and at 50% anthesis for FHB using the application procedures described in Chapters 3 and 4. All the test plants were inoculated with BYDV by virus-infected *R. padi.* The experiment was conducted as a randomized complete block design with three replications. A replicate consisted of four plants per DH line grown in a 17 cm diameter fibre pot and two spikes per line per replicate were FHB spray-inoculated and two spikes per line were point- inoculated. One pot containing four plants was grown as a control. The growing conditions remained the same as in previous growth cabinet experiments. Plants were scored for BYD symptoms after six weeks of inoculation and FHB was evaluated at 7, 14 and 21 days after inoculation. FHB severity was calculated as percent infection of

spikelets on spikes. Plant height above the soil surface was recorded at plant maturity (from soil to spike tip excluding awns?) and spike mass was measured after harvest.

5.3.5 Statistical analysis

The statistical analyses including univariate analysis, least squares means comparison, analysis of variance, and correlation analyses on the 2003 and 2004 data performed as in Chapters 3 and 4 using SAS version 9.1, SAS Institute Inc., Cary, N.C. Broad-sense heritabilities for DH populations were estimated from ANOVA using the formulae $h^2 = \sigma_G^2 \big/ [\sigma_G^2 + (\sigma_e^2 / r)]$ for single location and greenhouse data,

$h^2 = \sigma_G^2 \big/ [\sigma_G^2 + (\sigma_{GL}^2 / l) + (\sigma_e^2 / rl)]$ for combined data of four locations, and

$h^2 = \sigma_G^2 \big/ [\sigma_G^2 + (\sigma_{GL}^2 / l) + (\sigma_{GY}^2 / y) + (\sigma_{GYL}^2 / yl) + (\sigma_e^2 / rly)]$ for combined data of four locations in two years, where σ_G^2 is the genotypic variance, σ_{GL}^2 is genotype x location variance, σ_{GY}^2 is genotype x year variance, σ_{GYL}^2 is genotype x location x year variance, σ_e^2 is residual variance, *r* is the number of replications (blocks), *l* is number of locations, and *y* is the number of years.

5.4 RESULTS

5.4.1 Fusarium head blight

The two parents differed significantly and consistently in disease severity in both greenhouse and field experiments. The means for Maringa were 50.63 ± 0.636 and 48.35 ± 1.012 and for Wuhan, 18.20 ± 0.429 and 22.56 ± 0.647 for greenhouse and field, respectively.

5.4.1.1 Analyses of variance, correlation and heritability

Results of analyses of variance for the field experiments for DH lines are provided in Table 5.4.1.1. The ANOVA for combined site-years for the population indicated all sources of variation for field DI, DS, FHBI and FDK were significant except replication within location at $\alpha = 0.05$ (Table 5.4.1.2). Genotype was one of the largest sources of variation for disease severity ratings in all experiments indicating the diversity of variation in the DH lines. The other larger source of variation for disease severity rating was the genotype/location interaction presenting the importance of separate locations; FHB disease severity is highly influenced by environmental conditions.

The mean FHB disease severity in the greenhouse for the MW population was 45.62, ranging from 5.26 to 94.44 and for the WM population 52.20, ranging from 9.52 to 100. The mean infection level for all FHB traits of DH lines in the field trials did not differ among locations. Means and ranges for all traits including DON level for the selected 20 lines are presented in Table 5.4.3. Some lines showed lower accumulation of DON than Wuhan.

Table 5.4.1.1 Analysis of variance for disease incidence, disease severity, fusarium head blight index (FHBI)and *Fusarium*-damaged kernels (FDK) from four field environments (Carman, Glenlea, Portage la Prairie, and Ottawa) for a Wuhan/Maringa spring wheat population, 2003.

Dependent variable: Disease incidence					
Source	**df**	**SS**	**MS**	**F Value**	**Pr > P**
Genotype	148	227866	1539.63	2.60	<.0001
Loc	3	28101	9367.05	13.68	<.0001
Rep(loc)	4	813.31	203.33	1.95	0.1016
Loc*Geno	391	231794	592.82	5.67	<.0001
Residual	533	55712	104.52	.	.

Dependent variable: Disease severity					
Source	**df**	**SS**	**MS**	**F Value**	**Pr > P**
Genotype	148	198877	1343.76	2.58	<.0001
Loc	3	75000	25000	25.24	<.0001
Rep(loc)	4	2399.29	599.82	5.33	0.0006
Loc*Geno	391	204109	522.02	4.64	<.0001
Residual	533	59951	112.48		

Dependent variable: FHBI					
Source	**df**	**SS**	**MS**	**F**	**Pr > P**
Genotype	148	189630	1281.28	2.40	<.0001
Loc	3	52746	17582	18.89	<.0001
Rep(loc)	4	1987.58	496.89	5.97	0.0001
Loc*Geno	391	208813	534.05	6.42	<.0001
Residual	533	44364	83.23		

Dependent variable: FDK					
Source	**df**	**SS**	**MS**	**F**	**Pr > P**
Genotype	148	187234	1265.09	1.54	0.0014
Loc	2	54509	27255	16.80	0.0009
Rep(loc)	3	2972.94	990.98	7.42	<.0001
Loc*Geno	248	204177	823.30	6.16	<.0001
Residual	390	52099	133.59		

Table 5.4.1.2 Analysis of variance for disease incidence, disease severity, fusarium head blight index (FHBI) and *Fusarium*-damaged kernels (FDK) from four field environments (Carman, Glenlea, Portage la Prairie, and Ottawa) for a Wuhan/Maringa spring wheat population, 2003 and 2004

Dependent variable: Disease incidence					
Source	**df**	**SS**	**MS**	**F Value**	**Pr > P**
Genotype	19	9288.55	488.87	3.23	0.0017
Year	1	12522	12522	13.61	0.0019
Loc	3	698,76	232.92	1.54	0.2234
Rep(Loc)	4	21.74	5.44	0.07	0.0019
Year*Loc	3	372.48	124.16	0.91	0.4439
Year*Geno	19	17806	937.14	6.84	<.0001
Loc*Geno	32	4843.80	151.37	1.84	0.0076
Year*Loc*Geno	55	7531.69	136.94	1.67	0.0078
Residual	154	12641	82.08		

Dependent variable: Disease severity					
Source	**df**	**SS**	**MS**	**F Value**	**Pr > P**
Genotype	19	8992.55	362.24	1.66	.0996
Year	1	3929.99	3929.99	2.44	0.1842
Loc	3	1878.23	626.08	2.87	0.0515
Rep(Loc)	4	281.98	70.50	0.85	0.4965
Year*Loc	3	3854.95	1284.98	7.14	0.0004
Year*Geno	19	9564.49	503.39	2.80	0.0015
Loc*Geno	32	6973.78	217.93	2.62	<.0001
Year*Loc*Geno	55	9896.76	179.94	2.17	0.0001
Residual	154	12795	83.08		

Dependent variable: FHBI					
Source	**df**	**SS**	**MS**	**F**	**Pr > P**
Genotype	19	4012.02	211.16	1.25	0.2787
Year	1	962.79	962.79	1.21	0.3170
Loc	3	974.20	324.73	1.93	0.1448
Rep(Loc)	4	50.75	12.69	0.57	0.6846
Year*Loc	3	1703.19	567.73	4.09	0.0108
Year*Geno	19	6953.64	365.98	2.64	0.0027
Loc*Geno	32	5388.51	168.39	7.57	<.0001
Year*Loc*Geno	55	7628.71	138.70	6.23	<.0001
Residual	154	3426.28	22.25		

Table 5.4.1.3 Means and ranges of fusarium head blight (FHB) disease incidence (DI), disease severity (DS), index (FHBI), damaged kernels (FDK), and deoxynivalenol (DON) values after inoculation with *Fusarium graminearum* under field conditions from single environments (year/location) and combined environments over years (2003/2004) and locations (Carman, Glenlea, and Portage la Prairie in Manitoba and Ottawa) in a sub-population of 20 lines of a doubled haploid spring wheat population of Wuhan/Maringa.

	Parent means		Population	Range of
Trait	Maringa	Wuhan	mean	DHLs
DI_2003	30.56	19.44	32.25	5.00 - 90.00
DI_2004	21.25	8.75	20.63	5.00 - 95.00
DI_2003/2004	26.18	14.41	26.36	5.00 - 95.00
DS_2003	33.89	17.78	24.54	5.00 - 100
DS_2004	22. 50	8.13	18.97	5.00 - 60.00
DS_2003/2004	28.53	13.24	21.71	5.00 - 100
FHBI_2003	8.00	3.44	9.38	0.25 - 95.00
FHBI_2004	5.00	0.75	7.19	0.25 - 26.00
FHBI _2003/2004	6.59	2.18	8.27	0.25 - 95.00
FDK_2003	22.25	12.5	29.56	4.10 - 25.00
FDK_2004	15.35	6.52	11.25	2.10 - 15.20
FDK_2003/2004	18.80	9.53	20.44	2.10 – 25.00
DON_2004	10.1	3.73	5.61	2.93 - 11.96

The frequency distribution on the means of all locations for FHB severities for the Maringa/Wuhan DH populations showed a continuous distribution (Figure 5.4.1) indicating the quantitative nature of inheritance. DH lines segregated with values that covered the entire parental range. In addition, transgressive segregation within the DH population was observed at most locations. Several lines had disease values lower than the resistant parent. However, the majority of the segregants were inferior. A high range of variation was also observed in FDK among the DH lines showing a continuous frequency distribution and indicating a quantitative nature of inheritance of FHB resistance.

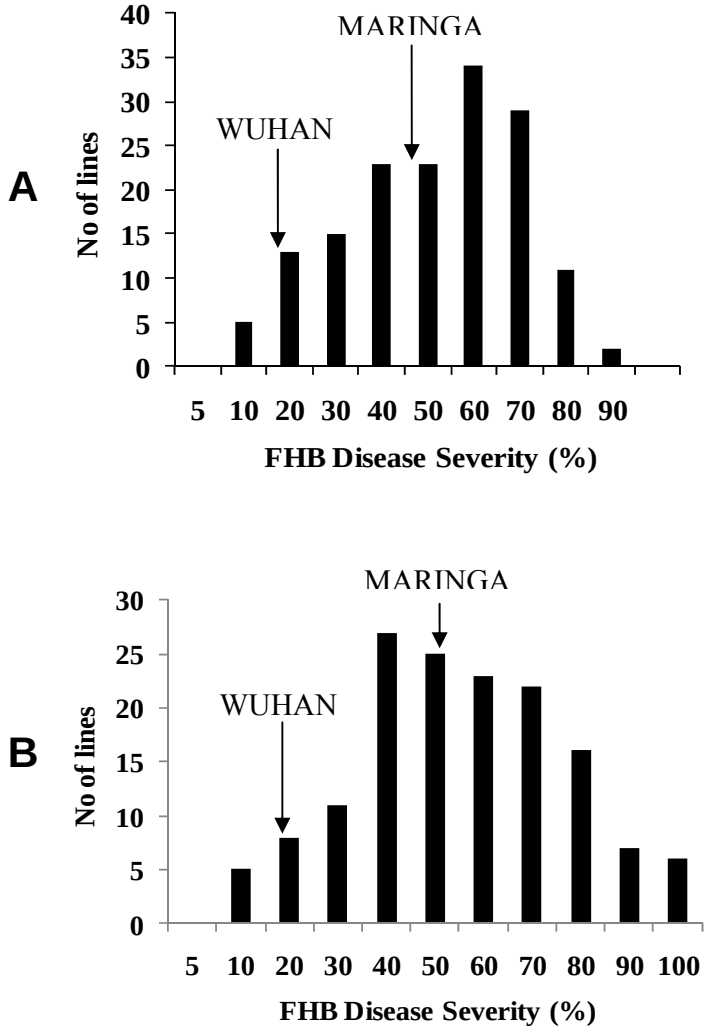


Figure 5. 4.1.1 Phenotypic distribution on means of fusarium head blight (FHB) severity across locations with Type II resistance in a doubled haploid population derived from the cross Wuhan/Maringa in A – Greenhouse and B – Field.

5.4.2 Barley yellow dwarf

Given the complexity involved for BYD inoculation, a single location was available for the experiment, the Glenlea Cereal Research Farm in Manitoba. Selection was based on values obtained from a quantitative assay (Haber and Comeau, 1998).Data

collected included heading date, plant height, leaf symptom score and spike mass from virus-inoculated plants. The latter recommend that parameters such as length of delay of heading, plant height and spike mass are the most critical quantitative assay parameters for predicting field performance under BYD pressure, whereas, the virus titres assayed by ELISA are not useful in predicting performance under pressure from seedling infection with BYDV.

Differences in days to heading of the parent cultivars in the greenhouse were about six days while in the field it was ten days. Maringa had earlier spike emergence than Wuhan. The earliest and the latest spike emergence for the DH lines spanned 15 days in the greenhouse and 30 days in the field. Some lines exhibited a grassy phenotype.

Disease symptoms were evident on some plots as early as four weeks after virus inoculation as indicated by yellowing and stunting. All of the plots showed evidence of good inoculation and good infection.

In both 2003 and 2004, infection rates were very high and 100% percent of the DH lines were infected in both controlled and field environments (Figure 5.4.2.1). All genotypes (Maringa, Wuhan and doubled haploid lines) inoculated with BYDV showed symptoms of the disease to different levels. Visual symptoms were scored at the heading stage of plant development, six weeks after inoculation. The 150 doubled haploid lines from the reciprocal crosses in the 2003 greenhouse trials had symptom scores ranging from 2 to 8 and the disease symptom rating scores for the field experiments ranged from 3.5 to 9 The mean symptom scores for the parental controls in the greenhouse were 3.63 for the tolerant parent Maringa and 6.95 for Wuhan. For the field experiment the disease symptom rating scores were 4.5 and 6.8 for the two parents, respectively. The analyses of variance on symptom rating scores and plant height and spike mass from the infected

plants are shown in Tables 5.4.2.1 and 5.4.2.2 for greenhouse and field, respectively. The significant effect of genotype on all three traits is evident from the ANOVA results. Analyses of variance for 2003 and 2004 for individual and combined years are presented in Table 5.4.2.3.

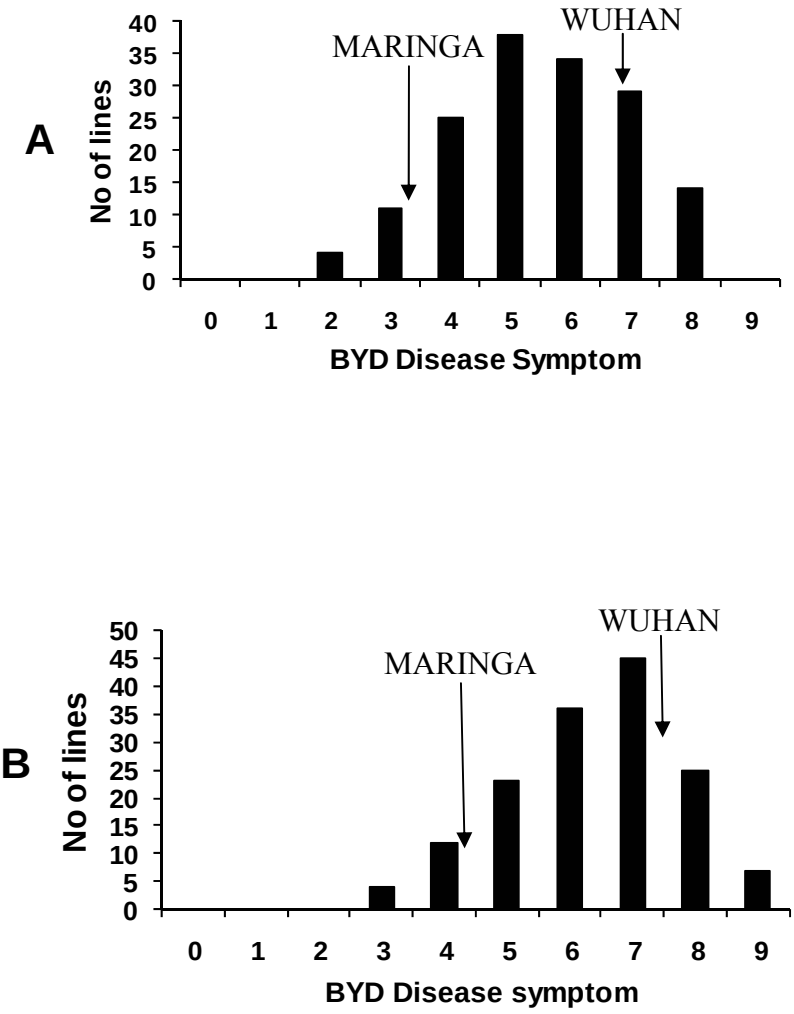


Figure 5.4.2.1 Phenotypic distribution of barley yellow dwarf disease tolerance in a doubled haploid population derived from the cross Wuhan/Maringa - based on a scale of 0-9, where 0=immune and 9 = extreme dwarfing with no spike . A – Greenhouse B – Field

Table 5.4.2.1 Analysis of variance for barley yellow dwarf (BYD) severity in Maringa/Wuhan reciprocal crosses artificially inoculated under controlled conditions with viruliferous aphids, *Rhopalosiphum padi* with BYDV-PAV isolate 9301PAV.

Dependent Variable: BYD symptom rating score

Source	**DF**	**SS**	**MS**	**F Value**	**Pr > F**
Genotype	149	324.286	2.176	3.24	<.0001
Error	150	100.75	0.671		
Total	299	425.037			

Dependent Variable: Spike Mass of infected plant

Source	**DF**	**SS**	**MS**	**F Value**	**Pr > F**
Genotype	149	5748.84	2.59	3.86	<.0001
Error	150	2231.66	0.671		
Total	299	7980.51			

Dependent Variable: Height of BYDV inoculated plant

Source	**DF**	**SS**	**MS**	**F Value**	**Pr > F**
Genotype	149	122.27	0.821	2.59	<.0001
Error	150	47.5	0.317		
Total	299	169.77			

Table 5.4.2.2 Analysis of variance for barley yellow dwarf (BYD) disease symptom rating scores from 2003 summer field environments for Maringa/Wuhan reciprocal crosses artificially inoculated with viruliferous aphids, *Rhopalosiphum padi,* with BYDV-PAV isolate 9301PAV.

Dependent Variable: BYD symptom rating score

Source	DF	SS	MS	F Value	Pr > F
Rep	1	20.021	20.0208	18.9	<.0001
Genotype	149	338.334	2.2701	2.14	<.0001
Residual	149	157.854	1.0594		

Dependent Variable: Spike Mass of infected plant

Source	DF	SS	MS	F Value	Pr > F
Rep	1	8.90	8.9014	0.57	0.4497
Genotype	149	3786.66	25.7596	1.66	0.0015
Residual	149	2043.73	15.4828		

Dependent Variable: Height of BYDV inoculated plant

Source	DF	SS	MS	F Value	Pr > F
Rep	1	0.003	0.003	0.01	0.9261
Genotype	149	113.417	0.761	1.97	<.0001
Residual	149	57.496	0.386		

Table 5.4.2.3 Analysis of variance for barley yellow dwarf (BYD) disease symptom rating scores from 2003 and 2004 field environment (Glenlea) combined for twenty selected DH lines from a Maringa/Wuhan cross artificially inoculated with viruliferous aphids, *Rhopalosiphum padi* L.*,* infected with BYDV-PAV isolate 9301PAV.

Variable: BYD symptom score

Source	**DF**	**SS**	**MS**	**F Value**	**Pr > F**
Genotype	18	39.28	2.18	6.77	<.0001
Year	1	1.38	1.38	0.32	0.6181
Rep	1	2.63	2.63	8.16	0.0069
Year* Rep	1	2.63	2.63	8016	0.0069
Year*Genotype	19	39.31	2.07	6.42	<.0001
Residual	38	12.24	0.32	.	.

Variable: Height of inoculated plant

Source	**DF**	**SS**	**MS**	**F Value**	**Pr > F**
Genotype	18	12.43	0.69	4.25	<.0001
Year	1	0.31	0.31	0.39	0.5587
Rep	1	0.31	0.31	1.92	0.1736
Year* Rep	1	0.31	0.31	1.92	0.1736
Year*Genotype	19	12.44	0.65	4.03	<.0001
Residual	38	6.18	0.16	.	.

In the greenhouse experiment the differences in plant height ranged from – 5 to + 20%, based on height of test plants versus control plants. In the field trials there were no control plots consequently plant height at harvest was recorded and compared to the infected Maringa plant, the best option available in the field (as relative height comparisions were already made between uninfected Maringa plants and DH lines in the greenhouse during seed- increase and greenhouse BYDV experiments which consisted of virus-infected and non-infected test plants). The height of the DH lines was greatly affected by virus infection: 12% of the DH plant lines were equal to or better than the tolerant parent Maringa, 48 % were of medium height and 40% were severely stunted.

There was a significant correlation between the greenhouse and the field experiments for all traits *(*height $r = 0.74$, symptom score $r = 0.80$). The correlation between spike mass and disease scores was low but statistically significant $(r = 0.22)$ Estimates of broad sense heritability on entry means for disease symptom scores were 0.93 and 0.77 for the greenhouse and field, respectively.

5.4.3 Association between FHB resistance and BYDV tolerance evaluation traits

The correlation between FHB resistance and BYDV tolerance traits are presented in Table 5.4.3.1. BYDV disease parameters showed a positive and significant correlation with the FHB disease traits. BYD disease symptom scores in the greenhouse (GSS) and the field (FSS) were moderately correlated (0.44) with field FHB disease severity (FDS) and were higher than height (GHT/FHT) and head mass ((FHM) 0.28 and 0.22, respectively.

Table 5.4.3.1 Correlation among traits associated with barley yellow dwarf virus (BYDV) tolerance and fusarium head blight (FHB) resistance in doubled haploid lines of the spring wheat cross Wuhan/Maringa. Correlation coefficients (r) were calculated using mean values for greenhouse and field data measurements.

	GDS	FDI	FDS	FHBI	FDK	FHT	FSS	FHM	GHT	GSS
GHM	ns	ns	ns	ns	ns	0.22*	0.32**	0.22*	0.26*	0.36**
GSS	ns	0.42**	0.44**	0.41**	0.44**	0.68**	0.80**	0.30*	0.81**	
GHT	ns	0.30*	0.28*	0.29*	0.31*	0.74**	0.63**	0.21*		
FHM	ns	ns	0.22*	0.18*	0.20*	0.28*	0.47**			
FSS	ns	0.37**	0.44**	0.40**	0.44**	0.76**				
FHT	ns	0.28*	0.28*	0.28*	0.32**					
FDK	ns	0.87**	0.88**	0.95**						
FHBI	ns	0.89**	0.91**							
FDS	ns	0.68**								
FDI	0.17*									

*** and ** i**ndicate significance at $P < 0.05$ and $P < 0.01$ respectively.
ns indicates no significant correlation.

For FHB:
GDS = greenhouse disease severity, FDI = disease incidence (field), FDS = field disease severity, FHBI = FHBI (field), and FDK = *Fusarium*-damaged kernels (field)

For BYD:
GHM = infected plant spike mass (greenhouse), GSS = disease symptoms score (greenhouse), GHT = plant height (greenhouse), FHM = spike mass (field), FSS = disease symptom score (field), and FHT = plant height (field)

Table 5.4.3.2 Entry means of fusarium head blight (FHB), disease severity (DS), damaged kernels (FDK), and deoxynivalenol (DON) values after inoculation with *Fusarium graminearum*, and barley yellow dwarf (BYD) symptoms under controlled and field conditions in two years (location combined) in a sub-population of a doubled haploid spring wheat population of Wuhan/Maringa.

DH lines	2002[1] GH-DS	2003[2] F-DS	2003[2] F-FDK	2004[3] F-DS	2004[3] F-FDK	2004[4] DON	2002[5] GH-	2003[6] F- BYD	2004[7] F-BYD
DHMWB 27	19.05	20	23.56	5	17.91	3.2	5	5.5	5
DHMWB 35	24.5.	60	34.2	20	25.99	7.72	5.5	5.5	4.5
DHMWB 55	26.45	20	30.4	5	15.25	5.58	5.5	4	5
DHMWC18a	21.5	20	9.12	15	6.93	4.52	5.5	5.5	6
DHMWC27	14.3	20	12.92	5	9.82	4.03	5	5.5	6
DHMWE 2	19.5	30	35.65	25	24.65	5.52	5	5.5	5
DHMWE60	14.81	40	40.28	10	23.5	5.78	5	.3.5	3.5
DHWMB21	14.81	15	17.48	5	10.8	2.93	4	4	4
DHWMB29	10.53	10	5.32	5	4.04	3.6	5	4	4.5
DHWMB39	22.5	20	15.96	20	12.13	6.63	4.5	5	4.5
DHWMB49	20.25	15	18.24	15	13.86	7.31	4	5	4.5
DHWMD39	28.65	20	19	35	14.44	12.06	5	5	5
DHWMD76	25.5	20	10.64	5	8.09	3.64	5	5.5	5
DHWME 41	17.65	20	15.96	5	12.13	3.16	5	4	4.5
Maringa	51.3	33.89	22.25	22.50	15.35	10.11	4	4	4
Wuhan	17.42	17.78	12.5	8.13	6.52	3.73	6	6.5	6.5

1= 150 lines in two greenhouse experiments

2= 150 lines in four locations

3= 20 lines in four locations

4= 20 lines in 3 loc

5= 150 lines in greenhouse

6= 150 lines in 1 location

7 = 20 lines one location

5.4.4 Successive inoculation of FHB and BYDV on 14 DH lines

Analyses of variance for disease severity of both FHB and BYDV on selected plants inoculated under controlled conditions showed significant differences among DH lines (Table 5.4.4.1). The disease severity of DH lines in the growth cabinet were low to high ranging from 0 - 60 % for FHB spray inoculation and 8 to 50 % for point inoculation. Barley yellow dwarf disease symptoms were low to medium and ranged from 2 – 5 on a 1- 9 scale (Table 4.11).

Table 5.4.4.1 Analysis of variance for fusarium head blight (FHB) disease severity resulting from spray (SP) and double floret injection (DFI) and barley yellow dwarf (BYD) disease symptom rating scores under controlled conditions for 14 Maringa/Wuhan DH lines artificially inoculated with *Fusarium graminearum* and viruliferous aphids, *Rhopalosiphum padi,* infected with BYDV-PAV isolate 9301PAV.

Dependent Variable: spray inoculation (SP)

Source	**DF**	**SS**	**MS**	**F Value**	**Pr > F**
Rep	2	0.0002	0.0001	1.47	0.2488
Genotype	13	3.8334	0.2248	3851.69	<.0001
Residual	26	157.854	1.0594		

Dependent Variable: Double floret injection (DFI)

Source	**DF**	**SS**	**MS**	**F Value**	**Pr > F**
Rep	2	0.00005	0.00002	0.08	0.9208
Genotype	13	1.20017	0.09232	259.45	<.0001
Residual	26	0.00925	0.00036		

Dependent Variable: BYD symptom rating score

Source	**DF**	**SS**	**MS**	**F Value**	**Pr > F**
Rep	2	0.00037	0.00019	1.84	0.1796
Genotype	13	0.02405	0.00185	18.36	<.0001
Residual	26	0.00262	0.00010		

The means of the 14 DH lines given in Table 5.4.4.1 shows that there are lines which exhibit excellent performance under disease pressure. Those lines are described in the following paragraphs. All the DH lines are awned like their parents, Maringa and Wuhan.

To facilitate the understanding of data collected in this study, the data are categorized into groups for spray-inoculation (SP), point-inoculation (PI) and barley yellow dwarf (BYD) based on parental checks.

For SP and PI

A value of 1-10 = resistant (R); 10.1 – 20 = moderately resistant (MR); 20.1 – 40 = moderately susceptible (MS) and >40 = S

For BYD

1 –4 = Tolerant; 4.1 – 6 = Intermediate, and >6 = susceptible

DH line MW B-27**:** This line has good seed and resembles the Maringa parent. Only point-inoculated florets were infected and no disease developed until 7 days after inoculation. The spray inoculation technique failed to produce any infection. The line has moderate height, about 70 cm, a little taller than the spring wheat Roblin. The flag leaf turned 50% yellow at wheat spike maturity. This line has a tolerant reaction to BYDV and a resistant reaction to FHB. Although there was no disease until 7 days after inoculation the line developed moderate levels of FDK, but low DON. It is recommended as resistant for both diseases.

MW B-35: Reaction to BYDV was better than for Maringa. Plant height was 79 cm, a good height. The flag leaf was still green at spike maturity; FHB did not extend to the rachis. This line has a full, long spike containing plump seed. No spike tip sterility resulted from BYDV. Infection by FHB spread 2 spikelets up and two down. This line is

MR for combined disease reaction. Spike mass was lower, 1.6 g/spike compared to 1.8 g for many other resistant lines. Levels of FDK and DON were moderate.

MW B-55: Plants are shorter strawed than most of the DH lines, slightly taller than Roblin. This line produces good plump Wuhan type seeds. Flag leaves are long and stay green until spike maturity, about 5 % tip sterility was observed. The plants had uniform height and produced higher spike mass than many other selected lines. It had low FDK and DON content. This is a possible candidate for recommendation as a good source of BYDV tolerance and FHB resistance.

MW C-18: Plants are tall at 85 cm in height, with loosely arranged spikelets, in long spikes. The line contains good plump seed. There was no spike tip sterility. Disease spread for FHB point inoculation included two up and two down from the point of inoculation, however it had about 30 % infection after FHB spray inoculation. This line had moderate values for FDK and DON content. This line can be recommended as a BYDV source of tolerance.

MW C-27: the line is of medium height at 69 cm with plump Wuhan type seed. The penultimate leaf remained green until spike maturity. Only the FHB-inoculated florets were infected, but about 10% of the spike tip was sterile. No infection was observed after FHB spray-inoculation. Low content of DON and FDK were observed. It is a good source of combined BYDV tolerance and FHB resistance.

MW E-2: The line had tall plants with long spikes and was tolerant to BYDV; no spike tip sterility was observed. The flag leaf and penultimate leaves remain green until spike maturity, however, FHB infection was over 50%. High levels of FDK developed although DON content was low. This line may be used as a source of BYDV tolerance.

MW E-60: The BYDV reaction of this line is similar to that of Maringa. The flag leaf and penultimate leaf stay green until wheat spike maturity. No spike tip sterility was observed. Rachis became purple with FHB infection, and the line is susceptible to FHB.

WM B-21: The plant height is 81 cm, and only point-inoculated florets were infected with FHB. More disease developed after spray inoculation. This line has plump seed and a long spike with numerous spikelets. Low FDK and DON content were observed. This line is recommended as a candidate for good sources of BYDV tolerance and FHB resistance although FHB reaction is MR.

WM B-29: This line possesses a good long wheat spike with plump seed. No spike tip sterility was observed. Spikelets are loosely packed and just one floret was infected after FHB spray-inoculation and only the inoculated florets were infected after point- inoculation. Plant height is medium, 70 cm and spikes are uniform. Peduncle elongation is good, over 12 cm in length, and the plant remains green until spike matures. Spike filling was not decreased by BYD. Rachis was not infected with FHB. Low FDK and DON content were reported. It is a possible candidate for good sources of BYDV tolerance and FHB resistance.

WM B – 39: In this line only the florets that were point-inoculated with FHB were infected. Two spikelets were infected after spray inoculation. This line has good plump seed and possesses a good compact spike arrangement. Low FDK and DON content were observed. This line is a possible candidate as a good source of resistance to both diseases.

WM B – 49: This line exhibited a grassy phenotype different from the previous experiments in the field and greenhouse. Spike sterility was observed, with only a few

good wheat-like spikes. There was no peduncle elongation and the wheat spike was enclosed inside the leaf sheath.

WM D-39: this line was susceptible to FHB and had an intermediate reaction to BYDV. The plant height was 80 cm, a taller line. Over 40% of the spike had infection with both spray- and point- inoculation although the infection did not develop until 7 days after inoculation. Most portions of the spike were sterile and the FHB infection moved up and down the spike. This line is not a good candidate for resistance germplasm when infected with both diseases. Higher FDK and DON content were observed.

WM D-76: The plants are tall, 82 cm. Some sterility on tip of the spike was observed. The flag leaf senesced, but penultimate leaf remained green until spike maturity. Rachis was infected after FHB infection and turned purple. The line showed early spike emergence with long spikes. Low FDK and DON content were reported.

WM E-41: This line has good plump seed. This line is early maturing with good peduncle elongation. The rachis was not infected. Only the inoculated florets were infected with FHB point-inoculation. Low levels of FDK and DON were reported for this line. This line is a good candidate for development of germplasm with resistance to FHB and tolerance to BYDV.

Table 5.4.4.2 Means of 14 Maringa/Wuhan DH lines and the parents for fusarium head blight (FHB) disease severity resulting from spray (SP) and point inoculation (PI), and barley yellow dwarf (BYD) symptoms under controlled conditions. The experiment was conducted once with three replications. The DH lines were artificially inoculated with viruliferous aphids, *Rhopalosiphum padi,* infected with BYDV-PAV isolate 9301PAV and *Fusarium graminearum*.

DH Line	SP	PI	BYD	HT	HM	Comments
MW B-27*	0.0	9.0	4.0	69.8	1.7	BYD – T FHB – R
MW B-35	20.0	11.5	2.0	78.8	1.6	BYD – T FHB – MR
MW B-55*	0.0	8.7	3.0	67.0	1.8	BYD – T FHB – R
MW C-18	29.2	17.5	3.0	84.7	1.7	BYD – T FHB – MS
MW C-27	15.0	11.7	3.0	68.8	1.8	BYD – T FHB – MR
MW E-2	60.0	50.0	3.3	81.5	1.5	BYD – T FHB – S
MW E-60	60.0	40.0	3.5	81.5	1.5	BYD – T FHB – S
WM B-21	20.0	11.3	2.0	81.2	1.8	BYD – T FHB – MR
WM B-29*	5.0	9.0	3.0	69.8	1.8	BYD – T FHB – R
WM B-39*	10.0	8.8	3.0	69.8	1.8	BYD – T FHB – R
WM B-49	0.0	9.0	5.0	69.8	1.8	BYD – I FHB – R
WM D-39	41.7	45.0	4.3	79.7	1.4	BYD – T FHB – S
WM D-76	20.0	18.8	3.0	82.3	1.8	BYD – T FHB – MR
WM E-41*	0.0	9.7	3.0	68.3	1.6	BYD – T FHB – R
Maringa	60.0	50.0	3.5	83.8	1.7	BYD tolerant
Wuhan	17.5	14.2	5.0	60.0	1.7	FHB resistant
Roblin	83.3	87.5	5.0	67.8	1.5	Susceptible

SP = FHB spray inoculated, PI = FHB point inoculated, BYD = BYD symptoms, HT = height HM = spike mass

R = resistant, MR = moderately resistant, MS = moderately susceptible, S = susceptible

T = tolerant, I = intermediate

* indicates line with FHB resistance and BYD tolerance

5.5 Discussion

Confounding environmental effects and inheritance of multiple traits have a significant impact on selection for FHB resistance (Anderson, 1948; Miedaner et al 2001) and an accurate assessment of resistance requires multiple tests over locations and years (Parry et al., 1995). In this study we decreased some of the variablility by using the doubled haploid (DH) technology which generated a homozygous population in a relatively short time and phenotyping in replicated greenhouse experiments and trials at multiple field locations. The analyses of variance (Tables 5.4.1.1, 5.4.1.2, 5.4.2.1, 5.4.2.2 and 5.4.2.3) showed that the phenotypic distribution of the genotypes (DH lines) was highly significant for all field trials. The experimental design and data collection methods were effective in removing the sources of variation from the entry effects. Statistically, the most significant factors affecting FHB ratings in the population were location and genotype. The significant location effect for all traits in the field experiments may be due to varying disease pressure because of different methods of inoculation (macroconidial spray or corn and barley grain spawn), differences in isolates used and differences in

irrigation system used in different locations. The genotype*location interaction for DI, DS, FHBI and FDK for all locations was equal or greater than genotype effect.

Disease severity was measured both in the greenhouse using point inoculation (double floret inoculation) and in the field using spray inoculation to test for reaction to both Type I and Type II resistance. The overall rating results were not significantly different and moderately correlated. The inheritance studies of FHB and BYDV reactions in Wuhan and Maringa have been described in the previous chapters of this thesis and indicated that the smaller number of genes controlling FHB reaction caused by *F. graminearum* in Wuhan can be used as a source of resistance to FHB.

Buerstmayr et al. (2000) concluded that the development of FHB resistant cultivars should be possible by phenotypic selection under epidemic conditions, and should be largely independent of plant height, flowering date, awnedness and genotype of the maternal parent within a cross. Both disease severity and FHBI have been reliable traits to consider as selection criteria for FHB reaction as reported in earlier studies (Miedaner et al., 2006). Selection may possibly be practised on a highly heritable trait that is correlated to a more complex trait. Some QTL work has been conducted on FHB resistance associated with plant height with Wuhan–1/Nyubai derivatives (Somers et al., 2003; McCartney et al., 2007). This study provided insight on the genetic control of FHB resistance in Wuhan which is thought to be different from Sumai 3, and complements this resistant source. It may well have value for pyramiding FHB resistance in spring wheat.

In terms of BYDV tolerance, Maringa had a visual symptom rating of 3.63 (greenhouse) and 4.35 (field) which is in line with results reported by Veskrna et al. (2009). They reported Maringa to be the variety (out of 63 varieties evaluated from different parts of the world) that showed the best resistance/tolerance to BYDV-PAV infections. The visual symptoms of BYDV attack are related especially to yellowing of

leaves and reduction in plant height above the soil surface and tillering in the infected plants. In this study DH lines that were less tolerant displayed greater loss in spike mass. However, there were lines which showed more tolerance or fewer disease symptoms with lower spike mass; in others the spike mass was greater in infected plants than in controls. A study conducted on winter and spring wheat by Veskrna et al. (2009) reported a higher susceptibility on average in spring wheat compared to winter wheat. They suggested the cause may be the more pronounced negative effect of stresses such as drought and higher temperature for spring crops than for winter crops. Maringa is a later variety than most of the adapted Canadian varieties; however some of the DH lines showed an earlier maturity with lower percent reduction on spike mass (≤ 20%) and symptom rating (≤ 4.5).

The high environmental variance observed in the field experiments may be due to temperature and light in the field compared to that in a growth cabinet/greenhouse under controlled conditions. BYD infection is a complicated event in all graminaceous hosts as disease outbreaks depend on several factors, such as the virus, the vector, the plant and the environmental conditions (Jensen and D'Arcy, 1995).Yield losses are greater in field experiments than in the greenhouse due to host responses to BYDV infections which cannot be controlled. This contributes to interactions between environmental stress and the damage caused by the virus (Riedell et al., 1999).

Considering the general reaction of the 150 Maringa/Wuhan DH lines to *R. padi* BYDV – PAV, it is clear that the vast majority of them are susceptible. In wheat, systemic disease spread seems to be more problematic than in other hosts, particularly when compared to barley and oat. BYDV infection modifies the physiology of cereals resulting in phloem disruption leading to reduced translocation. The diseased plants

differ physically from healthy plants providing a basis for measurement. There are a few other studies on interactions between BYDV and FHB conducted with simultaneous inoculation of FHB and BYDV. It is reported that BYDV infection significantly diminished the resistance response to FHB and enhanced DON content in the grain by interfering with the induction of both structural and chemical plant defence reactions (Liu and Buchenauer, 2005; Liu et al., 2006). In this study there were 14 DH lines that were good candidates for resistance when tested separately, however that number was reduced to six when the disease pressure was applied successively. Some lines exhibited earlier maturity, possibly due to virus infection coupled with environmental pressures in the experiments. All the recommended lines (Table 5.4.4.2) might enrich germplasm for either FHB resistance, BYDV tolerance, or both. BYDV and FHB are complex diseases which causes difficulties when attempting to generate germplasm with resistance or tolerance to them. Promising lines from this study included MW B-27, MW B-55, WM B-29, WM B-39, WM E-41. In addition, WM B-21 has good seed size, shape and colour with moderate resistance to FHB and tolerance to BYDV. Total immunity to these diseases may not be possible, but promising materials which demonstrate moderate resistance or tolerance may help protect crops at times of natural epidemics.

The analyses of a segregating population in the present study suggest that significant progress can be achieved by using Wuhan- and Maringa- derived DH lines that have combined both BYDV tolerance and FHB resistance when crossed with a suitable Canadian cultivar.

CHAPTER 6

GENERAL DISCUSSION

6.0 General Discussion

Disease resistance breeding encompasses and integrates several research areas, such as plant pathology, breeding and genetics. In North America, breeding for disease resistance in wheat and other cereal crops has been in practice for many decades and is directly correlated with lowered cost to farmers, protection of the environment from excessive use of pesticides, a minimization of toxic residues, a boost in availability of safe food, and other benefits humans and animals. Breeding for resistance for single diseases has been successful in many crops; for example, stem rust on wheat was successfully controlled with resistance gene *Sr31* until the discovery of race Ug99 with virulence for *Sr31*. Wheat is susceptible to many diseases. McIntosh (1998) reported that although common wheat may be attacked by a large number of diseases and pests, less than 20 diseases and about five insect or mite pests are of major significance. Some of these have a global distribution and occur in most wheat-growing areas, whereas others are restricted to certain geographic regions or climatic zones. Breeding for combined resistance to major diseases of local importance has become an important strategy in disease resistance breeding.

Fusarium head blight and barley yellow dwarf are two of the most devastating diseases of wheat in Canada and many other countries. Although, there are mechanisms for managing the two diseases, their control either alone or in combination has been difficult. Pyramiding host resistance genes may be the most efficient and cost effective method in the long term. Conventional and molecular plant breeding techniques have been used to pyramid fungal resistance genes such as *Fhb1* and *Fhb2,* for control of FHB, (Cuthbert et al., 2007). For BYDV, partial resistance genes *Bdv1*, present in wheat

cultivar Frontana, and *Bdv2* derived from *Thinopyrum intermedium* (Singh et al.,1993, Jahier et al., 2009), along with other disease resistance genes have been used to develop cultivars of spring wheat with suitable levels of BYDV tolerance.

Ideal sources of resistance are those present in closely related, commercial genotypes as lines that contain transferred chromatin with resistance alleles from related species and genera may exhibit poor agronomic characteristics. In general, exploitation of resistance based on alien genetic material declines with reduced genetic relatedness between the recipient and donor species (McIntosh 1998). The aim of modern resistance breeding is to attain sufficient resistance to all major diseases rather than high resistance to a single disease (Sip et al., 2005). A number of sources exhibiting partial resistance have been identified, but no single line has been identified with complete tolerance to BYD or resistance to FHB. A quantitatively inherited BYDV tolerance has been identified in several wheat lines but there is no cultivar in the wheat primary gene pool that is immune (Fedak et al., 2001; Comeau and Haber 2002; Veskrna et al., 2009). In this study we used Wuhan, a Chinese spring wheat line as a source of FHB resistance and Maringa a derivative of Frontana for BYDV tolerance.

The ability to select desirable individuals in a breeding population based on phenotype requires a homogeneous population. Doubled haploid lines are homozygous at all or the majority of all gene loci (Burr et al., 1988). Self pollination of these lines will generate offspring that are genetically identical or nearly identical to the parent. The use of a DH population can increase the efficiency of recurrent selection methods intended to improve performance of lines that can be derived from a given population. Lines may directly be used as parents of a hybrid, or every doubled haploid line produced could be a potential cultivar (Bouchez and Gallais, 2000; Bordes et al., 2006). These lines can be

tested for an unlimited number of traits in an unlimited number of environments. Also, a more 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 (Burr et al., 1988). In general, DH populations are favoured in species where efficient protocols for DH line development are available because of their rapid advance to homozygosity. Development of six doubled haploid populations (399 available DH lines) by corn- mediated doubled-haploid technology (wheat x maize), from reciprocal crosses of Roblin (FHB and BYDV susceptible), Maringa (BYDV tolerance) and Wuhan (FHB resistance), facilitated disease assessment in this study.

Understanding the genetics of a particular source of resistance is critical to the efficient and effective deployment of that source of resistance by plant breeders. Wuhan is a newer source of type II FHB resistance, and Maringa, known to be moderately susceptible to FHB, was used as a source of BYDV tolerance. While these genotypes are used in some breeding programs in Canada, little is known about the genetics of their resistance or tolerance. This work was designed to both investigate the inheritance of these sources of resistance/tolerance and to combine them into one breeding line. The large DH populations and the parents were extensively phenotyped in field locations and in a greenhouse environment for FHB resistance (disease incidence (DI), disease severity (DS), disease index (FHBI), and *Fusarium*-damaged kernels (FDK)) and for BYDV tolerance (disease symptoms, plant height and spike mass). The results indicated that there was substantial genetic variation for all traits evaluated.

Resistance to FHB and tolerance to BYDV are both complex quantitative traits. Estimates of gene number controlling resistance/tolerance based on conventional analysis of FHB and BYDV reactions of DH lines indicated that two or more genes control FHB

disease severity in both Wuhan and Maringa. Multiple genes are involved in resistance to disease severity and FDK. Ban and Suenaga (2000) using both DH lines and RILs determined that resistance in Sumai 3 was conditioned by two major genes with additive effects. The gene numbers here may be underestimated because some of the assumptions of the analyses outlined by (Wright 1968) and Falconer (1989) may not have been met.

The mean analyses for parents and DH lines indicate that FHB resistance in Wuhan and Maringa is mainly conditioned by additive genes for both DS, and FDK. Other plant breeders working with other sources of FHB resistance have reported that FHB resistance in Sumai 3 (Waldron et al., 1999) and Frontana (Singh et al., 1995) is controlled by additive genetic effects. However, Bai et al. (2000) and Liu et al. (2005) have reported an additive-dominance model for gene action conditioning FHB resistance.

Broad sense heritability estimates for FHBI are more precise as they include both DI and DS. The values were high, ranging from 0.71 to 0.94 for all six crosses, calculated from the variance components of different field environments. This high heritability estimate for FHB (type II) resistance agrees with previous studies conducted by Shen et al. (2003) 0. 87 and Liu et al. (2005) reported 0.78. The high heritability estimate for the Wuhan/Roblin cross indicates possible involvement of one or more dominant genes. For BYDV tolerance in Maringa the heritability estimate for the greenhouse and field was 0.80 and 0.66, respectively.

Segregating populations usually exhibit phenotypes that are extreme relative to the parental lines, this phenomenon is known as transgressive segregation, and it provides a mechanism by which hybridization might contribute to adaptive evolution (Rieseberg et al., 2003). Transgressive segregants are very important in breeding for FHB resistance. Sumai 3, the most commonly used source of FHB resistance in spring

wheat breeding programs is derived as a transgressive segregant from a cross involving two moderately – susceptible lines. This study, and other research has shown that additive effects are the most important genetic component conditioning FHB resistance and BYDV tolerance in most commonly-used adapted germplasm. As the resistance and tolerance studied in this research are primarily conditioned by additive genes, it may be possible to develop varieties with high levels of BYDV tolerance and FHB resistance by selecting for transgressive segregants.

The main goal of this project was to combine BYDV tolerance derived from wheat cultivar Maringa and FHB resistance conferred by Wuhan. The result from the analyses of 150 DH lines indicated that the combination of desirable characters for BYDV tolerance and FHB resistance from different sources by production of DH through corn pollen-mediated technology is a valuable aid to producing BYDV tolerant and FHB resistant lines and eventually a cultivar.

Wuhan resistance can be used either as an alternative or supplement to the Sumai 3 resistance and may allow breeders to develop FHB resistant spring wheat lines. The cultivar Maringa was found to exhibit a high degree of tolerance to BYDV (Kosova et al 2008) and was employed to contribute this tolerance into some lines in this study. Maringa is a derivative of Frontana. The *Bdv1* BYDV gene for partial resistance is derived from Frontana, however, no studies have been conducted to determine if Maringa possesses the *Bdv1* gene or some other gene for the basis of its high tolerance unlike in the North American wheat cultivar Anza into which the *Bdv1* gene was introduced by Singh at al. (1993). It was later discovered that the *Bdv1* gene was located on 7DS in linkage with genes *Lr34* and *Yr18* conferring adult plant resistance to leaf and stripe rust. Lower levels of resistance with established durability of resistance based on a number of

genes may be preferred. In many situations, resistance with moderate effectiveness will contribute significantly to crop protection.

The DH population was extensively phenotyped in the greenhouse and in four field locations under FHB disease pressure and in a greenhouse and one field location for BYDV. The performance of the parents, as well as the range of values observed for the DH population, indicated there was substancial genetic variation for all traits evaluated. The majority of the DH lines producing higher infection displayed greater loss in spike mass. However, there were lines showing a lower infection rate with lower spike mass while in some the spike mass was greater in BYDV infected plants than in controls. Some DH lines showed an early maturity with lower percent reduction on spike mass although both Wuhan and Maringa are late- maturing cultivars. Fourteen DH lines were found to perform well under disease pressure when tested independently on separate plants; however, only six lines achieved the same level of BYDV tolerance and FHB resistance when inoculated successively on the same plant. These lines have combined FHB resistance and BYDV tolerance.

This study suggests that significant progress can be achieved by using Wuhan- and Maringa- derived DH lines that have combined both BYDV tolerance and FHB resistance when crossed with a suitable Canadian parent. Increases in knowledge and technology, molecular biology and genomics have opened new possibilities for plant breeding and pyramiding of resistance genes. The ground work has been laid by detecting and developing germplasm with combined acceptable resistance and tolerance to two economically important diseases. These enriched DH populations will be helpful for use in marker-assisted selection programs, and such work has been initiated. The DH lines that have demonstrated combined BYDV tolerance and FHB resistance may be

used in cultivar development until we generate a genotype that is fully resistant to both diseases. The most advantageous strategy is still to look for germplasm possessing an acceptable level of resistance to all diseases of importance at the local or regional level.

CHAPTER 7

REFERENCES

References

Abramson, D., Clear, R. M. and Nowicki, T.W. 1987. *Fusarium* species and trichothecene mycotoxins in suspect samples of 1985 Manitoba wheat. Can. J. Plant Sci., 67, 611-619.

A 'Brook, J. 1981. Vectors of barley yellow dwarf virus. In EURAPHID 1980: Aphid Forecasting and Pathogens and a Handbook for Aphid Identification, ed. L. R. Taylor, p. 21. Harpenden, England: Rothamsted Exp. Stn.

Ajayi, O. 1986. The effect of barley yellow dwarf virus on the amino acid composition of spring wheat. Annals of Appl. Biol., 108, 145-149.

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

Anderson, A.L. 1948. The development of *Gibberella zeae* head blight of wheat. J. Phytopathol., 38, 595-611.

Anderson, J.A., Stack, R.W., Liu, S., Waldron, B.L., Fjeld, A.D., Coyne, C., Moreno-Sevilla, B., Fetch, J.M., Song, Q.J., Cregan, P.B. and Frohberg, R.C. 2001. DNA markers for Fusarium head blight resistance QTLs in two wheat populations. Theor. Appl. Genet., 102, 1164-1168.

Angerer, A., Lengauer, D., Steiner, B., Lafferty, J., Loeschenberger, F. and Buerstmayr, H. 2003. Validation of molecular markers linked to two *Fusarium* head blight resistance QTLs in wheat. *In* Proceedings of 10th International Wheat Genetics Symposium, Paestum, Italy. pp. 1096-1098.

Aoki, T. and O'Donnell, K. 1999a. Morphological and molecular characterization of *Fusarium pseudograminearum* sp. nov., formerly recognized as the group 1 population of *F. graminearum*. Mycologia, 91, 597-609.

Arthur, J.C. 1891. Wheat scab. Indiana Agric. Exp. Stn. Bull., 36, 129-138.

Atanassov, Z., Nakamura, C., Mori, N., Kaneda, C., Kato, H., Jin, Y.-Z., Yoshizawa, T. and Murai, K. 1994. Mycotoxin production and pathogenicity of *Fusarium* species and wheat resistance to Fusarium head blight. Can. J. Bot., 72, 161-167.

Atanasoff, D. 1920. *Fusarium* blight (scab) of wheat and other cereals. J. Agric. Res., 20, 1-32.

Aung, T., 1998, Development and Use of Doubled Haploids in Oats. In J. Chong (ed.), Oat Newsletter 44.

Ayala, L., Ginkel, M., Khairallah, M., Keller, B. Henry, M. 2001. Expression of *Thinopyrum intermedium*-derived barley yellow dwarf virus resistance in elite bread wheat backgrounds. J. Phytopathol., 91, 55–62.

Ayala, L., Ginkel, MV., Khairallah, M., Keller, B. and Henry, M. 2002. Identification of QTLs for BYDV tolerance in bread wheat. Euphytica, 128, 249-259.

Ayala, L.N., Bariana H.S., Singh, R.P., Gibson, J.M., Mechanicos A.A. and Larkin, P.J. 2007. Trigenomic chromosomes by recombination of *Thinopyrum intermedium* and *Th. ponticum* translocations in wheat. Theor. Appl. Genet., 116, 63–75.

Ayala, L.N., Tourton, A.A., Mechanicos A.A. and Larkin, P.J. 2009. Comparison of *Thinopyrum intermedium* derivatives carrying barley yellow dwarf virus resistance in wheat. Genome, 52, 537–546.

Badea A., Eudes F., Graf R.J., Laroche A., Gaudet D.A. and Sadasivaiah R.S. 2008. Phenotypic and marker-assisted evaluation of spring and winter wheat germplasm for resistance to fusarium head blight. Euphytica, 164, 803-819.

Bai, G.H. 1995. Scab of wheat: epidemiology, inheritance of resistance, and molecular markers linked to cultivar resistance. Purdue Univ., West Lafayette, IN, USA, Pp. 179.

Bai, G.H. and Shaner, G.E. 1994. Scab of wheat: prospects for control. Plant Dis., 78, 760-766.

Bai G. H. and Shaner, G. 1996. Variation in *Fusarium graminearum* and cultivar resistance to wheat scab. Plant Dis., 80, 975–979.

Bai, G. H., Kolb, F.L., Shaner, G. and Domier, L.L. 1999. Amplified Fragment length polymorphism markers linked to a major quantitative trait locus controlling scab resistance in wheat. J. Phytopathol., 89, 343-348.

Bai, G.H., Shaner, G. and Ohm, H.W. 2000. Inheritance of resistance to *Fusarium graminearum* in wheat. Theor. Appl. Genet., 100, 1-8.

Bai, G.H., Desjardins, A.E., and Plattner, R.D. 2001a. Deoxynivalenol-nonproducing *Fusarium graminearum* causes initial infection, but does not cause disease spread in wheat spikes. Mycopathologia, 153, 91-98.

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

Bailey, K.L., Gossen, B.D., Gugel, R.K. and Morrall, R.A.A. 2003. Diseases of Field Crops. Can. Phytopathol. Society, 98-99.

Baird, R.E., Mullinix, B.G., Peery, A.B., and Lang, M.L. 1997. Diversity and longevity of the soybean debris mycobiota in no-tillage system. Plant Dis., 81, 530-534.

Ban, T. 2000. Review-study on the genetics of resistance to *Fusarium* head blight caused by *Fusarium graminearum* in wheat. *In* Proceedings of the International Symposium on Wheat Improvement for Scab Resistance, Suzhou and Nanjing, China. pp. 82-93.180.

Ban, T. and Suenaga, K. 2000. Genetic analysis of resistance to Fusarium head blight caused by *Fusarium graminearum* in Chinese wheat cultivar Sumai 3 and the Japanese cultivar Saikai 165. Euphytica, 113, 87-99.

Ban, T. and Watanabe, N. 2001. The effects of chromosomes 3A and 3B on resistance to Fusarium head blight in tetraploid wheat. Hereditas, 135, 95-99.

Banks, P.M., Larkin, P.J., Bariana, H.S., Lagudah, E.S., Appels, R. and Waterhouse, P.M. 1995. The use of cell culture for subchromosomal introgressions of barley yellow dwarf virus resistance from *Thinopyrum intermedium* to wheat. Genome, 38, 395–405.

Barbieri, R. L., Carvalho, F. I. F. de., Barbosa Neto, J. F., Caetano, V. da R., Marchioro, V. S., Azevedo, R. de. and Lonrecetti, C. 2000. Diallel analysis for tolerance to barley yellow dwarf virus in Brazilian cultivars of bread wheat. Pesquisa Agropecuaria Brasileira, 36, 131-135.

Bechtel, D.B., Kaleikau, L.A., Gaines, R.L. and Seitz, L.M. 1985. The effects of *Fusarium graminearum* infection on wheat kernels. Cereal Chem., 62, 191-197.

Bekele, G.T. 1984. Head scab screening methods used at CIMMYT. Pages 169-173 in, Wheat for more tropical environments. A. R. Klatt, ed, CIMMYT, DG, Mexico.

Bennetzen J.L., SanMiguel, P., Chen, M., Tikhonov, A., Francki, M. and Z. Avramova. 1998. Grass Genomes. Proc. Natl. Acad. Sci., 95, 1975-1978.

Bernardo, A., Bai, G., Guo, P., Xiao, K., Guenzi, A.C. and Ayoubi, P. 2007. *Fusarium graminearum*-induced changes in gene expression between Fusarium head blight-resistant and susceptible wheat cultivars. Functional and Integrative Genomics, 7, 69–77.

Bisby and Bailey. 1923. Ear and root rots. Page 33 In Fourth Annual Report of the Survey of the prevalence of plant diseases in the Dominion of Canada. Ottawa, Ont.

Bissonnette, S. M., D'Arcy, C. J. and Pedersen, W.L. 1994. Yield loss in two spring oat cultivars due to *Puccinia coronata f. sp*. a*venae* in the presence or absence of barley yellow dwarf virus. J. Phytopathol., 84, 363-371.

Bonin C.M. and Kolb F.L. 2009. Resistance to Fusarium head blight and kernel damage in a winter wheat recombinant inbred line population. Crop Sci., 49, 1304-1312

Bordes, J., Charmet, G., Dumas de Vaulx, R., Pollacsek, M., Beckert, M. and Gallais, A. 2006. Doubled haploid versus S_1 family recurrent selection for testcross performance in a maize population. Theor. Appl. Genet., 112, 1063-1072.

Bouchez , A. and Gallais, A. 2000. Efficiency of the use of doubled-haploids in recurrent selection for combining ability. Crop Sci., 40, 23-29.

Brettell, R.I.S., Banks, P.M., Cauderon, Y., Chen, X., Cheng, Z.M., Larkin, P.J. and Waterhouse, P.M. 1988. A single wheatgrass chromosome reduces the concentration of barley yellow dwarf virus in wheat. Ann. Appl. Biol., 113, 599–603.

Bruehl, G.W.and Toko, H.V. 1957. Host range of two strains of the cereal yellow-dwarf virus. Plant Dis. Rept., 41, 730-734.

Bruehl, G.W. 1958. Comparison of eastern and western aphids in the transmission of barley yellow dwarf virus. Plant Dis. Rept, 42, 909-11.

Bruins, M.B.M., Karsal, I., Schepers, J. and Snijders, C.H.A. 1993. Phytotoxicity of deoxynivalenol to wheat tissue with regard to *in vitro* selection for Fusarium head blight resistance. Plant Sci., 94, 195-206.

Buerstmayr, H., Lemmens, M., Fedak, G. and Ruckenbauer, P. 1999. Back-cross reciprocal monosomic analysis of Fusarium head blight resistance in wheat (*Triticum aestivum* L.). Theor. Appl. Genet., 98, 76-85.

Buerstmayr, H., Steiner, B., Lemmens, M. and Ruckenbauer, P. 2000. Resistance to Fusarium head blight in winter wheat: heritability and trait associations. Crop Sci., 40, 1012-1018.

Buerstmayr, H., Lemmens, M., Hartl, L., Doldi, B., Steiner, B., Stierschneider, M. and Ruckenbauer, P. 2002. Molecular mapping of QTL for Fusarium head blight resistance

in spring wheat I: Resistance to fungal spread (Type II resistance). Theor. Appl. Genet., 104, 84-91.

Buerstmayr, H., Stierschneider, M., Steiner, B., Lemmens, M., Griesser, M., Nevo, E. and Fahima, T. 2003. Variation for resistance to head blight caused by *Fusarium graminearum* in wild emmer (*Triticum dicoccoides*) originating from Israel. Euphytica, 130, 17-23.

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

Burgess, L.W., Wearing, A.H. and Toussoun, T.A. 1975. Survey of Fusaria associated with crown rot of wheat in eastern Australia. Aust. J. Agric. Res., 26, 791-799.

Burgess, L.W., Summerell, B. A., Bullock, S., Gott, K.P. and Backhouse, D. 1994. Laboratory manual for Fusarium research, 3rd ed. University of Sydney, Dept. Crop Sciences and Royal Botanic Gardens, Sydney, Australia. 133p.

Burnett P.A., Ed. 1984. Barley yellow dwarf, a proceedings of the workshop. December 6-8, 1983. CIMMYT Mexico, D.F., Mexico. 209pp.

Burnett P.A., Ed. 1990. World perspectives on barley yellow dwarf. CIMMYT, Mexico, D.F., Mexico. 511pp.

Burnett, P.A., Qualset, C.O. and Comeau, A. 1995. Host plant tolerance or resistance for control of barley yellow dwarf. Pages 321-343. In D'Arcy, C. and Burnett, P.A. Barley yellow dwarf: 40 years of progress. Am. Phytopath. Soc. Press, St-Paul, Minn., USA.

Burr, B., Burr, F.A., Thompson, K.H., Albertson, M.C. and Stuber, C.W. 1988. Gene mapping with recombinant inbreds in maize. Genetics, 118, 519-526.

Bushnell, W. M. R., Hazen, B. E. and Pritsch, C. 2003. Histology and physiology of *Fusarium* head blight. Pages 44-83 In Fusarium Head Blight of wheat and barley. Leonard, K. J. and Bushnell, W. R., eds. APS Press, St Paul, MN, USA.

Cai, X., Chen, P. D., Xu, S. S., Oliver, R. E. and Chen, X. 2005. Utilization of alien genes to enhance Fusarium head blight resistance in wheat-a review. Euphytica, 142, 309-318.

Campbell, A. W., W. B. Griffin, A. J. Conner, J. S. Rowarth, and D. J. Burritt, 1998: The effect of temperature and light intensity on embryo numbers in wheat doubled haploid production through wheat x maize crosses. Annals Bot., 82, 29-33.

Campbell, K. A. G. and Lipps, P. E. 1998. Allocation of resources: Sources of variation in Fusarium head blight screening nurseries. J. Phytopathol., 88, 1078-1086.

Carleton M.A. 1916. Cereal Pest. The Small Grains. p536 Macmillan Co. New York.

Carrigan, L. L. , Ohm, H . W. and Foster, J. E. 1980. Barley yellow dwarf virus translocation in wheat and oats. Crop Sci., 23, 6 1 1-12.

Charmley, L.L., Rosenberg, A. and Trenholm, H.L. 1994. Factors responsible for economic losses to *Fusarium* mycotoxin contaminated grain, foods, and feedstuffs. Pages 47-486. In Mycotoxins in grain: compounds other than aflatoxins. Eds. Miller, J.D. and Trenholm, H.L. Eagan Press, St. Paul, Minn.

Chen, P. D., Liu, D. J. and Sun, W. X. 1997. New countermeasures of breeding wheat for scab resistance. Pages 59-165. In Fusarium Head Scab: Global Status and Future Prospects. Dubin, H. J., Gilchrist, L., Reeves, J., and McNab, A., eds. CIMMYT, Mexico D.F., Mexico.

Cheng, Z.M., He, X.Y., Wu, M.S. and G.H. Zhou. 1996. Nucleotide sequence of coat protein gene for GPV isolate of barley yellow dwarf virus and construction of expression plasmid for plant. Science in China. Series C. Life Sci., 39, 534-543.

Cheour F., Comeau A. and Asselin, A. 1993. Barley yellow dwarf virus multiplication and host plant tolerance in durum wheat. J. Phytopathol., 139, 357-366.

Chrpova, J., Sip, V., Ovesna, J., Kundu, J.K., Marik, P., Veskrna, O. and Horcicka, P. 2006. The use of resistance genes in breeding of barley and wheat for resistance to BYDV. In Tagungsband der 57. Jahrestagung der Vereinigung der Pflanzenzuchter and saatgutkaufleute. November 21-23, 2006, Reumburg-Gumpenstein 79-81 (in German)

Clear, R. M. and Abramson, D. 1986. Occurrence of fusarium head blight and deoxynivalenol (vomitoxin) in two samples of Manitoba wheat in 1984. Can. Plant Dis. Surv., 66, 9-11.

Clear, R.M. and Patrick, S.C. 1990. *Fusarium* species isolated from wheat samples containing tombstone (scab) from Ontario, Manitoba and Saskatchewan. Can. J. Plant Sci., 70, 1057-1069.

Clear, R.M. and Patrick, S.C. 2000. Fusarium head blight pathogens isolated from *Fusarium*- damaged kernels of wheat in western Canada, 1993-1998. Can. J. Plant Pathol., 22, 51-60.

Clear, R. M. and Nowicki, T. 2009. The impact of FHB on grain grading and handling in Canada. Sixth Canadian Workshop on Fusarium Head Blight, Ottawa, Ontario, November 1 - 4, 2009.

Comeau, A., Caetano, V., St-Pierre, C.A. M. and Haber, S. 2001. Accelerated breeding: dream or reality? In Bedo, Z. and Lang, L. (eds). Wheat in a global environment. Kluwer Acad. Publ., The Netherlands. Pp. 671-679.

Comeau, A. and Haber, S. 2002. Breeding for BYDV tolerance in wheat as a basis for a multiple stress tolerance strategy, Pp 82-92.

Comeau, A., and Pelletier, G.J. 1976. Predisposition to septoria leaf blotch in oats affected by barley yellow dwarf virus. Can. J. Plant Sci., 56, 13-19.

Coon, B. F. 1959. Aphid populations on oats grown in various nutrient solutions. Econ . Entomol., 52, 624-26.

Cooper, J. L, and Jones, A. T. 1983. Responses of plants to viruses: proposals for the use of terms. J. Phytopathol., 73, 127-128.

Cumagun, C.J.R., Bowden, R.L., Jurgenson, J.E., Leslie, J.F., and Miedaner, T. 2004. Genetic mapping of pathogenicity and aggressiveness of *Gibberella zeae* (*Fusarium graminearum*) toward wheat. J. Phytopathol., 94, 520-526.

Cuthbert, P.A., Somers, D.J., Thomas, J., Cloutier, S. and Brûlé-Babel, A. 2006. Fine mapping *Fhb1*, a major gene controlling Fusarium head blight resistance in bread wheat (*Triticum aestivum* L.). Theor. Appl. Genet., 112, 1465-1472.

Cuthbert, P.A., Somers, D.J. and Brûlé-Babel, A. 2007. Mapping of *Fhb2* on chromosome 6BS: a gene controlling Fusarium head blight field resistance in bread wheat (*Triticum aestivum* L.). Theor. Appl. Genet., 114, 429-437.

D' Arcy, C.J. 1995. Symptomatology and host range of barley yellow dwarf. In D Arcy CJ, Burnett PA (eds) Barley yellow dwarf. Forty years of progress. pp 9-28. St Paul MN: APS Press.

D'Arcy, C.J. and Domier, L.L. 2005. Family Luteoviridae. In Fauquet CM, Mayo MA, Maniloff J, Desselberger U, Ball LA(eds) Virus Taxonomy. Classification and nomenclature of viruses. Eighth report of the international committee on taxonomy of viruses. Elsevier, Academic press, Amsterdam, Pp 891-900.

D'Mello, J.P.F., Placinta, C.M. and Macdonald, A.M.C. 1999. Fusarium mycotoxins: a review of global implications for animal health, welfare and productivity. Anim Feed Sci Technol., 80, 183-205.

Dardis, J. V. and E. J. Walsh. 2002. Control of fusarium head blight in wheat under Irish growing conditions: Current situation and future prospects. Biology and Environment: Proceedings of the Royal Irish Academy Vol. 102B, No. 2 (Nov., 2002), Pp. 93-103.

del Blanco, I.A., Frohberg, R.C., Stack, R.W., Berzonsky, W.A. and Kianian, S.F. 2003. Detection of QTL linked to Fusarium head blight resistance in Sumai 3-derived North Dakota bread wheat lines. Theor. Appl. Genet., 106, 1027-1031.

del Ponte, E.M., Shah, D.A. and Bergstrom, G.C. 2002. Spatial patterns of Fusarium head blight in New York wheat fields in 2002. In Proceedings of 2002 National Fusarium 189 Head Blight Forum. Edited by S. M. Canty;. Lewis, J; Siler, L. and R.W. Ward, Erlanger, KY, USA. Pp. 136.

Desjardins, A.E., Proctor, R.H., Bai, G., McCormick, S.P., Shaner, G., Buechley, G. and Hohn, T.M. 1996. Reduced virulence of trichothecene-nonproducing mutants of *Gibberella zeae* in wheat field tests. Mol. Plant Microb. Int., 9, 775-781.

Dickson, J.G., Johann, H., and Wineland, G. 1921. Second progress report on the Fusarium blight (scab) of wheat. J. Phytopathol., 11, Pp 35.

Dill-Macky, R. 2003. Inoculation methods and evaluation of Fusarium head blight resistance in wheat. Pages 184-210 In: Fusarium Head Blight of Wheat and Barley. Leonard, K. J. and Bushnell, W. R., eds. APS Press, St Paul, MN, USA.

Domier L.L. 1995. Genome Structure and function of barley yellow ywarf viruses. In D Arcy CJ, Burnett PA (eds) Barley yellow dwarf. Forty years of progress. APS Press, St. Paul, pp 181-201.

Douglas, A.E. 1993. The nutritional quality of phloem sap utilised by natural aphid polulations. Eco. Entomol., 18, 31-38.

Dusabenyagasani, M., Hamelin, R.C., Collin, J. and Dostaler, D. 1997. Effect of cultivar and strain interactions in the screening of resistance against wheat scab caused by Fusarium graminearum. Phytoprotection, 78, 53-60.

El, Yamani. and Hill, J.H. 1990. Identification and importance of barley yellow dwarf virus in Morocco. Plant Dis., 74, 291-294.

Esau, K. 1957. Phloem degeneration in gramineae affected by the barley yellow dwarf virus. Am. J. Bot., 44, 245-251.

Falconer, D. S. 1989. Introduction to Quantitative Genetics, Ed. 3. Longmans Green/John Wiley & Sons, Harlow, Essex, UK/New York.

Falconer, D. S., and T. F. C. MacKay. 1996. Introduction to quantitative genetics. 4th edition. Longman Scientific & Technical, Burnt Mill, Harlow, United Kingdom.

Fauzi, M.T. and Paulitz, T.C. 1994. The effect of plant growth regulators and nitrogen on Fusarium head blight of the spring wheat cultivar Max. Plant Dis., 78, 289-292.

Fedak, G. 2000. Sources of resistance to Fusarium head blight. In Proceedings of the International Symposium on Wheat Improvement for Scab Resistance, Suzhou and Nanjing, China. Pp 4.

Fedak, G., Chen, Q., Conner, R., Laroche, A., Petroski, R. and Armstrong, K.C. 2000. Characterization of wheat-*Thinopyrum* partial amphiploids by meiotic analysis and genomic *in situ* hybridization. Genome, 43, 712-719.

Fedak, G., Chen, Q., Conner, R., Laroche, A., Comeau A. and ST.Pierre, C. 2001. Characterization of wheat-*Thinopyrum* partial amphiploids for resistance to barley yellow dwarf virus. Euphytica, 120, 373–378.

Fedak, G., Cao, W., Han, F., Savard, M., Gilbert, J. and Xue, A. 2004. Germplasm enhancement for FHB resistance in spring wheat through alien introgression. In Proceedings of 2nd Internationa Symposium on Fusarium Head Blight, East Lansing, MI, USA. pp. 56-57.

Fedak, G., Cao, W., Gilbert, J., Xue, A.G., Savard, M.E. and Voldeng, H.D. 2009. Two new sources of FHB resistance in bread wheat. In 6th Canadian workshop on fusarium head blight. Edited by T. Ouellet, D. Leger, and J. Thompson, Ottawa, Ontario, Canada Pp 62.

Fereres, A., Lister, R.M., Araya, J.E. and Foster, J.E. 1989. Development and reproduction of the English grain aphid (Homoptera: Aphididae) on wheat cultivars infected with barley yellow dwarf virus. Environ. Entomol., 18, 388-393.

Fereres, A., Araya, J.E., Housley, T.L. and Foster, J.E. 1990. Carbohydrate composition of wheat infected with barley yellow dwarf virus. J. Plant Dis. Protect., 97, 600-608.

Fernandez, M.R. and Fernandez, J.M.C. 1990. Survival of wheat pathogens in wheat and soybean crop residues in southern and central Brazil. Can J Plant Pathol. 12, 289-294.

Fernandez, M.R. 1992. The effect of *Trichoderma harzianum* on fungal pathogens infesting wheat and black oat straw. Soil Biol. Biochem., 24, 1031–1034.

Fernando, W. G. D., Paulitz, T. C., Seaman, W. L., Dutilleul, P. and Miller, J. D. 1997. Head blight gradients caused by *Gibberella zeae* from area sources of inoculum in wheat field plots. J. Phytopathol., 87, 414-421.

Fernando, W.G.D., Miller, J.D., Seaman, W.L., Seifert, K. and Paulitz, T.C. 2000. Daily and seasonal dynamics of airborne spores of *Fusarium graminearum* and other *Fusarium* species sampled over wheat plots. Can. J. Bot., 78, 497-505.

Fiebig, M., Poehling, H.M. and Borgemeister, C. 2003. Barley yellow dwarf virus, wheat, and *Sitobion avenae*: a case of trilateral interactions. The Netherland Entomol. Society, 110, 11-21.

Fitzgerald, P.J. and Stoner, W.N. 1967. Barley yellow dwarf studies in wheat (*Triticum aestivum* L.). Yield and quality of hard red winter wheat infected with barley yellow dwarf virus. Crop Sci., 7, 337-341

Flanders, K. A., Herbert, D., Buntin, D., Johnson, K., Bowen, J.F., Murphy, J.W. and Hagan, A. 2006. Barley yellow dwarf in small grains in the Southeast ANR-1082, Coop Ext system, Auburn. AL.

Forbes, A.R. 1977. The mouthparts and feeding mechanisms of aphids. In Aphids as Virus Vectors, ed. KF Harris, K Maramorosch, pp. 83-103. New York: Academic.

Francki, M.G., Ohm, H.W. and Anderson, J.M. 2001. Novel germplasm providing resistance to barley yellow dwarf virus in wheat. Aust. J. Agric. Res., 52, 1375-1382.

Francis, R.G. and Burgess, L.W. 1977. Characteristics of two populations of *Fusariumm roseum* '*graminearum*' in eastern Australia. Trans. Br. Mycol. Soc., 68, 421-427.

Gale, M.D. and Devos, K.M. 1998. Comparative genetics in the grasses. Proc. Natl. Acad. Sci., USA, 95, 1971-1974.

Gamborg, O. L., R. A. Miller, and K. Ojima. 1968. Nutrient requirement of suspension cultures of soybean root cells. Exp. Cell Res., 50, 151-158.

Gao, L., Ma Q., Y. Liu., Xin, Z. and Zhang, Z. 2009. Molecular characterization of the genomic region harboring the BYDV-resistance gene *Bdv2* in wheat J. Appl. Genet., 50, 89–98.

Gervais, L., Dedryver, F., Morlais, J.Y., Bodusseau, V., Negre, S., Bilous, M., Groos, C. and Trottet, M. 2003. Mapping of quantitative trait loci for field resistance to Fusarium head blight in European winter wheat. Theor. Appl. Genet., 106, 961-970.

Gilbert, J. 1998. Comparison of inoculation methods for screening tetraploid wheat lines for reaction to fusarium head blight. In Proceedings of the International Congress of Plant Pathology, 9-16 Aug. 1998, Edinburgh, Scotland. Offered Papers, Vol. 3: themes 3, 4, 5, and miscellaneous (abstr. 3.4.12).

Gilbert, J., Tekauz, A., Mueller, E., and Kromer, U. 1994. Occurrence of Fusarium head blight in Manitoba in 1993. Can. Plant Dis. Surv., 74, 77-78.

Gilbert, J. and Tekauz, A. 1995. Effects of fusarium head blight and seed treatment on germination, emergence, and seedling vigour of spring wheat. Can. J. Plant Pathol., 17, 252-259.

Gilbert, J. Tekauz, A., and Woods, S. M. 1997. Effect of storage on viability of Fusarium head blight–affected spring wheat seed. Plant Dis., 81, 159-162.

Gilbert, J., Fedak, G., Procunier, J.D., Aung, T., and Tekauz, A. 1997. Strategies for breeding for resistance to Fusarium head blight in Canadian spring wheat. In Fusarium Head Scab: Global Status and Future Prospects. Edited by H. J. Dubin, L. Gilchrist, J. Reeves, and A. McNab. CIMMYT, Mexico D.F., Mexico. Pp. 47-51.

Gilbert J., Procunier, J. D. and Aung. T. 2000. Influence of the D genome in conferring resistance to Fusarium head blight in spring wheat. Euphytica, 114, 181-186.

Gilbert, J. and Tekauz, A. 2000. Review: Recent developments in research on fusarium head blight of wheat in Canada. Can. J. Plant Pathol., 22, 1-8.

Gilbert, J., Abramson, D., McCallum, B. and Clear, R. 2001. Comparison of Canadian *Fusarium graminearum* isolates for aggressiveness, vegetative compatibility, and production of ergosterol and mycotoxins. Mycopathologia, 153, 209-215.

Gilbert, J., Woods, S.M. and Kromer, U. 2008. Germination of ascospores of *Gibberella zeae* after exposure to various levels of relative humidity and temperature. J. Phytopathol., 98, 504-508.

Gilbert, J., Clear, R. M., Ward, T.J., Gaba, D., Tekauz, A., Turkington, S., Woods, S., Nowiki, T. and O'Donnell, K. 2010. Relative aggressiveness and production of 3- or 15-acetyl deoxynivalenol and deoxynivalenol by *Fusarium graminearum* in spring wheat. Can. J. Plant Pathol., 32, 146-152.

Gilchrist, L., Rajaram, S., Mujeeb-kazi, A., van Ginkel, M., Vivar, H. and Pfeiffer, W. 1997. *Fusarium* scab screening program at CIMMYT. In Fusarium Head Scab: Global Status and Future Prospects. *Edited by* H. J. Dubin, L. Gilchrist, J. Reeves, and A. McNab. CIMMYT, Mexico D.F., Mexico. Pp. 7-12.196

Gildow, F.E. 1990. Current status of barley dwarf in the United States: A regional situation report. In P.A. Burnett (ed.) World perspective on barley yellow dwarf Proc. Intl. Workshop. Udine. Italy. 6 – 11 July 1987.

Gildow, F.E. 1999. Luteovirus transmission and mechanisms regulating vector specificity.Smith HG, Barker H, eds. *The Luteoviridae*.Wallingford: CAB International Publ. Pp.88–112.

Gill, C. C. 1975. An epidemic of barley yellow dwarf in Manitoba and Saskatchewan in 1974. Plant Dis. Rept., 59, 814-818

Golemboski, D.B., Lomonossoff, G.P. and Zaitlin, M. 1990. Plants transformed with a tobacco mosaic virus nonstructural gene sequence are resistant to the virus. Proc. Natl. Acad. Sci. USA, 87, 6311-6315.

Gordon, W.L. 1933. Species of *Fusarium* isolated from field crops in Manitoba. Vol. 2, Proceedings of the World's Grain Exhibition and Conference, Canada.

Gordon, W.L. 1944. The occurrence of *Fusarium* species in Canada. I. Species of Fusarium isolated from farm samples species of cereal seed in Manitoba. Can. J. Cereal Res., 22, 282-286.

Gordon, W.L. 1952. The occurrence of *Fusarium* species in Canada. II. Prevalence and taxonomy of *Fusarium* species in cereal seed. Can. J. Bot., 30, 209-251.

Goulart, L.R., H.Q. Ohm. and Foster, J.E. 1989. Barley yellow dwarf symptom severity in oat affected by plant growth stage at infection and plot type. Crop Sci. 29,1412-1416.

Gray, S. M., A.G. Power, D.M. Smith, A.J. Seaman, and Altman, N.S. 1991. Aphid transmission of barley yellow dwarf: virus acquisition access periods and virus concentration requirements. J. Phytopathol., 81,539-545.

Gray, S.M. and Banerjee, N. 1999. Mechanisms of arthropod transmission of plant and animal viruses. Microb. Mol. Biol. Reviews, 63, 128-148.

Gray, S. and Gildow, F.E. 2003. Luteovirus-aphid Interactions. Annu. Rev. Phytopatholo., 41, 539-566.

Groth, J. V., Ozmon, E. A. and Busch, R. H. 1999. Repeatability and relationship of incidence and severity measures of scab of wheat caused by *Fusarium graminearum* in inoculated nurseries. Plant Dis., 83, 1033-1038.

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

Haber, S. 1990. Situation review of barley yellow dwarf in Canada. . Pages 7-10 In World perspective on barley yellow dwarf. Proc. Intl. Workshop. Udine. Italy. 6 – 11 July 1987.

Haber, S. 1995. Interactions of barley yellow dwarf viruses: cross-protection and interactions with other pathogens and with abiotic factors. In Barley Yellow Dwarf: 40 years of progress. Eds C.J. D'Arcy and P.A. Burnett. Am. Phytopath. Soc. Press, St-Paul, Minn., USA . Pp 145-161.

Haber, S., R.I.H. Mckenzie, and A. Comeau. 1997. Barley yellow dwarf virus (BYDV)

tolerance in wheat as a predictor of yield. Can. J. Plant Pathol., 19, 324.

Haber, S. and Comeau, A. 1998. "Quantitative indoor assay"(QI assay), a tool to identify select and develop cereal germplasm that performs well under barley yellow dwarf (BYDV) disease pressure and under other biotic and abiotic stress. In Int. Congr. Plant Pathol. Edinburgh. Pp. 9-16.

Halbert, S. and D. Voegtlin. 1995. Biology and taxonomy of vectors of barley yellow dwarf viruses. In Barley Yellow Dwarf: 40 years of progress. Eds C.J. D'Arcy and P.A. Burnett. Am. Phytopath. Soc. Press, St-Paul, Minn., USA . Pp 217-258.

Hall, M.D., and van Sanford, D.A. 2003. Diallel analysis of Fusarium head blight resistance in soft red winter wheat. Crop Sci., 43, 1663-1670.

Halsted, B.E. and Gill, C.C. 1971. Transmission of barley yellow dwarf virus by different stages of the greenbug. J. Phytopathol., 61, 749-750.

Hammond, J. and Kamo, K.K. 1995. Effective resistance to potyvirus infection conferred by expression of antisense RNA in transgenic plants. Mol. Plant Microbe Interact., 5, 674-682.

Han, F. and Fedak, G. 2003. Molecular characterization of partial amphiploids from *Triticum durum* x tetraploid *Thinopyrum elongatum* as novel sources of resistance to

wheat fusarium head blight. In Proceedings of 10th International Wheat Genetics Symposium, Paestum, Italy. pp. 1148-1150.

Henriksen, B. and Elen, O. 2005. Natural *Fusarium* grain infection level in wheat , barley and oat after early application of fungicides and herbicides. J. Phytopathol., 153, 214-220.

Henry, M., Posadas G., Segura J. and Rajaram, S. 2002. Evaluating resistence to BYDV-PAV, BYDV-MAV, and CYDV-RPV in *Thinopyrum intermedium*-derived wheat lines. In Henry M., McNab A. (eds): Proc. Int. Symp. Barley Yellow Dwarf Disease: Recent Advances and Future Strategies. September 1–5, 2002, El Batan, Texcoco, 64–66.

Hewings, A.D. and Eastman, C.E. 1995. Epidemiology of barley yellow dwarf in North America. In Barley Yellow Dwarf: 40 years of progress. Eds C.J. D'Arcy and P.A. Burnett. Am. Phytopath. Soc. Press, St-Paul, Minn., USA. Pp. 75–106.

Holzapfel J., Voss H.-H., Miedaner T., Korzun V., Haberle J., Schweizer G., Mohler V., Zimmer-mann G. and Hartl, L. 2008. Inheritance of resistance to Fusarium head blight in three European winter wheat populations. Theor. App. Genet., 117,1119-1128.

Humphreys, D.G., Brown, P.D., Fox, S.L., Townley-Smith, T.F. and Gilbert, J. 2001. Breeding for Fusarium head blight resistance in spring wheat (*Triticum aestivum* L.*)* 2001 National Fusarium head blight forum proceedings. Erlanger, KY. December 8-10.

Inch, S.A. and Gilbert, J. 2003. Survival of *Gibberella zeae* in *Fusarium*-damaged wheat kernels. Plant Dis., 87, 282-287.

Inch, S., Fernando, W.D.G. and Gilbert, J. 2005. Seasonal and daily variation in the airborne concentration of *Gibberella zeae* (Schw.) Petch spores in Manitoba. Can. J. Plant Pathol., 27, 357-363.

Inch, S. A. 2009. Screening and evaluation of potential fungal antagonists for the biological control of fusarium head blight incited by *Gibberella zeae.* PhD Thesis, University of Manitoba, Canada.

Ittu, M., Saulescu, N.N., Ittu, G. and Moldovan, M. 2002. Approaches in breeding wheat for resistance to Fusarium head blight in Romania. Petria, 12, 67-72.

Ittu, M., Saulescu, N.N., Ittu, G. and Ciuca, M. 2008. Contributions to make modern Romanian bread winter wheat more resistant to FHB. Cereal Res Commu., 36B, 73-76.

Irwin, M. E. and Thresh, J.M. 1990. Epidemiology of barley yellow dwarf: A study in Ecological Complexity. Annu. Rev. Phytopathol., 28, 393-424.

Jahier, J., Chain, F., Barloy, D., Tanguy, A.M., Lemonine, J., Riault, G., Margale, E., Trottet, A. and Jacquot, E. 2009. Effect of combining two genes for partial resistance to

barley yellow dwarf virus-PAV (BYDV-PAV) derived from *Thinopyrum intermedium* in wheat. Eur. J. Plant Pathol., 58, 807 – 814.

Jensen, S.G. 1968. Photosynthesis, respiration and other physiological relationships in barley infected with the barley yellow dwarf virus. J. Phytopathol., 58, 204-208.

Jensen, S.G. 1970. Metabolism and carbohydrate composition in barley yellow dwarf virus-infected wheat. J. Phythopathol., 62, 587-592.

Jensen, S.G., Fitzgerald P. J. and J.R. Thysell. 1971. Physiology and field performance of wheat infected with barley yellow dwarf virus. Crop Sci., 11, 775-780.

Jensen, S.G. and van Sambeek, J.W. 1972. Differential effects of barley yellow dwarf virus on the physiology of tisues of hard red spring wheat. J. Phytopathol., 62, 290-293.

Jensen, S.G. and D'Arcy, J.C. 1995. Effects of barley yellow dwarf on host plants. In Barley yellow dwarf: 40 years of progress. Eds C.J. D'Arcy and P.A. Burnett. Am. Phytopath. Soc. Press, St-Paul, Minn., USA. Pp 55- 74.

Jiang, G.L., Dong, Y.H., Lewis, J.M., Silver, L. and Ward, R.W. 2006. Characterization of resistance to *Fusarium graminearum* in a recombinant inbred line population of wheat: Resistance to fungal spread, mycotoxin accumulation, and grain yield loss, and trait relationships. Crop SCi., 46, 2590-2597.

Jimenez-Martinez, E.S., Bosque-Perez, N. A., Berger, P.H., R.S. Zemetra, Ding, H. and Eigenbrode, S. 2004. Volatile cues influence the response of *Rhopalosiphum padi* (homoptera: Aphididae) to Barley yellow dwarf virus-infected transgenic and untransformed wheat. Environ. Entomol., 33, 1207-1216.

Kang, Z and H. Buchenauer. 2000a. Cytology and ultrastructure of the infection of wheat spikes by *Fusarium culmorum*. Mycol. Res., 104, 1083-1093.

Kang, Z and H. Buchenauer. 2000b. Ultrastructural and immunocytochemical investigation of pathogen development and host responses in resistant and susceptible wheat spikes infected by *Fusarium culmorum*. Physiol. Mol Plant Pathol., 57, 255-268.

Kimura, M., Kaneko, I., Komiyama, M., Takatsuki, A., Koshino, H., Yoneyama, K., and Yamaguchi, I. 1998. Trichothecene 3-*O*-acetyltransferase protects both the producing organism and transformed yeast from related mycotoxins. Cloning and characterization of *Tri101*. J. Biol. Chem., 273, 1654-1661.

Klahr, A., Zimmermann, G., Wenzel, G. and Mohler, V. 2007. Effects of environment, disease progress, plant height and heading date on the detection of QTLs for resistance to Fusarium head blight in a European winter wheat cross. Euphytica 154, 17-28.203.

Knox, R. E., J. M. Clark and R. M. DePauw. 2000. Dicamba and growth condition effects on doubled haploid production in durum wheat crossed with maize. Plant Breed., 119, 289-298.

Koch, N. and Huth, W. 1997. Interaction of barley yellow dwarf virus and *fusarium culmorum* (W.G. Sm) Sacc. In winter wheat. J phytopathol., 145, 425-428.

Kolb, F. L., Bai, G. H., Muehlbauer, G. J., Anderson, J. A., Smith, K. P. and Fedak, G. 2001. Host plant resistance genes for Fusarium head blight: mapping and manipulation with molecular markers. Crop Sci., 41, 611-619.

Kosova, K., Chrpova, J. and Sip, V. 2008. Recent advances in breeding of cereals for resistance to barley yellow dwarf virus – a review. Czech. J. Genet. Plant Breed., 44, 1-10.

Kosova, K., Chrpova, J. and Sip, V. 2009. Cereal resistance to Fusarium head blight and possibilities of its improvement through breeding – a review. Czech. J. Genet. Plant Breeding, 45, 87-105.

Lacey, J., Bateman, G. L. and Mirocha, C. J. 1999. Effects of infection time and moisture on development of ear blight and deoxynivalenol production by *Fusarium* spp. in wheat. Ann. Appl. Biol., 134, 277-283.

Lamboy, J.S., Lamboy, W.F., D'Arcy, C.J. and Shaw, P.D. 1991. Differences in sucrose stress and responses to salicylic acid in tolerant and susceptible sister oat lines inoculated with barley yellow dwarf virus strain MAV-NY. J. Phytopathol., 81, 1 154 -1155.

Lapidot, M., Gafny, R., Ding, B., Wolf, S., Lucas, W.J. and Beachy, R.N. 1993. A dysfunctional movement protein of tobacco mosaic virus that partially modifies the plasmodesmata and limits virus spread in transgenic plants. Plant J., 2, 959-970.

Larkin, P.J., Young, M.J., Gerlach, W.L. and Waterhouse, P.M. 1991. The Yd2 resistance to barley yellow dwarf virus is effective in barley plants but not in their leaf protoplasts. Ann. Appl. Biol., 118, 115-125.

Laurie, D.A. and Reymondie, S. 1991. High frequencies of fertilization and haploid seedling production in crosses between commercial haploid wheat varieties and maize. Plant Breed., 106, 182- 189.

Lister, R.M. and R. Ranieri. 1995. Distribution and economic importance of barley yellow dwarf. In C.J. D'Arcy and P.A. Burnett (ed.) Barley yellow dwarf: 40 years of progress. Am. Phytopath. Soc. Press, St-Paul, Minn., USA. Pp 29–53.

Liu, S. and Anderson, J.A. 2003. Targeted molecular mapping of a major wheat QTL for Fusarium head blight resistance using wheat ESTs and synteny with rice. Genome, 46, 817 - 823.

Liu, G., Liu, D., Qi, L., Zhang, P., Friebe, B. and Gill, B.S. 2005. Development and characterization of wheat- *Leymus racemosus* translocation lines with resistance to Fusarium Head Blight. Theor. Appl. Genet., 111, 941-948.

Liu S., Abate Z.A., and Mc Kendry A.L. 2005. Inheritance of Fusarium head blight resistance in the soft red winter wheat Ernie. Theor. Appl. Genet., 110, 454–461.

Liu, S., Zhang, X., Pumphrey, M.O., Stack, R.W., Gill, B.S. and Anderson, J.A. 2006. Complex microcolinearity among wheat, rice, and barley revealed by fine mapping of the genomic region harboring a major QTL for resistance to Fusarium head blight in wheat. Funct. Integr. Genomics, 6, 83-89.

Liu, S., Abate, Z.A., Lu, H., Musket, T., Davis, G.L. and McKendry, A.L. 2007. QTL associated with Fusarium head blight resistance in the soft red winter wheat Ernie. Theor. Appl. Genet., 115, 417-427.

Liu. Y. and Buchenauer, H. 2005. Interaction between barley yellow dwarf virus and *Fusarium* spp. affecting development of Fusarium head blight of wheat. Eur. J.Plant Pathol., 113,283-295.

Ma, H.X., Bai, G.H., Zhang, X. and Lu, W.Z. 2006a. Main effects, epistasis, and environmental interactions of quantitative trait loci for fusarium head blight resistance in a recombinant inbred population. J. Phytopathol., 96, 534-541.

Maldonado-Ramirez, S.L., Schmale III, D.G., Shields, E.J. and Bergstrom, G.C. 2005. The relative abundance of viable spores of *Gibberella zeae* in the planetary boundary layer suggests the role of long distance transport in regional epidemics of Fusarium head blight. Agric. Forest Meteorol., 132, 20-27.

Manns T.F. 1909. The Blade Blight of Oats – a Bacterial Disease. Ohio Agr. Expt. Sta. Bul. 210

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

Markkula, M. and Laurema, S. 1964. Changes in the concentration of free amino acids in plants induced by virus diseases and the reproduction of aphids. Annals Agriculturae Fenniae 3, 265-271.

Martin, R.R. and D'Arcy,C.J. 1995. Taxonomy of barley yellow dwarf viruses. In Barley yellow Dwarf: 40 years of progress. Eds C.J. D'Arcy and P.A. Burnett. Am. Phytopath. Soc. Press, St-Paul, Minn., USA. Pp 203-214.

Mayo, M.A. and Ziegler-Graf, V. 1996. Molecular biology of luteoviruses. Adv. Virus Res., 46, 413–60.

McCarthy, P.L., Hansen, J., Shiel, P.J., Zemetra, R.S.,Wyatt, S.D and Berger P.H. 1996. Coat protein-mediated resistance to wheat streak mosaic and barley yellow dwarf viruses in soft white winter wheats. Int. Congr. Virol., Jerusalem, 10th, p. 184.

McCartney, C.A., Somers, D.J., Fedak, G. and Cao, W. 2004. Haplotype diversity at fusarium head blight resistance QTLs in wheat. Theor. Appl. Genet., 109, 261-271.

McCartney, C.A., Somers, D.J., Fedak, G., DePauw, R.M., Thomas, J., Fox, S.L., Humphreys, D.G., Lukow, O., Savard, M.E., McCallum, B.D., Gilbert, J. and Cao, W. 2007. The evaluation of FHB resistance QTLs introgressed into elite Canadian spring wheat germplasm. Mol. Breed., 20, 209-221.

McGrath, P.F., Vincent, J.R., Lei,C.H., Pawlowski,W.P., Torbert, K.A., Gu,W., Kaeppler, H.F., Wan,Y., Lemaux, P.G., Rines, H.R., Somers, D.A., Larkins, B.A. and Lister, R.M. 1997. Coat protein-mediated resistance to isolates of barley yellow dwarf in oats and barley. Eur. J. of Plant Pathol., 103, 695-710.

McGuire, P.E. 1984. Status of an attempt to transfer the barley yellow dwarf virus resistance gene *Yd2* of barley to hexaploid wheat. Pages 113-119 in Barley yellow dwarf, a proceedings of the workshop. P.A. Burnett ed. CIMMYT. Mexico. D.F. Mexico.

McIntosh, R.A. 1998. Breeding wheat for resistance to biotic stresses. In, Braun, H. J., Altay, F., Kronstad, W.E., Beniwal, S.P.S. (eds): Wheat: Prospects for global improvement. Kulwer Academy Publ., Amsterdam, 71 – 86.

McKendry, A.L., Tague, D. N., Wright, R. L., Tremain, J. A. and Conley, S. P. 2005. Registration of 'Truman' wheat. Crop Sci. 45, 421-423.

McKirdy S.J. and Jones, R.A.C. 1996. Use of imidacloprid and newer generation synthetic pyrethroids to control the spread of barley yellow dwarf luteovirus in cereals. Plant Dis., 80, 895–901.

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

McMullen. M., Halley. S., Schatz, B., Meyer. S., Jordahl. J. and Ransom, J. 2008. Integrated strategies for Fusarium head blight management in the United States. Cereal Res. Commu., Vol 36 (Suppl B), 563-568.

Medina-Ortega, K.J., Bosque-Perez, N., Ngumbi, A., Edgardo, E., Jimenez-Martinez, S. and Eigenborde, S.D. 2009. *Rhopalosiphum padi* (Hemiptera: Aphididae) responses to volatile cues from barley yellow dwarf virus-infected wheat. Entomol. Society of America, 836-845.

Mesterhazy, A. 1995. Types and components of resistance to Fusarium head blight of wheat. Plant Breed., 114, 377-386.

Mesterhazy, A. 1997. Breeding for resistance to Fusarium head blight of wheat. Pages 79-85 In Fusarium Head Scab; Global Status and Future Propects. Dubin, H. J., Gilchrist, L., Reeves, J., and McNab, A., eds. CIMMYT, Mexico D.F., Mexico.

Mesterhazy, A. 2002. Role of deoxynivalenol in aggressiveness of *Fusarium graminearum* and *F. culmorum* and in resistance to Fusarium head blight. Eur. J. Plant Pathol., 2002, Volume 108, 7, 675-684.

Mesterhazy, A. 2003. Breeding wheat for Fusarium head blight resistance in Europe. Pages 211-240 In Fusarium Head Blight of Wheat and Barley. Leonard, K. J. and Bushnell, W. R., eds. APS Press, St Paul, MN, USA.

Miedaner, T. 1997. Breeding wheat and rye for resistance to *Fusarium* diseases. Plant Breed., 116, 201-220

Miedaner, T., Gang, G. and Geiger, H.H. 1996. Quantitative-genetic basis of aggressiveness of 42 isolates of *Fusarium culmorum* for winter rye head blight. Plant Dis., 80, 500-504.

Miedaner, T., Schilling, A.G. and Geiger, H.H. 2001. Molecular genetic diversity and variation for aggressiveness in populations of *Fusarium graminearum* and *Fusarium culmorum* sampled from wheat fields in different countries. J. Phytopathol., 149, 641-648.

Miedaner, T., Wilde, F., Steiner, B., Buerstmayr, H., Korzun, V. and Ebmeyer, E. 2006. Stacking quantitative trait loci (QTL) for Fusarium head blight resistance from non-adapted sources in a European elite spring wheat background and assessing their effects

on deoxynivalenol (DON) content and disease severity. Theor. Appl. Genet., 112, 562-569.

Miller, J.D. and Young, J.C. 1985. Deoxynivalenol in an experimental *Fusarium graminearum* infection of wheat. Can. J. Plant Pathol., 7, 132-134.

Miller, W. A., Waterhouse, P. A . and Gerlach, W. L. 1987. Sequence and organization of barley yellow dwarf virus genomic RNA . Nucleic Acids Res. 16, 97-111.

Miller, J.D. 1989. Effects of *Fusarium graminearum* metabolites on wheat cells. Pages 449-452 In Phytotoxins and plant Pathogenesis. Graniti, A., Durbin, R. D., and Ballio, A., Berlin.

Miller, W.A. and Rasochova. L A. 1997. Barley yellow dwarf viruses. Annu. Rev. Phytopathol., 35, 167–90.

Miller, J. D., Stack, R. W. and Joppa, L. R. 1998. Evaluation of *Triticum turgidum* L. var. *dicoccoides* for resistance to Fusarium head blight and stem rust. Pp 292-293 In Proceedings of 9th International Wheat Genetics Symposium. Saskatoon, Saskatchewan, Canada.

Miller, W. A.and Liu, S. 2002. CP-RTD genes of BYDV-PAV isolates affect aphid transmission efficiency and symptom severity. J. Phytopathol., 92, S48.

Mirocha, C. J., Abbas, H. K., Windels, C. E. and Xie, W. 1989. Variation in deoxynivalenol, 15-acetyldeoxynivalenol, 3-acetyldeoxynivalenol, and zearalenone production by *Fusarium graminearum* isolates. Appl. Environ. Microbiol., 55, 1315-1316.

Mohan, M., Nair, S., Bhagwat, A., Krishna, T.G., Yano, M., Bhatia, C.R. and Saski, T. 1997. Genome mapping, molecular markers and marker-assisted selection in crop plants. Mol. Breed., 3, 87-103.

Montllor, C.B. and Gildow, F.E. 1986. Feeding responses of two grain aphids to barley yellow dwarf virus-infected oats. Entomol. Exp. Appl., 42, 63-69.

Mowry, T.M. 1994 Russian wheat aphid (Homoptera: Aphididae) survival and fecundity on barley yellow dwarf virus infected wheat resistant and susceptible to the aphid. Environ. Entomol., 23, 326-330.

Muthomi, J.W., Schutze, A., Dehne, H.W., Mutitu, E.W., and Oerke, E.C. 2000. Characterization of *Fusarium culmorum* isolates by mycotoxin production and aggressiveness to winter wheat. Z. Pflanzenk. Pflanzen., 107, 113-123.

Nelson, P.E., Toussoun, T.A. and Marasas, W.F.O. 1983. *Fusarium* Species: An illustrated manual for identification. The Pennsylvania State University Press, University Park, IL, USA.

O'Donnell, K., Kistler, H.C., Tacke, B.K., and Casper, H.H. 2000. Gene genealogies reveal global phylogeographic structure and reproductive isolation among lineages of *Fusarium graminearum*, the fungus causing wheat scab. Proc. Natl. Acad. Sci. USA, 97, 7905-7910.

O'Donnell, K., Ward, T.J., Geiser, D.M., Kistler, H.C., and Aoki, T. 2004. Genealogical concordance between the mating type locus and seven other nuclear genes supports formal recognition of nine phylogenetically distinct species within the *Fusarium graminearum* clade. Fungal Genet. Biol., 41, 600-623.

O'Donnell, K., Ward, T.J., Aberra, D., Kistler, H.C., Aoki, T., Orwig, N., Kimura, M., Bjornstad, A., and Klemsdal, S.S. 2008. Multilocus genotyping and molecular phylogenetics resolve a novel head blight pathogen within the *Fusarium graminearum* species complex from Ethiopia. Fungal Genet. Biol., 45, 1514-1522.

Oliver, R.E., Stack, R.W., Miller, J.D., and Cai, X. 2007. Reaction of wild emmer wheat accessions to Fusarium head blight. Crop Sci., 47, 893-899.

Oswald, J.W. and Houston, B.R. 1951. A new virus disease of cereals, transmissible by aphids. Plant Dis. Rept., 35, 471-475.

Oswald, J.W. and Houston, B.R. 1953. Host range and epiphytology of the cereal yellow dwarf disease. J. Phytopathol., 43, 309-313.

Paillard, S., Schnurbusch, T., Tiwari, R., Messmer, M., Winzeler, M., Keller, B., and Schachermayr, G. 2004. QTL analysis of resistance to Fusarium head blight in Swiss winter wheat (*Triticum aestivum* L.). Theor. Appl. Genet., 109, 323-332.

Paliwal, Y. C. and Andrews, C. J. 1989. Barley yellow dwarf virus-host plant interactions affecting winter stress tolerance in cereals. In World Perspectives on Barley Yellow Dwarf International Workshop, Udine, Italy. Pp. 313-20.

Parry, D. W., Jenkinson, P. and McLeod, L. 1995. Fusarium ear blight (scab) in small grains-a review. Plant Pathol., 44, 207-238.

Paul, P.A., Lipps, P.E., Hershman, D.E., McMullen, M.P., Draper, M.A. and Madden, L.V. 2007. A quantitative review of tebuconazole effect on Fusarium head blight and deoxynivalenol content in wheat. J. Phytopathol., 97, 211-220.

Paulitz, T.C. 1996. Diurnal release of ascospores by *Gibberella zeae* in inoculated wheat plots. Plant Dis., 80, 674-678.

Pearce, R.B., Strange, R.N. and Smith, H. 1976. Glycine –betane and choline in wheat: Distribution and relation to infection by *Fusarium graminearum*. Phytochemistry, 15, 953.

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

Powell, A.P., Nelson, R.S., Hoffmann, B. N., Rogers, S.G., Farley, R.T. and Beachy, R.N. 1986. Delay of disease development in transgenic plants that express the tobacco mosaic virus coat protein gene. Science, 232, 738-743.

Power, A.G., Seaman, A.J. and Gray, S.M. 1991. Aphid transmission of barley yellow dwarf virus: inoculation access periods and epidemiological implications . J. phytopathol., 81, 545-548.

Power A.G. and Gray SM. 1995. Aphid transmission of barley yellow dwarf viruses: Interactions between viruses, vectors, and host plants. In Barley Yellow Dwarf: 40 years of progress. Eds C.J. D'Arcy and P.A. Burnett American Phytopathol. Society. Pp 259–291. St Paul MN, APS Press.

Prelusky, D.B. 1994. Residues in food products of animal origin. In Miller JD, Trenholm HL, editors. Mycotoxins in grain – Compounds other than aflatoxin. Pp 405-19.

Proctor, R. H., Hohn, T. M., and McCormick, S. P. 1995. Reduced virulence of *Gibberella zeae* caused by disruption of a trichothecene toxin biosynthetic gene. Mol. Plant-Microbe Interact., 8, 593-601.

Pronk,M.E.J., Schothorst, R.C. and van Egmond H.P. 2002. Toxicology and occurrence of nivalenol, fusarenon X, diacetoxyscirpenol, neosolaniol and 3- and 15-acetyldeoxynivalenol: a review of six Trichothecenes. RIVM Report, 388802024.

Pugh, G.W., Johann, H., and Dickson, J.D. 1933. Factors affecting infection of wheat heads by *Gibberella saubinetii*. J. Agric. Res., 46, 771-797.

Pugsley, A. T. 1971. A genetic analysis of the spring-winter habit of growth in wheat. Aust. J. Agric. Res., 22, 21-31.

Pugsley, A. T.1972. Additional genes inhibiting winter habit in wheat. Euphytica, 21, 547-552.

Purss, G.S. 1969. The relationship between strains of *Fusarium graminearum* Schwabe causing crown rot of various gramineous hosts and stalk rot of maize in Queensland. Aust. J. Agric. Res., 20, 257-264.

Purss, G.S. 1971. Pathogenic specialization in *Fusarium graminearum*. Aust. J. Agric. Res., 22, 553-561.

Qualset, C.O., Williams, J.C. Topcu, M.A. and Vogt, H.E. 1973. The barley yellow dwarf virus in wheat: importance, sources of resistance and heritability. Pages 465-470. In proceedings of the fourth international wheat genetics symposium. Missouri Agricultural Experimental Station, Columbia, MO, U.S. A.

Quiroz, A. J. and Niemeyer, H.M. 1998. Olfactometer-assessed responses of aphid *Rhopalosiphum padi* to wheat and oat volatiles. J. Chem Ecol. 24,113-124.

Rasmusson D.C., and Schaller, C.W. 1959. The inheritance of resistance in barley to barley yellow dwarf virus. J. Agron., 54, 661-664.

Rhodes, J.D., Croghan, P.C. and Dixon, A.F.G. 1996. Uptake, excretion and respiration of sucrose and amino acids by the pea aphid *Acyrthosiphon pisum*. J. Expt. Biology, 199, 1269-1276.

Riedell, W.E., Kieckhefer, R.W., Haley, S.D., Langham, M.A.C. and Evenson, P.D. 1999. Winter wheat responses to bird cherry-oat aphids and barley yellow dwarf virus infection. Crop Sci., 39, 158-163.

Rieseberg, L.H., Widmer, A., Arntz, M. A. and Burke, B. 2003. The genetic architecture necessary for transgressive segregation is common in both natural and domesticated populations. Phil. Trans. R. Soc. Lond. B. 358, 1141-1147.

Rochow, W. F. 1958. The role of aphids in vector-specificity of barley yellow dwarf virus. Plant Dis. Rept. 42, 905-8.

Rochow, W. F., Jedlinski, H., Coon, B. F. and Murphy, H. C. 1965. Variation in barley yellow dwarf of oats in nature. Plant Dis. Rep., 49, 692-95.

Royer, T.A., L Giles, K., Nyamanzi, T., Hunger, R.M. Krenzer, E.G., Elliott Kindler, N.C. and Payton, M. 2005. Economic evaluation of the effects of planting dates of application rate of imidacloprid for management of cereal aphids and barley yellow dwarf on winter wheat. J. Econ. Entomol., 98, 95-102.

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

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

Salas, B., Steffenson, B.J., Casper, H.H., Tracke, B., Prom, L.K., Fetch, T.G. Jr. and Schwarz, P.B. 1999. *Fusarium* species pathogenic to barley and their associated mycotoxins. Plant Dis., 83, 667-674.

Schaafsma, A.W., Tamburic-Ilincic, L. and Hooker, D.C. 2005. Effect of previous crop, tillage, field size, adjacent crop, and sampling direction on airborne propagules of

Gibberella zeae/Fusarium graminearum, fusarium head blight severity, and deoxynivalenol accumulation in winter wheat. Can. J. Plant Pathol., 27, 217–224.

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

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

Schroeder, H. W. and Christensen, J. J. 1963. Factors affecting resistance of wheat to scab caused by *Gibberella zeae*. J. Phytopathol., 53, 831-838.

Scott, D.H. 1968. Effect of barley yellow dwarf virus infection on the development of root rot caused by *Cochliobolus sativus* in *Avena sativa* and *Triticum durum*. Ph. D. Thesis, .University of Illinois, Urbana. USA.

Scott, P.M. 1997. Multi-year monitoring of Canadian grains and grain-based foods for trichothecenes and zearalenone. Food Additives Contamin, 14, 333-339.

Seed Manitoba 2011. Variety Selection and growers' source guide. Manitoba Cooperator, Winnipeg, MB. Published 2010 Pp 122.

Seo, J.-A., Kim, J.C., Lee, D.H. and Lee, Y.W. 1996. Variation in 8- ketotrichothecenes and zearalenone production by *Fusarium graminearum* isolates from corn and barley in Korea. Mycopathologia, 134, 31-37.

Shaner, G. and Buechley, G. 2000. Sampling spores of *Fusarium graminearum*. In Proc. Of 2000 National Fusarium Head Blight Forum, Erlanger, KY, dec 10-12, Michigan State Univ., pp.182-186.

Shaner, G.E. 2003. Epidemiology of Fusarium head blight of small grain cereals in North America. Pages 84-119. In Fusarium Head Blight of Wheat and Barley. Leonard, K. J. and Bushnell, W. R., eds. APS Press, St Paul, MN, USA.

Sharma, H., Ohm, H., Goulard, L., Lister, R., Appels, R. and Benlhabib O. 1995. Introgression and characterization of barley yellow dwarf virus resistance from *Thinopyrum intermedium* into wheat. Genome, 38, 406–413.

Shen, X., Ittu, M., and Ohm, H.W. 2003. Quantitative trait loci conditioning resistance to Fusarium head blight in wheat line F201R. Crop Sci., 43, 850-857.

Singh, R., Burnett, P., Albarran M. and Sanjaya, R. 1993. *Bdv1*: a gene for tolerance to barley yellow dwarf virus in bread wheats. Crop Sci., 33, 231–234.

Singh, R.P., Ma, H. and Rajaram, S. 1995. Genetic analysis of resistance to scab in spring wheat cultivar Frontana. Plant Dis., 79, 238-240.

Sip V., Bartos P., Chrpova J., Hanzalova A., Sirlova L., Sarova J., Dumalasova V., Cejka L., Hanisova A., Bobkova L., Bizova I. and Horcicka P. 2005. Theoretical bases and sources for breeding wheat for combined disease resistance. Czech J. Genet. and Plant Breed., 41, 127-143.

Sip V., Chrpova J., Vacke J. and Ovesna J. 2004. Possibility of exploiting the *Yd2* resistance to BYDV in spring barley breeding. Plant Breed., 123, 24-29.

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

Somers, D.J., Isaac, P. and Edwards, K. 2004. A high-density microsatellite consensus map for bread wheat (*Triticum aestivum* L.). Theoretical & Applied Genetics, 109, 1105-1114

SLYKHUIS, J. T. 1956. The first record of BYDV in Canada from barley in Alberta in 1955. In Can. Plant Disease Survey 35th Ann. Rept. Pp 14.

Slykhuis, J . T . , Zillinsky, F . J . , Hannah, A. E . and Richards, W. R. 1959. Barley yellow dwarf virus on cereals in Ontario. Plant Dis. Rept., 43, 849-54.

Slykhuis, J. T. 1962. An International survey for virus diseases of grasses. Food and Agriculture Organization of the United Nations: Plant Protection Bulletien 10, 1-16.

Snijders, C.H.A. 1990b. Response to selection in F_2 generations of winter wheat for resistance to head blight caused by *Fusarium culmorum*. Euphytica, 50, 163-169.

Snijders, C.H.A. 1990c. Genetic variation for resistance to Fusarium head blight in bread wheat. Euphytica, 50, 171-179.

Snijders, C.H.A. and Perkowski, J. 1990. Effects of head blight caused by *Fusarium culmorum* on toxin content and weight of wheat kernels. J. Phytopathol., 80, 566-570.

Snijders, C.H.A. 1994. Breeding for resistance to Fusarium head blight in Wheat and maize. In Mycotoxins in grain: compounds other than aflatoxin. Edited by JD Miller and HL Trenholm. Eagan Press. St. Paul. Minn. Pp. 37-58.

Spanic, V. Cosic, J. Dvojkovic, K. Maric, S. Guberac, V. Dreznr, G. 2008. Effect of fungicide and Fusarium head blight treatment on grain yield of winter wheat. Cereal Res. Commu., 36, Suppl. 6, 299-300.

Stack, R. W. 1989. A comparison of inoculum potential of ascospore and conidia of *Gibberella zeae*. Can. J. Plant Pathol., 11, 137-142.

Stack, R.W., Elias, E.M., Fetch, J.M., Miller, J.D. and Joppa, L.R. 2002. Fusarium head blight reaction of Langdon durum-*Triticum dicoccoides* chromosome substitution lines. Crop Sci., 42, 637-642.

Stack, R.W. 2003. History of Fusarium head blight with emphasis on North America. Pages 1-34 In Fusarium Head Blight of Wheat and Barley. Leonard, K. J. and Bushnell, W. R., eds. APS Press, St Paul, MN, USA.

Stack, R. W. and McMullen, M. I. 1985. Head blighting potential of Fusarium species associated with spring wheat heads. Can. J. Plant Pathol. 7, 79-82.

Stack, R.W. 1999. Fusarium head blight: Return of an old problem. APSnet feature, May 1999 at http://www.apsnet.org/feature/FHB (accessed May 25, 2006)

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

Statistics Canada. 2009. http://www.statcan.ca (accessed April 10, 2010).

Steiner, B., Lemmens, M., Griesser, M., Scholz, U., Schondelmaier, J. and Buerstmayr, H. 2004. Molecular mapping of resistance to Fusarium head blight in the spring wheat cultivar Frontana. Theor. Appl. Genet., 109, 215-224.

Suenaga, K. and Nakajima, K. 1993. Variation in doubled haploid plants of wheat obtained through wheat (*Triticum aestivum* L.) x maize (*Zea mays* L.) crosses. Plant Breed., 111, 120-124.

Suneson C.A. 1955. Breeding for resistance to yellow dwarf virus in barley. J. Agron., 47, 283.

Sutton, J. 1982. Epidemiology of wheat head blight and maize ear rot caused by *Fusarium graminearum*. Can. J. Plant Pathol., 4, 195-209.

Teich, A.H., and Hamilton, J.R. 1985. Effect of cultural practices, soil phosphorus, potassium, and PH on the incidence of Fusarium head blight and deoxynivalenol levels in wheat. Appl. Environ. Microbiol., 49, 1429-1431.

Tekauz, A., McCallum, B., and Gilbert, J. 2000. Review: Fusarium head blight of barley in western Canada. Can. J. Plant Pathol., 22, 9-16.

Toth, B., Mesterhazy, A., Horvath, Z., Bartok, T., Varga, M., and Varga, J. 2005. Genetic variability of central European isolates of the *Fusarium graminearum* species Complex. Eur. J. Plant Pathol., 113, 35-45.

Tschanz, A. T., Horst, R. K., and Nelson, P. E. 1976. The effect of environment on sexual reproduction of *Gibberella zeae.* Mycologia, 68, 327-340.

van der Broek, L.J. and Gill, C.C. 1980. The median latent periods for three isolates of barley yellow dwarf virus in aphid vectors. J. Phytopathol., 70, 644–46.

van Deynze, A.E., Nelson, J.C., Yglesias, E.S., Harrington, S.E., Braga, D.P., McCouch S.R.. and Sorrells, M.E. 1995. Comparative mapping in grasses: Wheat relationships. Mol. Gen. Genet., 248, 744-754.

van Eeuwijk, F.A., Mesterhazy, A., Kling, C. I., Ruckenbauer, P., Saur, L., Buerstmayr, H., Lemmens, M., Keizer L. C. P., Maurin, N. and Snijders, C.H.A. 1995. Assessing non-specificity of resistance in wheat to head blight caused by inoculation with European strains of *Fusarium culmorum*, *F. graminearum* and *F. nivale* using a multiplicative model for interaction. Theor. Appl. Genet., 90, 221-228.

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

van Ginkel, M, and Henry, M. 2002. Breeding for BYDV tolerance/resistance in CIMMYT bread wheats targeted to developing countries, barley yellow dwarf disease. In Henry M. McNab A, eds. Barley yellow dwarf disease: Recent advances and future strategies. CIMMYT, Mexico, D.F. Mexico, 34-39.

van Regenmortel, M.H.V**.** 2000. Introduction to the species concept in virus taxonomy. In Virus Taxonomy: Seventh Report of the International Committee on the Taxonomy of Viruses van Regenmortel, M.H.V., Fauquest, C.M., Bishop, D.H.L., Carstens, E.B.,

Estes, M.K., Lemon, S.M., Maniloff, J., Mayo, M.A., McGeoch, D.J., Pringle, C.R. and Wickner, R.B., (eds), pp. 3–16. Academic Press, San Diego.

Veskrna, O., Chrpova, J., Sip, V., Sed lacek, T. and Horcicka, P. 2009. Reaction of wheat varieties to infection with barley yellow dwarf virus and prospects for resistance breeding. Czech J. Gen. Plant breed., vol 45 part 2, pages 45-56.

Waalwijk, C., Kastelein, P., de Vries, I., Kerenyi, Z., van der Lee, T., Hesselink, T., Kohl, J. and Kema, G. 2003. Major changes in *Fusarium* spp. in wheat in the Netherlands. Eur. J. Plant Pathol., 10, 743-754.

Waldron, B.L., Moreno-Sevilla, B., Anderson, J.A., Stack, R.W. and Frohberg, R.C. 1999. RFLP mapping of QTL for fusarium head blight resistance in wheat. Crop Sci., 39, 805-811.

Walker, S. L., Leath, S., Hagler, W. M. and Murphy, J. P. 2001. Variation among isolates of *Fusarium graminearum* associated with Fusarium head blight in North Carolina. Plant Dis., 85, 404-410.

Wang, Y.Z. and Miller, J.D. 1988. Effects of *Fusarium graminearum* metabolites on wheat tissue in relation to Fusarium head blight resistance. J. Phytopathol., 122, 118-125.

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

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

Waterhouse, P.M., Gildow, F.E. and Johnstone, G.R. 1988. Luteoviruses. No. 339 In : Descriptions of plant viruses. Commonw. Mycol. Inst. Assoc. Appl. Biol., Kew, Surrey, England.

Watson, M.A. and Mulligan, T. 1960. The manner of transmission of some barley yellow dwarf viruses by different aphid species. Ann. Appl. Biol., 48, 711-720.

Whitlow, L.W. and Hagler Jr,W.M. 2005. Mycotoxins in dairy cattle: Occurrence, toxicity, prevention and treatment. Proc. Southwest Nutr. Conf. pp. 124-138

Wilcoxson, R.D., Kommedahl, T., Ozmon, E.A. and Windels, C.E. 1988. Occurrence of *Fusarium* Species in scabby wheat from Minnesota and their pathogenicity to wheat. J. Phytopathol., 78, 586-589.

Wilcoxson, R.D., Bush, R.H. and Ozmon, E.A. 1992. Fusarium head blight resistance in spring wheat cultivars. Plant Dis., 76, 658-661.

Wilde, F., Korzun, V., Ebmeyer, E., Geiger, H. H. and Miedaner, T. 2007. Comparison of phenotypic and marker-based selection for Fusarium head blight resistance and DON content in spring wheat. Mol. Breed., 19, 357-370.

Wang, M.B., Abbot, D.C. and Waterhouse, P.M. 2000. A single copy of a virus-derived transgene encoding hairpin RNA gives immunity to barley yellow dwarf virus. Mol. Plant Pathol., 1, 347-356.

Wang, Y.Z. and Miller, J.D. 1988. Effects of *Fusarium graminearum* metabolites on wheat tissue in relation to Fusarium head blight resistance. J. Phytopathol., 122, 118-125.

Wong, L. S. L., Tekauz, A., Leisle, D., Abramson, D. and McKenzie, R. I. H. 1992. Prevalence, distribution, and importance of Fusarium head blight in wheat in Manitoba. Can. J. Plant Pathol. 14, 233-238.

Wright, S. 1968. Genetic and biometric foundations. Evaluation and the genetics of populations. A treatise in three volumes. Volume I: Genetics and biometric foundations. The University of Chicago Press. Chicago and London. P. 469.

Wu, A. B., Li, H. P., Zhao, C. S. and Liao, Y. C. 2005. Comparative pathogenicity of *Fusarium graminearum* isolates from China revealed by wheat coleoptile and floret inoculations. Mycopathologia 160, 75-83.

Yang, J., Bai, G. and Shaner, G.E. 2005a. Novel quantitative trait loci (QTL) for Fusarium head blight resistance in wheat cultivar Chokwang. Theor. Appl. Genet., 111, 1571-1579.

Yao, J., Ge, Y., Wang, S., Yao, G., Zhou, C. and Qian, C. 1997. Chromosomal location of genes for scab resistance in wheat cultivar Sumai 3. Acta Agron. Sin., 23, 450-453.

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

Yu, Y. J. 1982. Monosomic analysis for scab resistance and yield components in the wheat cultivar Sumai 3. Cereal Res. Commu,. 10, 185-189.

Zhang, X., Zhou, M., Ren, L., Bai, G., Ma, H., Scholten, O.E., Guo, P. and Lu, W. 2004. Molecular characterization of Fusarium head blight resistance from wheat variety Wangshuibai. Euphytica, 139, 59-64.

Zhang, Z., Xu, J., Xu, Q., Larkin P. and Xin, Z. 2004. Development of novel PCR markers linked to the BYDV resistance gene *Bdv2* useful in wheat for marker-assisted selection. Theor. Appl. Genet., 109, 433-439.

Zhang, M.Y., Xin, Z.Y., Cao, Y.P., Xu, J., Ning, D.X. and Chen, X, 2005. An elite winter wheat variety Lingkang 11 with multi-resistance and its cultivation skill. J. Triticeae Crops 25, 153.

Zhou, W., Kolb, F.L., Bai, G., Shaner, G. and Domier, L.L. 2002. Genetic analysis of scab resistance QTL in wheat with microsatellite and AFLP markers. Genome 45, 719-727.

Appendix

Appendix 1 Generation of Doubled haploid populations

Corn: Pollen donor

Maize (*Zea mays* L.) from the four-way cross, Seneca 60/Golden Bantam//Manitoba Sweet/Indian Flint corn, was planted in a growth cabinet each week to ensure a constant supply of pollen for doubled haploid (DH) production of wheat and oat. The corn plants were fertilized every week with a fertilizer solution of 20-20-20 (approx. 10 g/l) and 2g/L of ammonium sulphate (21-0-0). The plants were grown at 22°C light/20°C dark with a photoperiod of 16 hours, programmed at 7:00 am. Between one and two hours after the lights came on the pollen would start to shed (Campbell et all, 1998). The old anthers were removed by stripping off the tassels or shaking the plant in the morning. By removing all the old anthers, any new anthers that started to protrude were easily noticeable and a supply of fresh pollen was ensured. Fresh pollen was collected periodically at 30-min intervals by shaking pollen from tassels onto a sheet of paper (Figure A1.1).

Figure A1.1 Corn anthers ready for pollination

Parent Plants

F_1 seeds were generated from reciprocal crosses of wheat cultivars Roblin, Maringa and Wuhan (Table 3.3.1). Three seeds per cross were used to generate doubled haploid populations for this research. Seeds were germinated in Petri dishes lined with moistened filter paper. The seedlings were transplanted individually into 10" plastic pots containing two parts soil-less mix, one part sand and one part perlite. The plants were grown in a growth cabinet at 16°C light/15°C dark with a photoperiod of 16 hours. They were fertilized daily with a siphon mixer of a ratio 16:1 water to fertilizer (fertilizer water soluble NPK: 20-20-20, 200g/20L). Plants were sprayed with Pirliss (50% pirimicarb) at a rate of 1 g/l to control insect pests as and when required. At the time of emasculation and crossing Raid was used to control the pests.

Crossing

Emasculation

Wheat florets were emasculated 1–2 days before anthesis, when the anthers in the florets approximately 2/3 of the way up the spike started to change from a light green/yellow colour to a bright yellow colour and the stigma was fluffy. The awns were trimmed closed to the glumes. Central florets were discarded (Figure A1.2) and the anthers of the primary florets were removed with fine forceps starting at the bottom of the spike. Care was taken to minimize injury to the floral parts, in particular the stigma. The smaller top and bottom spikelets were removed. The emasculated spike was labeled with a crossing tag with date of emasculation, and then covered with a glassine bag and closed with a paper clip. The wheat heads were ready for pollination the next day.

Figure A1.2 Removal of centre floret

Pollination

Plump and fresh maize anthers were collected on a paper towel as they started to emerge from the corn tassel. The glassine bag was removed from the spike and the floret to be pollinated was selected. Using fine forceps to open the floret, maize pollen was dusted over the stigma. Once the entire spike was pollinated; the date of pollination was marked on the crossing tag and the glassine bag replaced. The crossing bag was left on the spike until the time of excision.

Hormone Spray

The day following the pollination, spikes were sprayed with a hormone dicamba (Sigma D-5417) solution (100 mg/L) prepared by dissolving 25 mg dicamba in 250 ml doubled distilled water. Although growth conditions, pollen source, method of handling plants and wheat genotype are important considerations, the type of hormone used is found to be the most significant factor. Dicamba has been found to improve the frequency of production of doubled haploid plants by increasing the number of successful caryopses generated for each emasculated spike, and for promoting germination of embryos to haploid plantlets (Knox et al., 2000). A fresh hormone solution was made weekly and was refrigerated at 4^0 C. The glassine bag was removed from the spike, and using a small hand-held pressurized sprayer, dicamba was liberally applied to the spike, (about 2 passes with the sprayer). The crossing tag was marked with the date of spraying and bag was replaced. The pollinated wheat plants were maintained in the growth cabinet at $24/20^0$ C light/dark with a 16 hour photoperiod until embryo rescue.

Caryopsis excision and embryo rescue

The developing caryopses were isolated from the florets sixteen days after pollination. The caryopses were carefully collected in a 10ml syringe and surface-sterilized with 70% ethanol for 40 seconds, followed by 10–15 ml of bleach (1% active chlorine) for 5 min, followed by two rinses in sterile water.

Caryopses were dissected under sterile conditions in a laminar flow hood. The white, somewhat translucent embryos were rescued and placed on B5 medium (Gamborg et al., 1968). They were incubated in the dark at 4^0 C for 2 days followed by 2 days in the dark at room temperature, and then placed under light banks (a combination of fluorescent and incandescent light) for a 16-hour photoperiod at room temperature (23 -25^0C) for 2–3 weeks for the embryos to grow.

Medium preparation for haploid plant growth: 20 g of sucrose + 8 g of agar were added to 1 litre of distilled water. The mixture was then heated to 85° F, stirring until completely dissolved. The temperature was reduced to 75° and 2.5 g of Gamborg's B-5 basal medium (SIGMA®) was added, stirring until completely dissolved. The pH level was adjusted to 6 by adding sodium hydroxide (NaOH 1N). The medium was dispensed into glass vials (8 ml/35 ml vial), and sterilized in an autoclave at 15 psi, 121°C for 20 min. From one litre of medium, 120 one-oz Quorpak vials were filled with approximately 8 ml of medium. The medium was autoclaved in the vials and cooled on a slant. A single rescued embryo was placed in each vial individually.

Transplanting Haploid Plants

When the embryos grew to a size of 1-1 ½" (almost touching the cap of the vial), haploids plantlets were transplanted into 3" plastic pots containing Sunshine 5 soilless

mix and watered. The pots were then set into a tray of water and then covered with a plastic lid to provide a humid environment. The lid was left on for 3 days or until the haploids were established. These haploid starts were kept at 15°C with a 16 hour light/8 hour dark cycle.

Colchicine Treatment-Doubling

Plant Preparation

When the haploids were at the three leaf stage, they were subjected to a colchicine treatment to promote chromosome doubling. The haploid plants were prepared for colchicine by trimming off about 1/3 of the leaf tissue. Next, the plants were removed from pots and the soil was stripped from the roots. The roots were cut back leaving about 1/8" and quickly rinsed (Figure A1.3).

Figure A1.3 Haploid plant preparation for colchicine

Colchicine Treatment and Rinse

The trimmed plants were placed in a beaker ready for colchicine treatment (Figure A1.4). Six drops (using an eye dropper) of DMSO (dimethyl sulfoxide) was added to 60 ml of colchicine. This solution was transferred to the beaker containing the trimmed haploids. An aerator, with a rubber tube leading from it, was placed in the beaker. The beaker was placed in a plastic pail and covered with a lid. A hole was made in the lid to allow the air tube to pass through. The covered pail was placed in a cupboard with the door ajar just enough to permit the air tube to run from the beaker to the air pump. This step ensured that the plants were exposed to as little light as possible as well as containing any chemical which might percolate from the beaker. The plants were treated for 3 1/2 hours at room temperature.

To rinse residual colchicine from the plants, the beaker was placed in the sink with both aeration tube and a rubber hose attached to the faucet to allow water to flow such that it trickled out of the beaker. The cold and hot water taps were adjusted so that the temperature of the rinse water was between 15-18°C. Plants were rinsed for a minimum of 2 hours.

Figure A1.4 Haploid plants in container for colchicine treatment

After rinsing, the haploid plants were replanted into 6" fibre pots filled with moistened Sunshine 5 mix. The pots were then set in a tub filled with water overnight to allow for sufficient water uptake by the Soil-less mix. The tub was covered with a lid for 3 days. The colchicined plants were placed in growth cabinets set at 15°C with a cycle of 16 hours of light and 8 hours of darkness. They were fertilized daily with a 20-20-20 fertilizer solution (approx. 10 g/L stock solution) siphoned into the watering line (diluted 16:1).

A total of 562 DH lines (Table A1.1) were generated during March 2001 – April 2002 from the F_{1s} of three reciprocal crosses.

Table A1.1 Generation of the first set of doubled haploid seeds from F1s of six crosses: a total of 562 lines were made available for bulk seeding.

Cross	Florets Pollinated	Caryopses Developed	Embryos Rescued	Haploids exposed to colchicine	No. (%) of DH plants surviving colchicine	Fertile DH plants
Roblin / Wuhan (RW)	683	445 (65%)	210 (43%)	109 (52%)	80 (73%)	63 (79%)
Wuhan / Roblin (WR)	610	403 (66%)	301 (75%)	123 (41%)	108 (88%)	96 (89%)
Maringa / Roblin (MR)	734	625 (85%)	303 (46%)	222 (73%)	170 (77%)	145 (85%)
Roblin / Maringa (RM)	640	512 (80%)	289 (58%)	190 (66%)	120 (63%)	103 (86%)
Maringa / Wuhan (MW)	649	370 (57%)	201 (54%)	112 (56%)	85 (76%)	72 (85%)
Wuhan / Maringa (WM)	637	302 (47%)	197 (65%)	112 (57%)	97 (87%)	83 (86%)
Total	3953	2657 (69%)	1501 (55%)	868 (58%)	660 (76%)	562 (85%)

Appendix 2 Phenotypic distribution of Fusarium head blight severity in doubled haploid populations

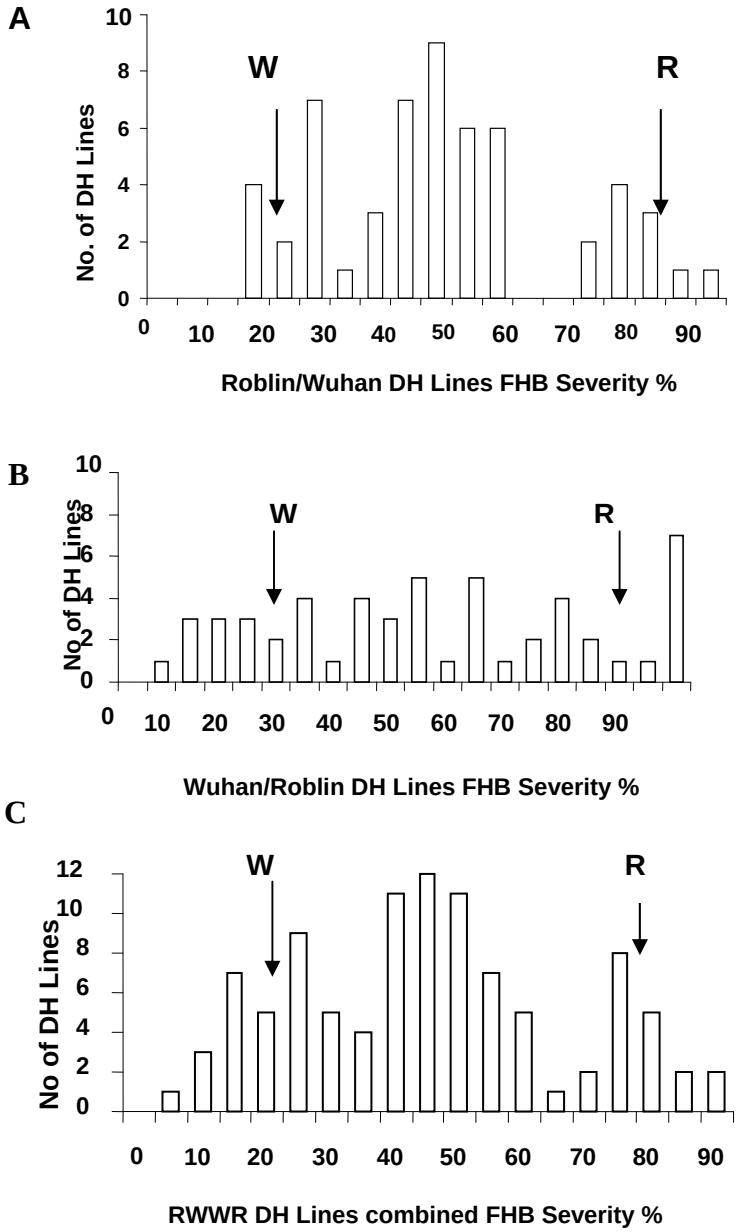


Figure A2.1 Phenotypic distribution of Fusarium head blight severity in doubled haploid populations developed from A. Roblin(R)/Wuhan(W), B. W/R and C for both populations combined. The data were obtained from greenhouse experiments (2002 – 2003). Transgressive segregation was observed at both ends of the histograms.

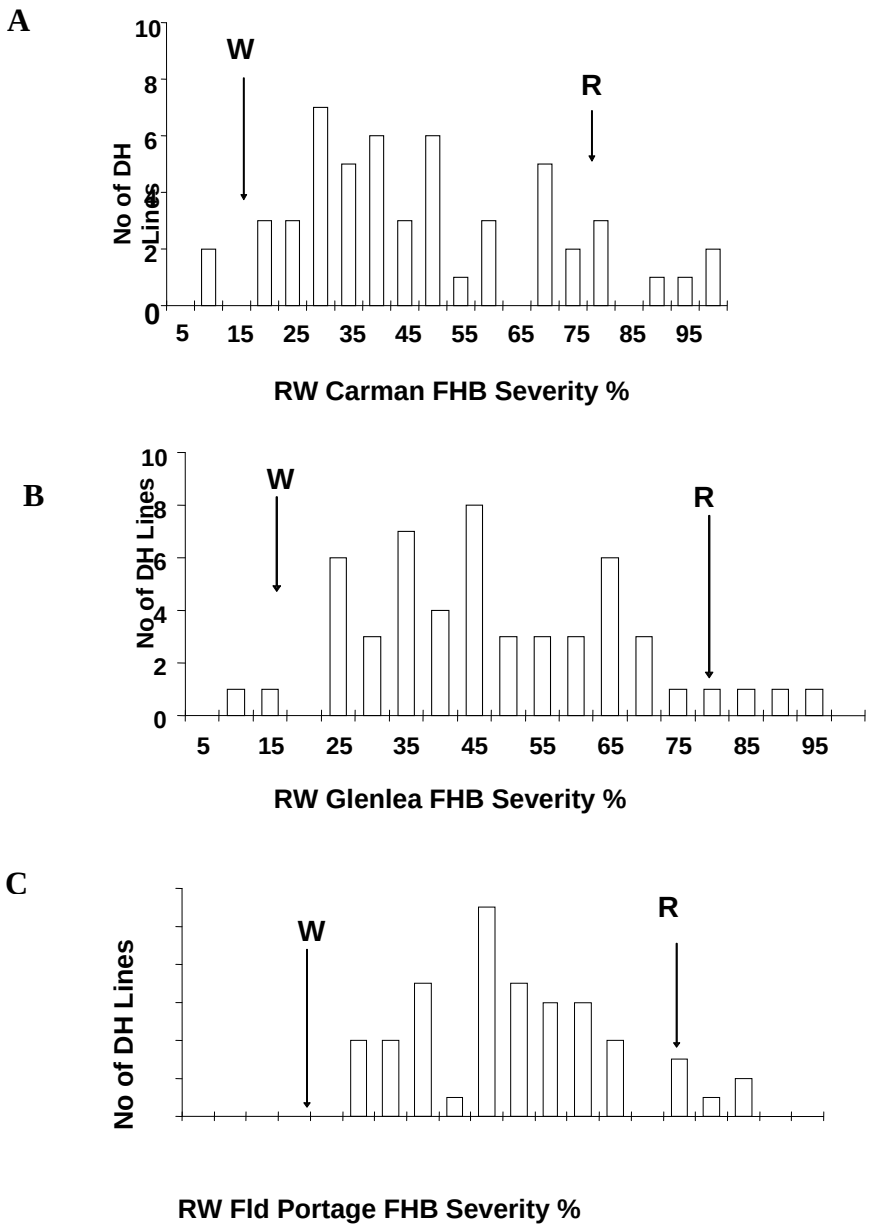


Figure A2.2 Phenotypic distribution of Fusarium head blight severity in a doubled haploid population developed from the cross Roblin(R)/Wuhan(W). The data were obtained from three Manitoba field experiments at A Carman, B. Glenlea, and C. Portage la Prairie in 2003. Transgressive segregation was observed at both ends of the histograms except at Portage la Prairie.

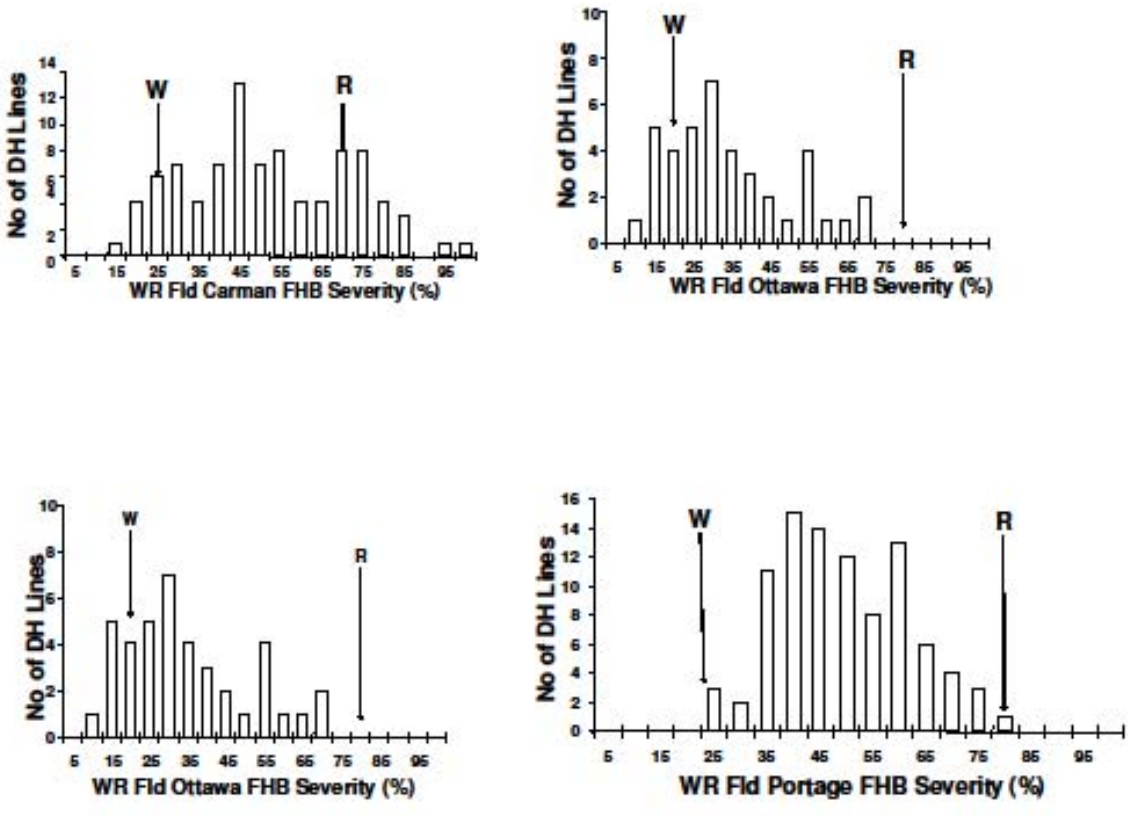


Figure A2.3 Phenotypic distribution of Fusarium head blight severity in a doubled haploid population developed from the cross Wuhan/Roblin. The data were obtained from three Manitoba field experiments at A Carman, B. Glenlea, and C. Portage la Prairie in 2003 and Ottawa. Transgressive segregation was observed at both ends of the histograms except at Portage la Prairie.

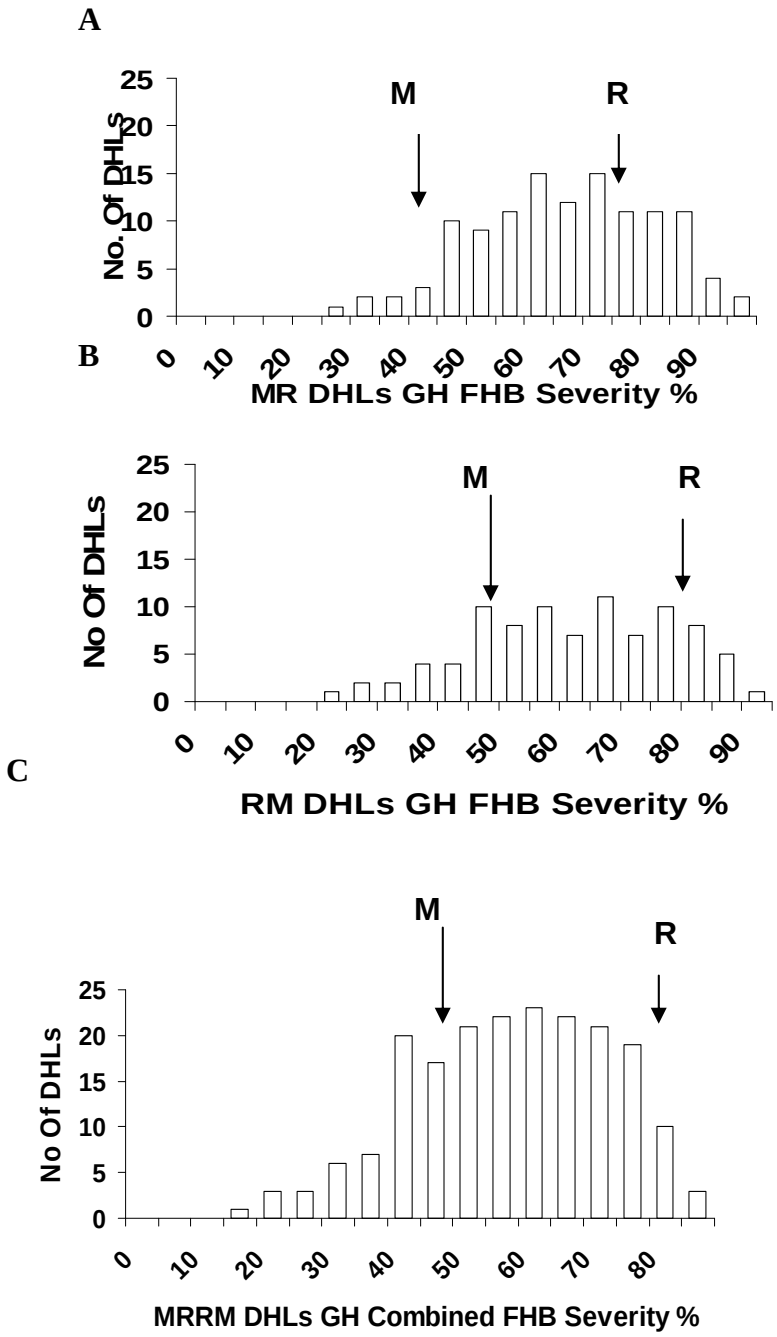


Figure A. 2. 4 A to C Phenotypic distribution of Fusarium head blight severity, a Type II resistance to FHB, in doubled haploid population developed from cross of Maringa (M) x Roblin (R). The data was obtained from the greenhouse experiments (2002 – 2003). Transgressive segregation was observed at both ends of the histograms.

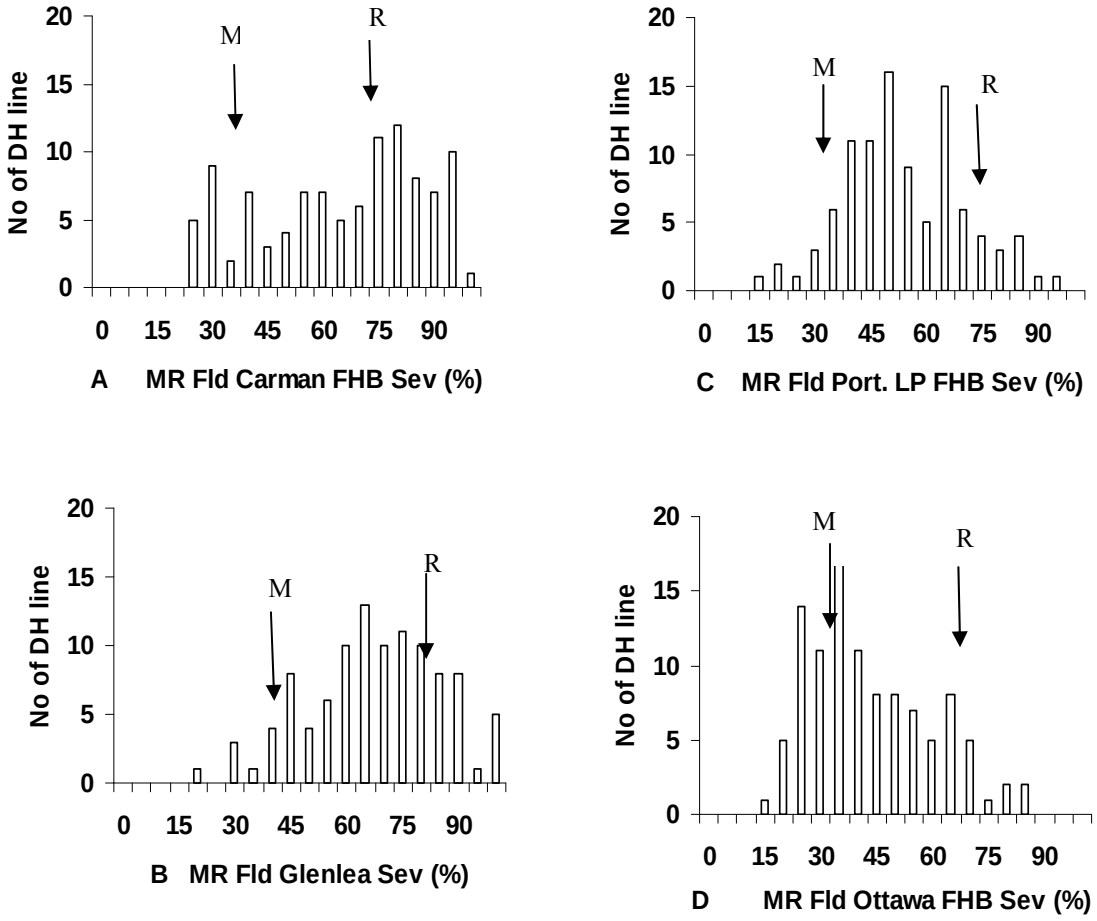


Figure A2.5 A to D Phenotypic distribution of Fusarium head blight severity, a Type II resistance to FHB, in doubled haploid population developed from cross of Maringa/Roblin . The data was obtained from the field experiments at four locations in summer of 2003. Transgressive segregation is observed at both ends of the histograms except for Roblin end in Ottawa.

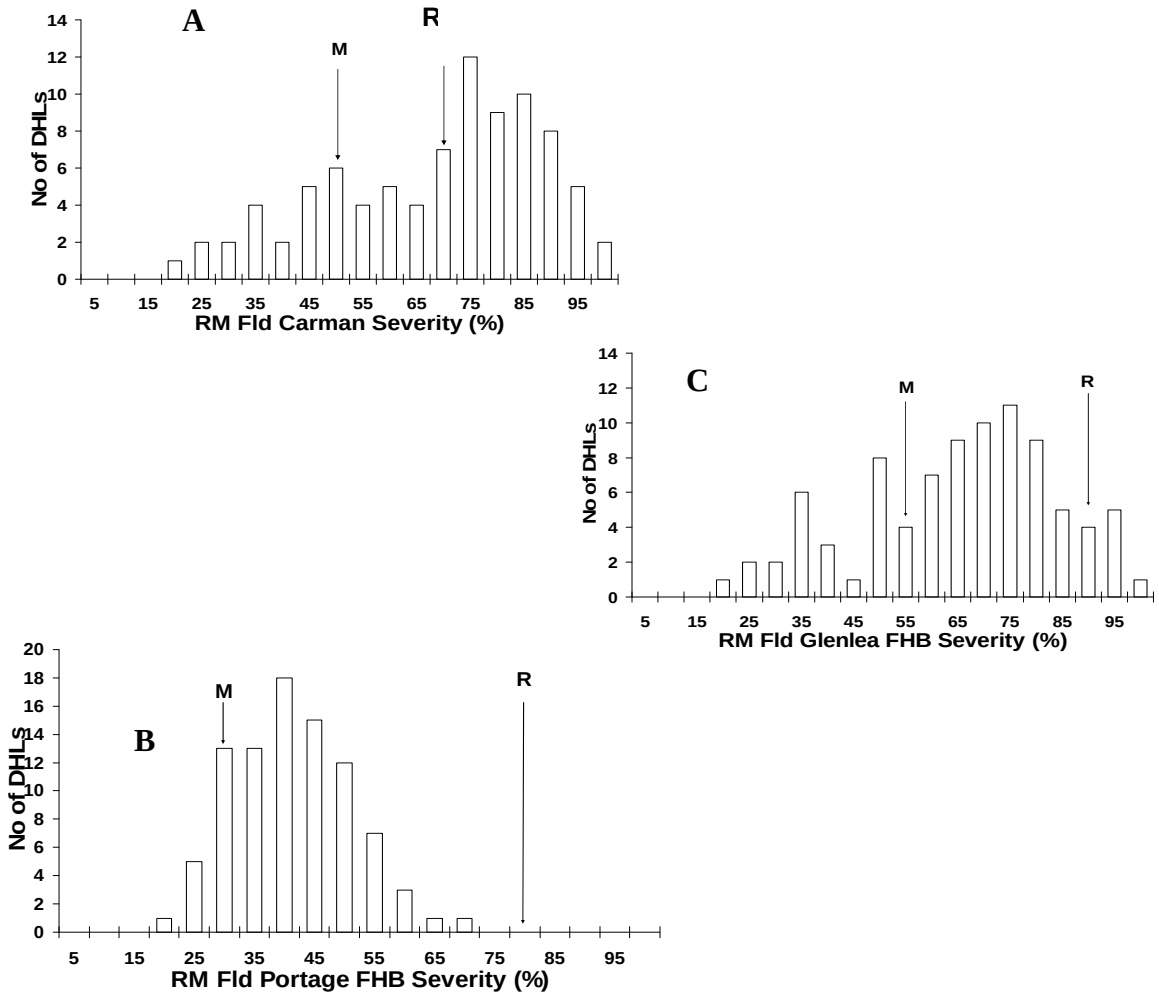


Figure A.2.6 A to C Phenotypic distribution of Fusarium head blight severity, a Type II resistance to FHB, in doubled haploid population developed from cross of Roblin (R) x Maringa (M). The data was obtained from the field experiments at four locations in summer of 2003. Transgressive segregation is observed at both ends of the histograms except for Roblin end in Portage FHB nursery.