CHARACTERIZING CHROMOSOMES FOR FUSARIUM HEAD BLIGHT

RESISTANCE IN A SPRING WHEAT (*Triticum aestivum* L.) CULTIVAR,

'FRONTANA'

A Thesis
Submitted to the Graduate Faculty
of the
North Dakota State University
of Agriculture and Applied Science

By

Dalitso Noble Yabwalo

In Partial Fulfillment of the Requirements
for the Degree of
MASTER OF SCIENCE

Major Department:
Plant Sciences

December 2009

Fargo, North Dakota

North Dakota State University
Graduate School

Title

Characterizing chromosome for Fusarium head blight resistance in a spring wheat

(Triticum aestivum) cultivar, 'Frontana'

By

Dalitso Noble Yabwalo

The Supervisory Committee certifies that this *disquisition* complies with North Dakota State University's regulations and meets the accepted standards for the degree of

MASTER OF SCIENCE

ABSTRACT

Yabwalo, Dalitso Noble; M.S.; Department of Plant Sciences; College of Agriculture, Food Systems, and Natural Resources; North Dakota State University; December 2009. Characterizing Chromosomes for Fusarium Head Blight Resistance in a Spring Wheat (*Triticum aestivum* L.) Cultivar, 'Frontana'. Major Advisor: Dr. Mohamed Mergoum.

Fusarium head blight (FHB), caused by the fungus *Fusarium graminearum*, is a major fungal disease of wheat and other cereal crops that causes both yield and quality losses due to shriveled kernels and accumulation of mycotoxins in the seed. 'Frontana', a Brazilian spring wheat cultivar, is a source of resistance genes to FHB, and it is believed to express resistance to both the establishment and spread of FHB (type I and type II resistance, respectively). Reciprocal backcross monosomic (RBCM) lines developed using Frontana and 'Chris', a susceptible spring wheat cultivar, and involving critical chromosomes 3A, 6A, and 4D from these parents were compared to characterize the type of resistance expressed by Frontana and confirm the chromosomes carrying genes for resistance. In four separate greenhouse tests, spray and single floret inoculation techniques were used to assess both types of resistance. Plants were inoculated when half of the plants in a pot were at anthesis (Feekes Growth Stage 10.5). Genotypes were evaluated for disease incidence, spread, deoxynivalinol (DON) content, Fusarium damaged kernels (FDK) and severity at 21 d after inoculation. Generally, RBCM lines with the critical Frontana chromosomes had low FHB incidence, spread, and severity. The RBCM line with chromosome 3A from Frontana exhibited the lowest FHB severity after spray inoculation, and the least spread after point inoculation. Frontana 3A lines had the lowest FHB incidence levels after spray inoculation amongst the RBCM lines that were tested. This implies the presence of major

resistance genes on chromosome 3A which are likely involved in both resistance to disease establishment and spread. However, resistance genes on 3A likely also interact with genes on other chromosomes to confer resistance to FHB because Frontana typically expressed a higher level of resistance to disease establishment and spread. Chromosome 4D also seems to play a significant role in Type I resistance while 6A contributes to Type II resistance.

ACKNOWLEDGEMENTS

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Fusarium head blight (FHB), primarily caused by *Fusarium graminearum* Schwabe teleomorph *Gibberella zeae* (Schwein), is a devastating disease of wheat (*Triticum aestivum* L.) and other small grains. Enormous resources have been mobilized to reduce the agronomic and economic losses caused by the disease. Dubin et al. (1997) stated that since 1993, FHB of wheat and barley *(Hordeum vulgare* L.) has had the greatest negative disease impact on US agriculture. The disease potentially causes loss of yield and poor kernel quality. Consequently, FHB can lower the market value of the crop. In the 1990s, over $2.6 billion US in losses have been attributed to FHB in wheat, and such losses have had a significant negative effect on farm communities in the Upper Midwest (McMullen et al., 1997). Furthermore, the disease has increasingly become a threat to the world's food supply due to outbreaks in Asia, Canada, Europe and South America (Dubin et al., 1997). FHB poses a two-fold threat. First, infested cereals have reduced quality and yield as a result of discolored, shriveled kernels, also called "tombstone" kernels. Second, scabby kernels are often contaminated with mycotoxins, particularly deoxynivalenol (DON) which makes it unsuitable for food or feed (McMullen et al., 1997).

In order to efficiently and effectively develop FHB resistant wheat cultivars, it is important to identify genes that are responsible for resistance and characterize their mode of action. This study used a subset of a previously produced group of reciprocal backcross monosomic (RBCM) lines to determine whether FHB resistance genes from the spring wheat cultivar, 'Frontana' (PI #500147, CItr 12470)

(Singh et al., 1995) exclude and/or limit the spread of FHB. Specific RBCM lines containing chromosomes that were earlier determined to be responsible for the expression

of FHB resistance in Frontana (Berzonsky et al., 2007) were evaluated for resistance to initial FHB infection (Type I resistance) or resistance to spread within the spike (Type II resistance).

'Alsen' (Frohberg et al., 2006) is known to exhibit a Type II resistance, while Frontana has been associated with both a Type I and II resistance (Singh et al., 1995). Six RBCM lines of interest were targeted from the previous study by Berzonsky et al. (2007) and each line has a particular or critical chromosome from either Frontana or 'Chris' (Heiner and Johnston, 1967). The chromosomes targeted were 3A, 6A and 4D. The RBCM were compared with Alsen and Frontana for their mechanism of resistance. The objective of this study was to determine which of the chromosomes carry genes that are critical for a Type I or Type II resistance to FHB in Frontana.

LITERATURE REVIEW

Background and Epidemiology

Fusarium head blight occurs widely on wheat throughout the world. The disease causes huge losses which are attributable to a reduction in grain yield and quality, including the production of mycotoxins, such as deoxynivalenol (DON), that can render the final wheat products unacceptable for food or feed (Gilbert and Tekauz, 2000). The disease is characterized by premature bleaching of spikelets, sterile spikelets and sometimes poorly filled kernels, resulting in what is referred to as tombstone kernels. Purple-black or pink mycelia of *F. graminearum* may appear on the spikelets (Sutton, 1982).

Fusarium graminearum, has a wide host range, affecting both cereals and non-cereal crops such as dry bean (*Phaseolus vulgaris* L.), canola (*Brassica rapa* L.), soybean (*Glycine max* L.), corn (*Zea mays* L.) and sugar beet (*Beta vulgaris* L.), among other crops (Burlakoti et al., 2007; Sutton, 1982). Nonetheless, the pathogen does not seem to cause serious economic problems in these crops as it does in wheat.

Burlakoti et al. (2007) demonstrated that the *F. graminearum* isolates from potato (*Solanum tuberosum* L) and sugar beet induced typical FHB symptoms in susceptible wheat cultivars and are capable of producing a wide range of trichothecenes that are hazardous to both human and animal health. Ear and stalk rots are both caused by *F. graminearum* in corn (Sutton, 1982). This fungal pathogen has been identified as the major cause of FHB or scab in North America (Parry et al., 1995).

Fusarium graminearum reproduces both sexually and asexually by way of ascospores and macroconodia, respectively. A study on the pathogenic effect of both ascospores and macroconidia on inoculated plant material using point inoculation technique found that

3

both spore types gave a quantitatively similar result, such that either spore type can be used to evaluate resistance (Stack, 1989). The pathogen overwinters in plant debris of a preceding infected crop, which can serve as a source of inoculum for the next crop (Atanosoff, 1920). Schaafsma et al. (2001) found that corn residue is a more favorable host for *F. graminearum* to overwinter. The pathogen can survive up to two years in the wheat debris especially if the plant material is not buried (Pereyra et al., 2004). Whenever the release of spores coincides with the flowering time of cereal crops, a FHB epidemic may be imminent (Atanosoff, 1920). Warm and moist conditions are favorable for the pathogen's colonization. Wind and splashing or wind driven rain are widely regarded as the major dispersal mechanisms for *F. graminearum* (Sutton, 1982).

Van Eewijk et al. (1995) evaluated 25 wheat genotypes from five European countries for resistance to 17 different strains of *F. graminearum, F. culmorum* and *F. nivale* at six locations across Europe. They found that different strains and species of *Fusarium* can induce FHB symptoms.

The pathogen produces many secondary metabolites, including the trichothecene mycotoxins; DON (vomitoxin), 3-acetyldeoxynivalenol and 15-acetyldeoxynivalenol. Samples with DON accumulation levels of 3 mg g^{-1} and higher are generally considered unacceptable because of the expected toxic effects to both humans and animals (Sutton, 1982). The *Tri5* gene of *G. zeae* is involved in the DON biosynthesis pathway and is responsible for production of the toxin during infection. When Desjardins et al. (1996) disrupted this pathway; the mutant fungus did not produce DON and had reduced virulence compared with the wild type. This was attributed to a reduced disease incidence and

4

severity. When the *Tri5* gene was restored, virulence of the pathogen increased, which lead to the conclusion that trichothecenes are essential for virulence.

Types of Resistance

Several types of resistance to FHB have been described in spring wheat (Schroeder and Christensen, 1963). Resistance to initial infection (Type I) is classified as the incidence of infection in the presence of natural or augmented inocula (e.g., spray inoculations or point inoculation); whereas, resistance to spread within the spike (Type II) is classified as the spread of infection within the spike following single floret infection (SFI). Resistance to DON accumulation is described as Type III (Miller et al., 1985); Type IV is resistance to kernel infection (Wang and Miller, 1988); whereas, tolerance to the disease is referred to as Type V (Mesterhazy, 1995). A number of wheat cultivars have been developed with Type II resistance which can be more easily selected. For example, Alsen (PI 615543) 'Glenn' (Mergoum et al., 2006), and 'Faller' (Mergoum et al., 2008), released by North Dakota State University have Type II resistance to FHB. However, Bai and Shaner (2004) noted that under optimum conditions and with abundant inocula, even cultivars with Type II resistance succumb to the disease.

Disease Management

Several strategies have been explored to control FHB. These include cultural practices aimed to reduce overwintering of the spores. Good agricultural practices advocate for an integrated approach to reduce the chance of disease. For instance, Schaafsma et al. (2001)

5

found that DON accumulation was lower in fields to which conventional tillage was applied compared with fields with minimum or no tillage. Bai and Shaner (1994) stated that agronomic and chemical control measures are only partly effective in limiting damage due to FHB. The use of resistant cultivars together with appropriate agronomic practices is the best method for controlling the disease. These practices include, but are not limited to crop rotation and management of nitrogen (N) application rates and tillage. Schaafsma et al. (2001) demonstrated that corn stubble is a primary source of *F. graminearum* inoculum, and DON concentration was lower in fields following soybean than in fields following corn or wheat during the second crop season. They recommended that wheat growers should plant resistant or less susceptible cultivars, avoid growing corn and/or wheat the previous year, use urea rather than ammonium nitrate as the N source, and practice a balanced N fertilization program.

Genetics of Resistance

Genetic variation for FHB resistance in wheat has been well documented (Buerstmayr et al., 1996; Mergoum et al., 2007). However, the number of genes involved in some genotypes and the types of resistance they express are not very well understood. Depending on the materials and methods used, the inheritance of resistance has been described as being monogenic, oligogenic and polygenic. It was determined that less than five genes are involved in the expression of FHB resistance in Chinese and South American wheat accessions (Buerstmayr et al., 1999). It is commonly believed that FHB resistance is polygenic (Bai and Shaner, 1994) and expression of resistance is highly influenced by the environment. Kolb et al. (2001) indicated that resistance to FHB exhibits quantitative

6

variation and its inheritance involves several loci on different chromosomes. This renders the phenotypic evaluation of FHB resistance difficult, and gene expression is complicated by genotype x environment (GxE) interactions. Thus, it is very difficult to reproduce phenotypic results when testing for FHB resistance. Consequently, screening for FHB resistance is time consuming, laborious, and costly (Steiner et al., 2004).

Researchers have attempted to analyze the genetic basis of FHB resistance in wheat using aneuploid stocks (e.g., intervarietal chromosome substitutions) and by mapping quantitative trait loci (QTL) based on high-density genetic maps. Molecular mapping and marker-assisted selection (MAS) have also been successfully used to combine or pyramid different sources of FHB resistance into a single genotype (Tumburic-Ilincic et al., 2006). Molecular markers have been linked to QTL associated with various types of FHB resistance, particularly in the Chinese accession 'Sumai3' (Anderson et al., 2001; Bai et al., 1999; Buerstmayr et al., 2002; Yang et al., 2003; 2005; Zhou et al., 2002).

Different chromosomes have been determined to carry genes that control FHB resistance in wheat. Chromosomes 3A, 5A, 7A, 3B, 6B, 4D and 6D, in particular, have been identified as important for resistance in several different genotypes, and these chromosomes may possibly carry genes for FHB resistance that are present in a range of scab-resistant genotypes (Buerstmayr et al., 1999; Waldron et al., 1999). Anderson et al. (2001) used a 'Sumai3/Stoa' population and a ND2603/Butte 86 population and found that FHB resistance was associated with QTL regions on chromosomes 3BS, and 6BS. The 3BS QTL region (*Qfhs.ndsu-3BS*) was responsible for 41.6% and 24.8% of the resistance to FHB in both the 'Sumai3/Stoa' and 'ND2603/Butte 86' populations, respectively. Thus, the conclusion was that a region on 3BS had a major effect on resistance to FHB.

7

It has also been reported that wheat chromosomes 3A and 5A carry QTL for FHB resistance (Steiner et al., 2004). Chromosome 3A from *Triticum macha* was identified as responsible for restricting FHB invasion or having a Type I resistance (Grausgruber et al., 1998). Han et al. (2005) mapped genes induced during FHB infection to chromosomes 3A, 6A, and 4D. Singh et al. (1995) noted that the Brazilian spring wheat cultivar Frontana is a widely used source for FHB resistance, and its resistance probably involves a minimum of two or three additive genes. Frontana is believed to express both Types I and II resistance to FHB (Singh et al., 1995), and Steiner et al. (2004) reported that although its resistance is primarily due to an inhibition of fungal penetration, Frontana also inhibits the spread of the fungus after infection (Type II).

Berzonsky et al. (2007) used a series of Chris monosomic lines to develop reciprocal backcross monosomic lines involving Frontana and they showed that chromosomes 3A, 6A and 4D likely carry genes for FHB resistance in this cultivar. They identified Frontana chromosomes involved in both the reduction in severity of FHB and the accumulation of DON, presumably due to a Type I mechanism since the evaluations were based exclusively on spray inoculation of the lines tested. A backcross reciprocal monosomic analysis enables individual wheat chromosomes to be analyzed for their effect on a specific trait while minimizing the influence of genetic background on the trait. Snape et al. (1983) explained the use of RBCM analysis in wheat and demonstrated its application to determine which chromosomes harbor QTL for height and grain yield. Heyne and Livers (1953) used the reciprocal backcross monosomic technique to study the inheritance of leaf rust and other agronomic characteristics in wheat. The method was also used to determine that chromosomes 5A, 1B, 4B, 6B and 6D from a FHB resistant wheat line reduced fungal

8

spread (Buerstmayr et al., 1999); whereas, homoeologous chromosome groups 2 and 6 also influenced DON content.

Identifying genes that confer resistance and understanding the complex genetic mechanism of FHB resistance can enhance breeding for resistance. However, knowledge is limited on which genes involved in FHB resistance function to either limit fungal invasion or spread. For example, do the putative Frontana genes act to exclude fungal invasion of the tissue (Type I resistance), do they act to prevent spread of the fungus (Type II resistance), or do they act in a combination of these ways? Evans et al. (2005) showed that FHB resistance was expressed in Frontana leaves but not in the leaves of genotypes purported to only express a Type II resistance. This might suggest that Frontana exhibits Type I resistance, since glumes of the spike can be considered modified leaves from an evolutionary standpoint.

As indicated previously, a number of chromosomes are associated with FHB resistance in general. This study was undertaken to determine the Frontana genes functioning to provide either Type I or Type II resistance to FHB.

MATERIALS AND METHODS

Plant Material

This study included six RBCM lines with critical chromosomes 3A, 6A, and 4D

derived from Frontana and Chris and four hard red spring wheat checks. These checks were

previously determined to be either highly resistant or susceptible to FHB. Frontana RBCM

lines with chromosomes 3A, 6A and 4D demonstrated a high level of resistance to FHB

infection while RBCM lines with similar chromosomes from Chris were determined to be

susceptible (Berzonsky et al., 2007). The development scheme for the RBCM lines, as was

illustrated by Berzonsky et al. (2007), is shown in Figure 1. The parents of the RBCM

lines, Frontana and Chris, including Alsen and 'Choteau' (Lanning et al., 2004) were

included as checks (Table 1).

Alsen is a resistant hard red spring wheat cultivar that exhibits a Type II resistance

inherited from the Chinese wheat cultivar 'Sumai3' (Frohberg et al., 2006; Mergoum et al.,

2007). Choteau is also a hard red spring wheat cultivar that was developed by the Montana

Agricultural Experiment Station in 2003. It is high yielding, resistant to wheat stem sawfly

(*Cephus cinctus* Nort.) (Lanning et al., 2004), but highly susceptible to FHB (W.A.

Berzonsky, personal commun.).

Critical chromosomes were categorized into three groups referred to as chromosome

groups, CG. Chromosome 3A was designated as chromosome group 1, 6A as chromosome

group 2, and 4D as chromosome group 3. Four separate greenhouse experiments were

carried out at North Dakota State University (46° N and 96° W) where day and night

temperatures were maintained between 16 ^{0}C to 21 ^{0}C with a 16h d length.
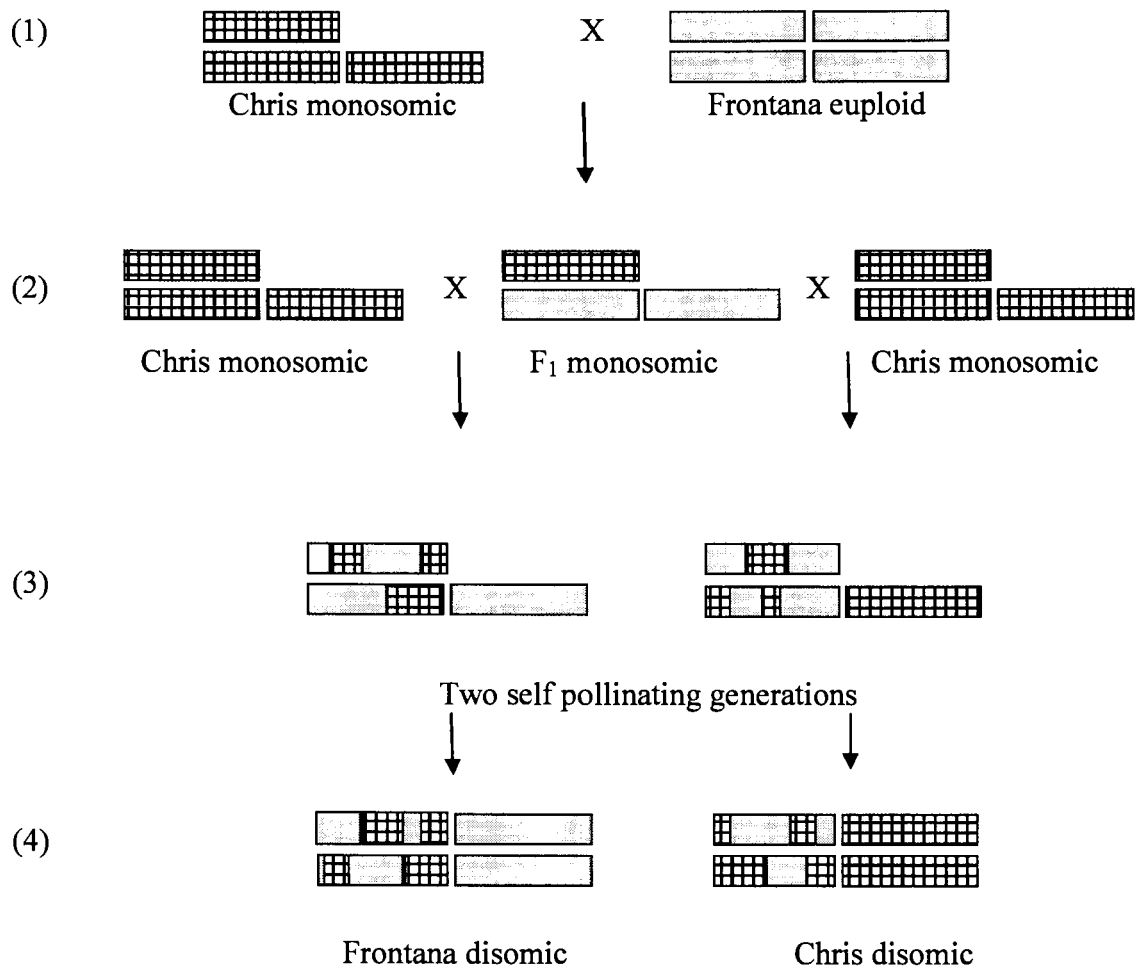
Figure 1. An illustration of the development process of the RBCM lines.

(1) A Chris monosomic line was crossed with a euploid Frontana variety; (2) the F_1 was

reciprocally backcrossed to the Chris monosomic line; (3) the monosomic progeny from

the crosses in (2) were selected and allowed to self-pollinate for two generations. (4)

Disomic plants carrying critical chromosomes from either Frontana or Chris were selected

and subjected to FHB evaluation (adapted from Berzonsky et al., 2007). As depicted in the

diagram, the genetic background of the disomic lines is similar (left side of each chromosome pair) except for the critical chromosome (right side of each pair).

Table 1: Genotypes included in the study, the description showing the critical chromosome carried, and Fusarium head blight reaction.

ID	Genotype	Description	FHB Reaction
1	Frontana(3A)	RBCM†	Resistant
2	Frontana(6A)	RBCM	Resistant
3	Frontana (4D)	RBCM	Resistant
4	Chris(3A)	RBCM	Susceptible
5	Chris(6A)	RBCM	Susceptible
6	Chris(4D)	RBCM	Susceptible
	Controls/checks:		
7	Frontana	Parent check	Resistant
8	Chris	Parent check	Susceptible
9	Alsen	Check	Resistant
10	Choteau	Check	Susceptible

†RCBM=Reciprocal backcross monosomic line.

Experimental Design

The experiment was set up as a nested block design with three replicates per greenhouse experiment. Each replicate was divided into two parts; T1 and T2 representing point and spray inoculation methods, respectively. Three CG were randomly assigned to T1 and T2 in each replicate. Finally, the genotypes were allocated to the CG, ensuring that the correct genotypes were placed in the appropriate CG. Thus, 3A, 6A and 4D were in different CG and the genotypes were nested with in CG. Each genotype was subjected to both point and spray inoculation in one replicate.

12

Greenhouse Planting and Management

To ensure germination of enough seeds of each type, kernels were germinated in petri dishes lined with two moist filter paper disks which were placed in a refrigerator for 3 to 4 days. Seeds with emerging radicals were selected and planted individually into 8.0 L plastic containers with an artificial soil mix, Sunshine[R] LC1 Mix. The artificial soil comprised of 70 to 80% Canadian sphagnum peat moss, perlite, dolomitic limestone, gypsum, and a wetting agent (Sun Gro[TM] mix Horticulture, Inc., Bellevue, WA). The same seed source was used for each planting and 8 plants were planted per pot.

Pots were typically watered every other day or as required depending on prevailing ambient conditions. A water soluble 20:20:20 granular fertilizer was dissolved in water and was applied to the plants at each watering. Fourteen days after emergence, seedlings were treated with Tilt (Propinoconazole 41.8%) as a precautionary measure against powdery mildew which is caused by *Erysiphe graminis* f. sp. *tritici* (Em. Marchal). Talster (Bifenthrin 7.9%) and Avid (Abamectin 2.0%) were periodically applied to plants to control thrips, *Frankliniella Californica* (Moulton) and aphids, *Diuraphis noxia*.

Inoculum Preparation

Fusarium graminearum inoculum was prepared from a field isolate (ALI-1). The isolate was cultured in petri dishes on a mung bean media at 4 ^{0}C for about 7 days. The macro-conidia were suspended in autoclaved double-distilled water and using a hemocytometer, spores were counted to achieve a concentration of 50,000 spores ml^{-1}. The final concentration was attained by diluting the spore suspension as needed with sterile double-distilled water. Prior to inoculation, a drop of Polyoxyethylene Sorbitan

13

Monooleate (Tween 80) was added to the inoculum to ensure a consistent distribution of spores in the water.

Inoculation

Plants were inoculated when 50% of the spikes per pot reached anthesis (10.5 Feekes stage). Ten plants per pot were inoculated, and all genotypes were treated to *F. graminearum* spores using both point and spray inoculation. For point inoculation, a Nichiryo Oxford Model 8100 Repetitive Syringe Dispenser was used to dispense 10 μL of a fungal spore suspension into the middle floret of a spike. This method initiates an infection site with the intent to assess the resistance to fungal spread within the spike or Type II resistance (Mesterhazy, 2003).

An atomizer was used to spray 2ml of the spore suspension onto the entire spike to assess the resistance to initial fungal colonization or Type I resistance. Inoculated spikes were immediately covered with glassine bags and misted with water once every day for 5 days to retain optimum humidity for fungal colonization and disease development.

Data Collection

Disease assessment: Visual scores

Visual evaluation of FHB incidence, severity and spread were based on evaluations conducted 21 d after inoculation. Disease incidence was determined by the number of spikes that exhibited disease symptoms after spray inoculation and expressed as a percentage of the total number of spikes inoculated. For incidence assessments, a spike

was considered diseased when it had at least one bleached spikelet due to FHB (Steiner et al., 2004). Disease severity was assessed by the number of spikelets per spike that exhibited FHB symptoms after spray inoculation (McMullen et al., 2008), and severity was determined as a percentage of the total number of spikelets on that spike.

Disease spread was assessed by inoculating a single spikelet on the middle part of a spike and counting the number of spikelets that developed disease symptoms beyond the initial inoculation point. Spread was determined as a percentage of spikelets with disease symptoms on the spike. Consequently, the total number of spikelets with disease symptoms per plot was expressed as a percentage to the total number of spikelets of inoculated spikes per pot.

Fusarium damaged kernels (FDK)

Inoculated spikes were harvested and threshed by hand, and kernels that appeared discolored and shriveled due to *F. graminearum* were counted and expressed as a percentage of the total number of kernels harvested from inoculated spikes. After the visual assessment, kernel samples were sent to a USDA Research facility in Manhattan, Kansas for another FDK evaluation using an automated single-kernel near-infrared (SKNIR) system. A SKNIR technique is nondestructive, more dependable as it generates rapid and objective FDK scores than visual assessment, and has the ability to predict DON content (Wegulo et al., 2008), although we did not use the DON results generated from this technique in this study.

Thousand kernel weight (TKW)

The weight of kernels was determined based on 100 kernels from inoculated heads and a simple proportion was calculated to determine thousand-kernel-weight. This data set relates to quality and flour extraction potential.

DON content

A coffee grinder was used to mill kernels from net pots into flour for DON content evaluation using a capillary gas chromatography with electron capture detection method (Tacke and Casper, 1996). Although DON testing by gas chromatography typically requires samples between 10 to 100 g of whole kernel or finely ground flour for DON tests, a minimum of 2.5g of flour is still acceptable for the evaluation. Thus, samples with less than 2.5g of flour cannot be accurately assessed for DON content. In this study, it was often difficult to meet the minimum requirements due to small sample sizes, especially considering the size of greenhouse plots. Therefore, DON evaluations for seasons 2, 3, and 4 were done at the University of Minnesota's DON testing lab using a technique called the Single Kernel DON analysis. The method detects DON content on whole ungrounded kernels and on sample sizes as small as 5mg. About 10 kernels were used for this DON analysis method.

Statistical Analysis

Means of collected data were analyzed for each greenhouse experiment using a mixed model and a PROC MIXED command with SAS 9.1 program (Cary, NC), and the experiment-wise error was set at $p \leq 0.05$. The effects of genotypes, chromosome groups,

and inoculation method were considered fixed; whereas, replicates, and seasons were considered random variables. A homogeneity test for the four greenhouse seasons was carried out using the Bartlett statistic (Gomez and Gomez, 1984) for unequal degrees of freedom at $p \leq 0.001$. Unequal degrees of freedom method was used because DON results had unequal number of observations. Means were separated by the Duncan Multiple Range Test (DMRT). ANOVA tables are presented in Appendix A.

Regression analyses of FHB incidence, severity, plant height, VFDK, SKNIR-FDK, DON and other related parameters were done using the same software (SAS 9.1) and graphs were generated using Microsoft Excel 2007.

RESULTS

A homogeneity test of residual mean squares for the four seasons for disease severity,

incidence, FDK using SKNIR (SKNIR-FDK), TKW, and plant height revealed that the

variances were homogeneous; therefore data for these parameters were combined for the

analyses of variances for all the parameters measured in the study. Data for visually

assessed FDK (VFDK) for seasons 1, 2, and 4 were also combined, while data for season 3

were analyzed independently (Appendices A and B). Analysis of variance (ANOVA) tables

for all evaluated parameters are also presented in appendices A and B.

Variances for DON tests for both gas chromatography and single kernel analyses were

not homogeneous; hence data from each season were analyzed separately. The DON

content tables are presented in appendix C.

Disease Severity

The FHB severity means, irrespective of inoculation method, are presented in Table 2.

Frontana 3A, Frontana, and Alsen exhibited low disease severity in CG1 and the scores

were not significantly different. However, Frontana 3A was significantly different from

Chris 3A. In CG2, Frontana and Alsen had the lowest FHB severity and the scores were not

significantly different. Fusarium head blight severity scores for Frontana 6A and Alsen

were not significantly different; Frontana 6A was significantly different from Chris 6A.

Frontana 6A was also significantly different from Frontana. In CG3, Frontana and Alsen

had the lowest FHB severity scores followed by Frontana 4D. Frontana 4D FHB severity

was not significantly different from Alsen but was significantly higher than Frontana, and

18

the difference between Frontana 4D and Chris 4D was significant. In general, Frontana, Frontana 3A and Alsen were the three genotypes with the lowest severity ratings. Overall, these genotypes had FHB severity levels of less than 20%. Across CG, Frontana 3A had lower FHB severity ratings than both Frontana 6A and Frontana 4D.

Table 2: Means of Fusarium head blight disease severity and incidence of genotypes in different chromosome groups evaluated on the 21 d after inoculation in four greenhouse seasons.

Genotype	CG†	Disease severity ‡		Disease incidence ‡	
		-------------------------------%------------------------------			
Alsen	1	15.83	*ab*	46.00	*cdef*
Choteau	1	51.79	*e*	62.75	*ef*
Chris	1	43.43	*de*	59.37	*ef*
Chris 3A	1	42.00	*de*	55.71	*def*
Frontana 3A	1	15.16	*ab*	29.96	*abc*
Frontana	1	5.41	*a*	13.75	*ab*
Alsen	2	17.77	*ab*	42.54	*cde*
Choteau	2	41.71	*de*	57.83	*def*
Chris	2	37.46	*cde*	53.50	*def*
Chris 6A	2	40.75	*de*	50.67	*cdef*
Frontana 6A	2	26.76	*bcd*	42.75	*cde*
Frontana	2	7.68	*a*	16.71	*ab*
Alsen	3	16.67	*ab*	43.14	*cde*
Choteau	3	51.22	*e*	69.15	*f*
Chris	3	43.50	*d*	57.08	*def*
Chris 4D	3	44.93	*de*	57.57	*def*
Frontana 4D	3	20.99	*bc*	35.29	*bcd*
Frontana	3	6.27	*a*	13.00	*a*

†CG=Chromosome group
‡Means followed by the same letter within the same column are not significantly different at P≤0.05

Disease Incidence

General disease incidence assessments for CG1 (Table 2) demonstrated that Frontana and Frontana 3A had the lowest FHB incidence scores and there was no significant

difference between the mean incidence scores of Frontana and Frontana 3A. Frontana 3A FHB incidence however, was significantly lower than Chris 3A. In CG2, Frontana had the lowest disease incidence, which was significantly difference from mean incidence for all other genotypes. The FHB incidence scores for Alsen, Frontana 6A, and Chris 6A were not significantly different. A similar trend was observed in CG3 with the lowest FHB incidence levels being Frontana followed by Frontana 4D. Mean incidence for Frontana and Frontana 4D were significantly different. Mean incidence scores for Alsen, Frontana 4D, and Chris 4D were not significantly different. Overall, Frontana and Frontana 3A had the lowest FHB incidence scores.

Table 3 shows FHB incidence levels expressed by the genotypes under investigation following spray inoculation alone. In CG1, Frontana had the lowest mean FHB incidence followed by Frontana 3A and Alsen. These means were significantly different compared with the incidence means for Choteau and Chris 3A. In CG2, Frontana and Alsen were the only genotypes with low FHB incidence levels. The mean incidence of Frontana 6A was not significantly different from the means for Chris 6A and Chris. In CG3, means for Frontana, Frontana 4D, and Alsen were not significantly different.

Results for FHB severity following spray inoculation alone are also presented in Table 3. In CG1, FHB severity means for Frontana, Frontana 3A, and Alsen were not significantly different and these genotypes expressed the least FHB incidence. Means for Frontana 3A and Chris 3A were significantly different, but those for Chris 3A and Chris were not. In CG2, means for Frontana and Alsen were the lowest and not significantly different. However, the mean FHB severity for Frontana 6A was not significantly different from the mean for Chris 6A. In CG3, means for FHB severity of Frontana, Frontana 4D,

Table 3: Means of genotypes for Fusarium head blight (FHB) incidence, severity, and spread in three chromosome groups evaluated during the four greenhouse seasons.

Genotype	CG†	FHB Incidence Mean‡		FHB Severity Mean‡		FHB Spread Mean‡	
		--------%--------					
Alsen	1	28.63	ab	10.82	a	20.83	b
Choteau	1	62.17	d	53.50	d	50.07	d
Chris	1	60.25	d	41.62	cd	46.12	d
Chris 3A	1	51.83	cd	40.69	cd	43.31	cd
Frontana 3A	1	27.67	a	17.70	ab	12.62	ab
Frontana	1	12.50	a	5.45	a	5.37	a
Alsen	2	17.67	a	7.76	a	27.78	bc
Choteau	2	55.83	d	41.50	cd	41.93	c
Chris	2	52.33	cd	36.24	c	38.68	c
Chris 6A	2	43.83	bcd	43.23	cd	38.26	c
Frontana 6A	2	32.92	bc	26.81	bc	26.71	b
Frontana	2	16.67	a	6.80	a	8.55	a
Alsen	3	15.50	a	8.73	a	24.61	b
Choteau	3	59.98	d	43.51	d	58.92	d
Chris	3	55.08	d	42.97	cd	44.03	cd
Chris 4D	3	53.14	cd	41.87	cd	47.99	d
Frontana 4D	3	29.08	ab	18.89	ab	23.09	b
Frontana	3	11.67	a	4.81	a	7.72	a

†CG = Chromosome group
‡Means followed by the same letter within column are not significantly different at
$p < 0.05$

and Alsen were not significantly different, but the mean of Frontana 4D was significantly different from both the means of Chris 4D and Chris.

In terms of disease spread (Table 3) following single floret inoculation (SFI), results for CG1 indicate that Frontana and Frontana 3A had the lowest mean FHB spread with their means not significantly different. Furthermore, means for Alsen and Frontana 3A were not significantly different. Mean FHB spread scores for Frontana 3A and Chris 3A were significantly different. For CG2, Frontana had the lowest mean FHB spread score and

21

was significantly different from the rest of the genotypes. Mean disease scores for Frontana 6A and Alsen were not significantly different, but the mean for Frontana 6A was significantly different from the mean for Chris 6A. In CG3, the mean FHB spread score for Frontana was significantly different from the means for the rest of the genotypes. However, mean scores for Frontana 4D and Alsen were not significantly different, while means for Frontana 4D and Chris 4D were significantly different.

Visual Fusarium Damaged Kernels (VFDK)

The results for VFDK for the combined analysis of seasons 1, 2, and 4 as well as for the individually analyzed season 3 are reported in Table 4. In CG1, the combined analysis showed that Alsen and Frontana had similar low VFDK scores followed by Frontana 3A with the next lowest VFDK score. In CG2, Frontana 6A, Frontana, Chris 6A, and Alsen had low VFDK and the differences in ratings were not statistically significant. However, in CG3 only Frontana and Alsen had similarly low VFDK and both had a mean VFDK which was significantly different when compared with the rest of the genotypes. Across CG, Frontana and Alsen had the lowest VFDK scores and the mean VFDK was not significantly different for the two genotypes. The second lowest VFDK was Frontana 3A, but the mean VFDK for Frontana 3A was not significantly different from that for Frontana, Frontana 6A and Alsen.

In season 3, Frontana, Frontana 4D and Alsen had the lowest VFDK scores and the mean VFDK scores for these four genotypes were not significantly different. However, Frontana 3A had a relatively high VFDK score compared to its performance in the other three seasons.

Table 4: Means of visual Fusarium damaged kernels (VFDK) of genotypes in different chromosome groups assessed in seasons 1, 2, & 4 and season 3, respectively.

Genotype	CG†	Seasons 1, 2, 4 VFDK ‡		Season 3 VFDK ‡	
		-----------------------------%-----------------------------			
Alsen	1	5.57	a	12.28	ab
Choteau	1	26.78	egh	49.55	ef
Chris	1	17.75	bcde	37.00	cde
Chris 3A	1	20.78	cdeg	42.62	def
Frontana 3A	1	11.36	abcd	31.35	cde
Frontana	1	3.57	a	0.80	a
Alsen	2	4.79	a	10.65	ab
Choteau	2	32.81	gh	22.28	bc
Chris	2	17.82	bcde	38.00	cde
Chris 6A	2	13.48	abcd	33.45	cde
Frontana 6A	2	13.81	abcde	30.20	cd
Frontana	2	5.60	a	0.15	a
Alsen	3	7.57	ab	4.92	ab
Choteau	3	37.54	h	38.98	de
Chris	3	26.12	egh	54.57	f
Chris 4D	3	21.95	deg	43.27	def
Frontana 4D	3	23.38	deg	4.27	ab
Frontana	3	9.14	abc	0.08	a

†CG = Chromosome group
‡Means followed by the same letter within season are not significantly different at $P \leq 0.05$

Single Kernel Near Infrared FDK (SKNIR-FDK)

Results for SKNIR-FDK analysis are presented in Table 5. Among CG1, Frontana had the lowest mean FDK score, which was significantly different from the rest of the genotypes whereas Frontana 3A, Chris 3A, and Alsen scores were similar. In CG2, Frontana had the lowest mean SKNIR-FDK score, which, except for Chris 6A was significantly different from the rest of the genotypes. However, means for Frontana 6A,

Chris 6A, Alsen, and Chris were not significantly different. Similarly, in CG3, the SKNIR-

FDK analyses indicated that Frontana had the lowest mean SKNIR-FDK followed by

Frontana 4D, but the means for the two genotypes were not significantly different. In

general, the SKNIR-FDK results were consistent throughout the four greenhouse seasons,

but scores were higher than those which were visually assessed for FDK.

Table 5: Means of Fusarium damaged kernels (FDK) from the single kernel near-infrared technique (SKNIR) and thousand kernel weight (TKW) for genotypes in different chromosome groups for four greenhouse seasons.

Genotype	CG†	SKNIR-FDK Mean‡		TKW Mean‡	
		---------------------------%---------------------------			
Alsen	1	41.35	*bcde*	32.43	*ef*
Choteau	1	61.27	*ef*	22.12	*ab*
Chris	1	49.91	*cdef*	26.02	*bcde*
Chris 3A	1	41.97	*bcde*	28.82	*cde*
Frontana 3A	1	36.34	*bcd*	32.16	*ef*
Frontana	1	15.98	*a*	38.34	*f*
Alsen	2	43.06	*bcde*	32.12	*ef*
Choteau	2	58.49	*def*	23.61	*abc*
Chris	2	42.00	*bcde*	26.99	*bcde*
Chris 6A	2	33.90	*abc*	29.82	*cde*
Frontana 6A	2	38.62	*bcde*	28.29	*Bcde*
Frontana	2	16.03	*a*	38.20	*f*
Alsen	3	46.46	*cde*	32.80	*ef*
Choteau	3	70.17	*f*	18.97	*a*
Chris	3	52.36	*cdef*	24.57	*abcd*
Chris 4D	3	44.47	*cde*	27.76	*bcde*
Frontana 4D	3	38.19	*bcd*	30.91	*de*
Frontana	3	22.19	*ab*	38.80	*f*

†CG=Chromosome group
‡Means followed by the same letter within season are not significantly different at P≤0.05;

Thousand-Kernel-Weight (TKW)

Among CG1, Frontana, Frontana 3A, and Alsen had the highest TKW, and their means were not significantly different (Table 5). Similarly, in CG2, Frontana and Alsen had the highest TKW but their means were not significantly different. Mean TKW for Frontana 6A was not significantly different from means for Alsen, Chris 6A, Choteau, and Chris. Among genotypes in CG3, Frontana and Alsen had the highest TKW, while the mean TKW for Frontana 4D was not significantly different from the mean for Alsen.

DON Accumulation

The accumulation of DON varied from one season to another and was not consistent among the genotypes tested over the four seasons. The DON content generated at NDSU using the gas capillary chromatography method were generally lower compared with levels measured by the single kernel method. Analysis of DON accumulation levels in season 1 did not result in significant differences among genotypes. However, there were significant differences in DON accumulation levels for season 2, but differences were not consistent across chromosome groups. Alsen and Frontana had the lowest DON accumulation levels for CG1, but in CG2, only the mean DON accumulation of Choteau was significantly different from the rest of the genotypes. In this group, it exhibited the highest level of DON accumulation. The DON levels for Frontana 4D, Alsen, and Frontana DON were not significantly different in CG3 (Table 6).

Table 6: Means of deoxynivalenol (DON) content of the genotypes in the
three chromosome groups for season 2 using the gas capillary
chromatography technique.

Genotype	Chromosome group	DON †	
		---------ppm---------	
Alsen	1	1.71	ab
Choteau	1	9.95	bcdefg
Chris	1	12.43	cdefgh
Chris 3A	1	19.62	fgh
Frontana 3A	1	15.91	efgh
Frontana	1	3.55	abcd
Alsen	2	1.11	a
Choteau	2	21.90	h
Chris	2	7.27	abcde
Chris 6A	2	3.74	abcd
Frontana 6A	2	0.46	a
Frontana	2	2.58	abc
Alsen	3	2.30	abc
Choteau	3	20.05	gh
Chris	3	13.75	defgh
Chris 4D	3	11.60	bcdefgh
Frontana 4D	3	9.28	abcdef
Frontana	3	4.28	abcd

†Means followed by the same letter within season are not significantly
different at P≤0.05

Deoxynivalenol results based on the single-kernel analysis technique for season 2

depict Frontana and Alsen as having the lowest DON accumulation among CG1 genotypes,

while Chris 3A had the highest. Frontana 3A had high DON content as well, but the mean

was not significantly different from DON accumulation for the susceptible lines. Among

CG2 genotypes, Frontana 6A, Alsen, Chris 6A, Chris, and Frontana were not significantly

different, while Choteau had a significantly higher mean DON content. Alsen had the

lowest mean DON accumulation in CG3; however, the mean DON content of Alsen was

not significantly different from the mean DON content of Frontana. Some susceptible

genotypes had unexpectedly low DON content. For instance, Chris 6A, and Chris 4D had

means which were not statistically different from the means for resistant genotypes (Table

7).

Table 7: Means of deoxynivalenol (DON) content of the genotypes in the three chromosome groups for season 2 using the single kernel DON analysis technique.

Genotype	Chromosome group	DON †	
		------ppm------	
Alsen	1	31.48	*a*
Choteau	1	198.43	*def*
Chris	1	253.25	*ef*
Chris 3A	1	273.13	*f*
Frontana 3A	1	185.00	*cdef*
Frontana	1	26.97	*a*
Alsen	2	32.40	*a*
Choteau	2	148.23	*bcde*
Chris	2	48.93	*ab*
Chris 6A	2	34.63	*a*
Frontana 6A	2	18.60	*a*
Frontana	2	10.82	*a*
Alsen	3	29.15	*a*
Choteau	3	206.12	*def*
Chris	3	115.15	*abcd*
Chris 4D	3	66.52	*ab*
Frontana 4D	3	80.47	*abc*
Frontana	3	34.78	*a*

†Means followed by the same letter within season are not significantly different at $P \leq 0.05$

Table 8: Means of deoxynivalenol (DON) content of the genotypes in the three chromosome groups for season 3 using the single kernel DON analysis technique.

Genotype	Chromosome group	DON †
		-------ppm--------
Alsen	1	16.60 *abc*
Choteau	1	54.87 *cde*
Chris	1	39.87 *abcde*
Chris 3A	1	44.33 *abcde*
Frontana 3A	1	84.02 *ef*
Frontana	1	0.48 *a*
Alsen	2	20.48 *abcd*
Choteau	2	41.23 *abcde*
Chris	2	41.83 *abcde*
Chris 6A	2	63.57 *de*
Frontana 6A	2	46.88 *bcde*
Frontana	2	0.85 *a*
Alsen	3	9.98 *abc*
Choteau	3	73.98 *e*
Chris	3	121.00 *f*
Chris 4D	3	79.88 *ef*
Frontana 4D	3	2.28 *ab*
Frontana	3	0.08 *a*

†Means followed by the same letter within season are not significantly different at $P \leq 0.05$

In season 3 (Table 8), DON content for CG1 showed that Frontana exhibited the lowest level of DON accumulation,while Frontana 3A exhibited the highest. In CG2, Frontana again had the lowest DON content, with Alsen having the next lowest. Genotypes with the lowest mean DON content in CG3 were Frontana, Frontana 4D and Alsen, but means among these genotypes were not significantly different. Chris had the highest mean DON content of 121ppm.

28

In season 4 and withing CG1, means for the DON content of genotypes Frontana,

Alsen, Frontana 3A, Chris 3A, and Chris were not significantly different. Only Choteau

had a significantly higher mean DON accumulation in among genotypes within this group.

In CG2, means for Frontana, Alsen, Chris 6A, and Chris were not significantly different.

However, Choteau had a significantly higher mean DON accumulation compared with the

other genotypes. Similar results were observed for CG3 (Table 9).

Table 9: Means of deoxylivalenol (DON) content of the genotypes in the three chromosome groups for season 4 using the single kernel DON analysis technique.

Genotype	Chromosome group	DON †
		------ppm--------
Alsen	1	1.98 *ab*
Choteau	1	38.82 *e*
Chris	1	17.55 *abcde*
Chris 3A	1	12.37 *abcd*
Frontana 3A	1	14.62 *abcd*
Frontana	1	3.55 *abc*
Alsen	2	1.69 *a*
Choteau	2	65.68 *f*
Chris	2	17.46 *abcde*
Chris 6A	2	13.15 *abcd*
Frontana 6A	2	25.68 *cde*
Frontana	2	4.36 *abc*
Alsen	3	4.72 *abc*
Choteau	3	40.29 *e*
Chris	3	24.40 *bcde*
Chris 4D	3	12.73 *abcd*
Frontana 4D	3	32.75 *de*
Frontana	3	6.94 *abc*

†Means followed by the same letter within season are not significantly different at P≤0.05

In general, DON accumulation for genotypes was lower for season 4 compared with the other seasons. Frontana and Alsen consistently had the lowest DON accumulation levels across the seasons. The Frontana derived lines generally accumulated more DON compared with Frontana itself, perhaps due to the expression of additional genes for resistance on other chromosomes of Frontana. This suggests that 3A might not carry all genes responsible for reducing DON accumulation or that there is a suppression of 3A resistance in the absence of other genes putatively influencing FHB in Frontana.

FHB Resistance Relationships with Other Traits

Linear relationships between agronomic traits and FHB resistance was performed and results are presented in Appendix D. Correlation and regression analyses show that there was a strong relationship between FHB severity and incidence, $r^2=0.77$ (Fig. 2) and a Pearson Correlation of $r=0.87$, P<.000. Disease severity and VFDK were also strongly related (Fig. 3), with a Pearson Correlation, $r=0.81$, P<.0001.

$$y = 1.017x + 12.35$$
$$r^2 = 0.768$$
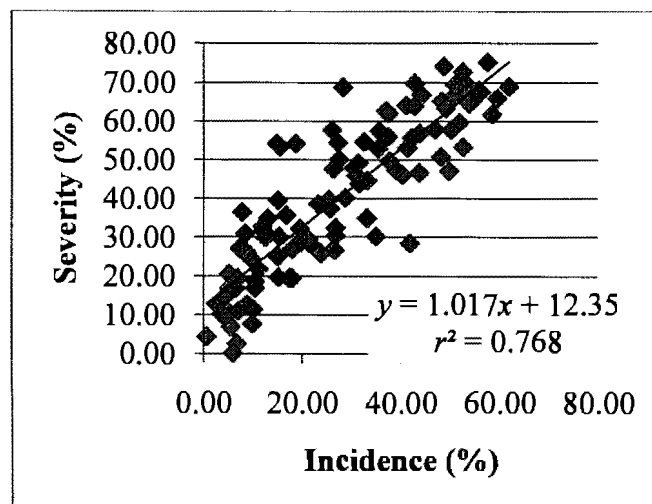
Figure 2: Linear relationship between FHB incidence and severity

In this study, the association between plant height and FHB severity was weak

[r^2=0.098, and r=3.1, P<.005] (Fig. C7). Disease severity was highly correlated to TKW

(Fig. C2) with r=0.81 (P<.0001) but the relationship was an inverse one.
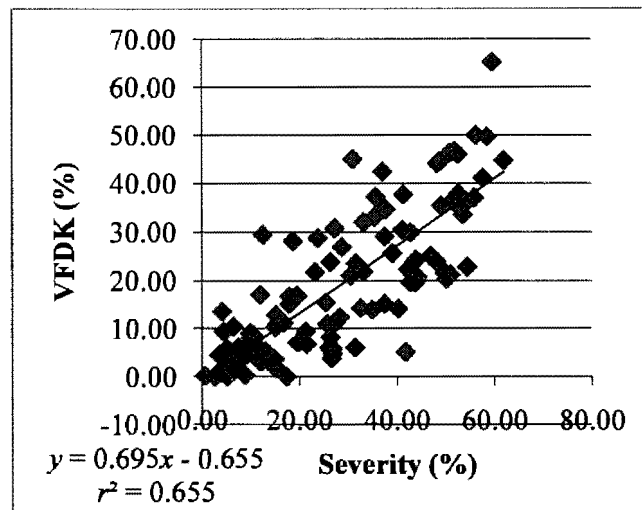
Figure 3: Linear relationship between FHB severity and VFDK

Thousand-kernel-weight and VFDK were inversely related (Fig. C6) with r=0.80, P<.0001.

There was also a negative correlation between TKW and SKNIR-FDK (Fig. C8). Fusarium

head blight spread and severity were also highly associated (Fig. C9) with a correlation of

r= 0.91, P<.0001. For spray inoculated pots, FHB incidence and severity were strongly

correlated at r=0.97, P<.0001 (Fig. C10), and FHB incidence and spread using point

inoculation were strongly correlated (Fig. C11) at r=0.93, P<.0001.

A simple linear regression analysis for DON and FHB severity was not significant

(Table 10). However, a quadratic regression model was significant at p>0.05, (r^2 =0. 215,

p=0.003). Because of the many factors relating to DON accumulation and FHB disease

progression, it is difficult to interpret the practical meaning of this quadratic relationship.

The correlations between DON accumulation and FHB incidence, VFDK, and SKNIR-FDK were significant. However, the linear relationship between DON and these parameters was weak. In season 2, there was a significant linear correlation between DON and VFDK. Based on the simple linear model, 38% of the mean variation in DON content can be attributed to VFDK while SKNIR-FDK explained 13% of the variation in DON content.

In season 3, had higher FHB severity and DON content correlation between (Table 10). The linear relationships between DON and FHB incidence, VFDK and SKNIR-FDK were also significant, but the correlation between VFDK and DON was higher than between SKNIR-FDK and DON.

In season 4, the correlation between FHB severity and DON content was not significant, while the linear correlation between DON content and FHB incidence was significant but weak. However, the linear relationships between DON content and VFDK and SKNIR-FDK were significant and comparatively strong.

Table 10: Coefficient of determination (r^2), correlation coefficients (r), and p-values for deoxynivalenol (DON) against Fusarium head blight severity, incidence, visual Fusarium damaged kernels (VFDK), and single kernel near infrared assessed Fusarium damaged kernels (SKNIR-FDK) for seasons 2, 3, and 4.

Season[†]	Statistic	Severity	Incidence	FDK	SKNIR-FDK
	r^2	0.090	0.118	0.383	0.129
2	P-value	0.863	0.0001	0.0001	0.008
	r	0.300	0.344	0.619	0.359
	r^2	0.383	0.347	0.738	0.149
3	P-value	0.0001	0.0001	0.0001	0.006
	r	0.619	0.589	0.859	0.386
	r^2	0.070	0.139	0.523	0.245
4	P-value	0.055	0.006	0.0001	0.0001
	r	0.265	0.373	0.723	0.495

†DON results for season 1 are not included because they were not significant

DISCUSSION

Numerous reports have emphasized that evaluation of FHB resistance demands a meticulous, time consuming approach which may be costly because resistance is quantitative and expression is affected by the environment. In this study, artificial point and spray inoculations conducted individually for each genotype at anthesis enabled a determination of the types of genes working to express FHB resistance. Replication of the experiment in time and space increased the precision of estimating the level of disease resistance or susceptibility of the various genotypes.

The inoculation methods used were to distinguish between Types I and Type II FHB resistance. Type I resistance, measured by the percentage of infected spikes after spray inoculation (Steiner et al. 2004), reduces the number of initial infection sites or colonization by *F. graminearum*. Fusarium head blight severity, determined following spray inoculation, can either be a result of many spikelets colonized at the time of initial infection or due to FHB spread after colonization of one spikelet. Therefore, spray inoculation assesses Type I resistance but can also be employed for an indirect assessment of Type II resistance (Loffler et al., 2009; Miedaner et al., 2003). Type II resistance was evaluated by looking at the FHB progression beyond an initial infection point at the middle of the spike.

Type I Resistance: Disease Incidence

Frontana had the lowest mean disease incidence scores and was often significantly different from means for the other genotypes. Frontana is believed to possess a Type I resistance (Singh et al., 1995; Steiner et al., 2004), while Alsen expresses a Type II

resistance (Frohberg et al., 2006; Mergoum et al., 2007). Based on the results presented herein, chromosome 3A from Frontana likely carries a major gene responsible for Type I resistance. However, the level of FHB infection was typically higher in Frontana 3A than it was in Frontana itself. This can perhaps be explained by the presence of important genetic interactions or additional genes for resistance that are present in Frontana but absent in the Frontana 3A RCBM line.

Chromosome 6A from Frontana expressed the highest disease incidence. In addition, the expressed level of resistance of Frontana 6A was not significantly different from the expressed level of the susceptible genotypes, demonstrating a lack of genes or the suppression of genes for FHB Type I resistance on chromosome 6A of Frontana. The results for Frontana chromosome 4D also suggest that this chromosome carries a gene or genes involved in Type I resistance although its effect seems to be less than chromosome 3A.

Disease Severity

Disease severity results demonstrated that Frontana 3A, Frontana and Alsen expressed very low FHB severity level. The low disease severity scores for Frontana 3A suggest a significant role of chromosome 3A in reducing FHB severity. It is possible that chromosome 3A plays a role in both reducing FHB incidence and spread. Steiner et al. (2004) indicated that chromosome 3A from Frontana was consistently associated with FHB severity, explaining 16% of the phenotypic variance. Throughout the present study, Frontana 3A demonstrated a high level of resistance to disease severity after spray inoculation, a strong indication that 3A most likely has QTL that play a major role in

34

conferring both resistance to *F. graminearum* colonization and spread (Steiner et al., 2004; Mardi et al., 2006). Additionally, since Frontana exhibited much lower disease severity than both Frontana 3A and Alsen, Frontana likely has genes for FHB resistance on other chromosomes apart from 3A or expression of the 3A QTL is partially suppressed in the absence of other genes for resistance.

Buerstmayr et al. (2003) mapped the first Type I FHB resistance QTL to chromosome 5A using the 'CM-82036/Remus' cross. CM-82036 was derived from a cross involving Sumai3, a spring wheat line from China, and a susceptible line. By inference, the Type I QTL on 5A probably was derived from Sumai3. Since Alsen traces its parentage to Sumai3 (Frohberg et al., 2006) and is described as possessing Type II resistance to FHB (Mergoum et al., 2007), Alsen may also express some level of Type I resistance. Furthermore, FHB severity scores for both Alsen and Frontana were often not significantly different, implying that they both might express Type I resistance. If Frontana and Sumai3, the major sources of FHB resistance, both express a Type I and II resistance, their derivative lines might be used in comparison with other genotypes to identify Types I and II resistance and possibly distinguish between unique sources of each type of resistance.

Our results illustrated that only Frontana and Alsen exhibited low FHB severity, suggesting that chromosome 6A from Frontana might not carry a major gene for reducing FHB severity. The mean FHB severity for Frontana 4D was low enough to support the idea that chromosome 4D restricts FHB severity, as was previously proposed by Berzonsky et al. (2007) and Loffler et al. (2009).

Type II Resistance: Disease Spread

Among genotypes in CG1, Frontana 3A and Frontana exhibited the lowest FHB spread within the spike and means for the two genotypes were not significantly different. This suggests the presence of Type II resistance genes on chromosome 3A in Frontana. However, Frontana had lower scores than Frontana 3A, possibly indicating that Frontana carries additional genes for resistance or that minor gene expression is suppressed, if such minor genes are even present in Frontana 3A. Singh et al. (1995) proposed that Frontana expresses both a Type I and Type II resistance and throughout this study, Frontana exhibited resistance to disease incidence, severity, and spread following both spray and point inoculation.

Results pertaining to CG2 illustrate that Frontana 6A might have a gene or genes expressing resistance to spread within the spike since its mean FHB spread was not significantly different from Alsen. In CG3, means for FHB spread of Frontana 4D and Alsen were not significantly different; however, means for FHB spread for both genotypes were significantly different from Frontana. It is likely that chromosome 4D from Frontana has a gene or gene that restricts FHB spread. Buerstmayr et al. (1998) also found that there was a gene for FHB resistance carried on chromosome 4D after they completed a backcross reciprocal monosomic analysis involving 'Hobbit' as a susceptible parent and 'U-136.1' as a resistant parent.

Association of FHB Resistance with Other Traits

Certain agronomic traits have been associated with FHB resistance. In this study, an assessment of linear relationships between such agronomic traits and FHB resistance was

performed. A strong correlation between FHB severity and incidence, suggests a similar genetic control for the two parameters (Groth et al., 1999). The same theory can be applied to the strong correlation between disease severity and VFDK.

Previous reports (Buerstmayr et al., 2000; Hilton et al., 1999; Mesterhazy, 1995) showed that plant height is a factor in resistance and that tall genotypes are more resistant to FHB than short genotypes. In this study, the observed association between plant height and FHB severity was weak. This is probably because of the different plant heights of the genotypes used in this study. For example, Frontana and all its derived RBCM lines are tall and have some level of resistance to FHB. Alsen is a semi-dwarf cultivar with Type II resistance, while Chris is a tall genotype, which is known to be susceptible to FHB. In this germplasm, plant height is possibly controlled by an independent set of genes from the ones that govern resistance to FHB. Consequently, the relationship between plant height and reaction to FHB is not a strong one in all cases. This was also shown by Steiner et al. (2004) who argued that breeders can select for FHB resistance regardless of plant height.

The high correlation between disease severity, TKW, and VFDK signifying that TKW or quality of the kernels decreases with increased disease severity and VFDK. Fusarium head blight spread and severity were also highly associated implying that the two parameters are influenced by the same genes under similar environmental conditions (Groth et al., 1999; Steiner et al., 2004). It seems that there is not much difference between Type I and Type II resistance since FHB spread, incidence, and severity are all highly correlated. Disease incidence and severity from spray inoculated pots were strongly correlated. Similarly, disease incidence and spread using point inoculation were strongly correlated.

The correlations between DON accumulation and FHB incidence, VFDK, and SKNIR-FDK were significant but weak. Fuentes et al. (2005) also calculated a significant but weak correlation between FHB incidence and DON content, while Schlang et al. (2008) reported no significant correlation between level of *F. graminearum* infection and DON content. Audenaert et al. (2009) did not report a significant correlation between FHB severity and DON content. However, season 3 for this study produced higher FHB severity and DON content correlation.

The correlation between FHB severity and DON content was not significant, while there was a significant but weak linear correlation between DON content and FHB incidence. However, the linear relationships between were significant and comparatively strong. These results for correlation between DON content and VFDK and SKNIR-FDK for season 4 are similar to those of Chen et al. (2006) who calculated a high linear correlation between DON content and FDK.

GENERAL CONCLUSIONS

Since a RCBM Frontana 3A exhibited reduced FHB severity and spread, there is likely a gene or genes on chromosome 3A of Frontana that expresses both a Type I and Type II resistance to FHB. Frontana chromosome 4D likely carries a gene or genes expressing a Type I resistance.

Colonization of a wheat spike by *F. graminearum* can occur on any spikelet following spray inoculation. Therefore, as a more precise means of studying spread of the disease was the use of a single floret inoculation technique. Results suggest that Frontana chromosome 3A has a gene or genes expressing resistance to spread in addition to resistance to FHB incidence and severity. A gene or genes on Frontana chromosomes 6A and 4D likely contribute to this resistance to spread of FHB in the spike.

As it represents resistance to FHB at the point of initial infection, incidence or Type I resistance precedes the spread of FHB in the spike. Therefore, in some of the Frontana genotypes studied, both low incidence as well as low FHB severity was observed. For example, Frontana 3A and Frontana 4D exhibited low FHB incidence and severity. Conversely, Frontana 6A did not exhibit resistance to FHB incidence and it expressed high severity. Rarely did Frontana 3A express a higher level of resistance to FHB than did Frontana. Thus, although chromosome 3A from Frontana carries a major source of both Types I and II FHB resistance, the genes on this chromosome do not represent the only genes for FHB resistance in this cultivar.

Strong associations were observed for FHB incidence, spread, severity, and other FHB related traits. Results also demonstrated that kernel quality is affected by FHB severity and

is directly related to FDK. In this study however, plant height was not important for FHB resistance.

The linear relationships between DON and other parameters were but weak, and therefore suggest more complex relationships than simply linear. Nonetheless, more work needs to be done to determine how DON correlates with other FHB related parameters.

REFERENCES

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

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

Atanasoff, D. 1920. Fusarium-blight (scab) of wheat and other cereals. J. Agric. Res. 20:1- 40.

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

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

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

Bai, G., and Q.P. Xiao. 1989. Genetic analysis on resistance to scab in six wheat cultivars. Acta Agric. Shanghai 5:17-23.

Berzonsky, W.A., B.L. Gebhard, E. Gamotin, G.D. Leach, and S. Ali. 2007. A reciprocal backcross monosomic analysis of the scab resistant spring wheat (*Triticum aestivum* L.) cultivar, 'Frontana'. Plant Breeding 126:234-239.

Buerstmayr, H., M. Lemmens, G. Fedak, and P. Ruckenbauer. 1999. Backcross

reciprocal monosomic analysis of Fusarium head blight resistance in wheat

(*Triticum aestivum* L.) Theor. Appl. Genet. 98:76-85.

Buerstmayr, H., M. Lemmens, H. Grausgruber, and P. Ruckenbauer. 1996. Scab

resistance of international wheat germplasm. Cereal Res. Commun. 24:195-

202.

Buerstmayr, H., M. Lemmens, L. Hartl, L. Doldi, B. Steiner, M. Stierschneider, and

P. Ruckenbauer. 2002. Molecular mapping of QTLs for Fusarium head blight

resistance in spring wheat: resistance to fungal spread (Type II resistance).

Theor. Appl. Genet. 104:84-91.

Buerstmayr, H., B. Steiner, L. Hartl, M. Griesser, N. Angerer, D. Lengauer, T.

Miedaner, B. Schneider, and M. Lemmens, 2003. Molecular mapping of QTLs for

Fusarium head blight resistance in spring wheat. II. Resistance to fungal penetration and

spread. Theor. Appl. Genet. 107:503-508.

Buerstmayr, H., B. Steiner, M. Lemmens, and P. Ruckenbauer. 2000. Resistance to

Fusarium head blight in two winter wheat crosses: heritability and trait

associations. Crop Sci. 40:1012-018.

Burlakoti, R.R., R. Estrada Jr., V.V. Rivera, A. Boddeda, G.A. Secor, and T.B.

Adhikari. 2007. Real-Time PCR quantification and mycotoxin production of

Fusarium graminearum in wheat inoculated with Isolates collected from   potato,

sugar beet, and wheat. Phytopathology 97:835-841.

Chen, J., C. A. Griffey, M. A. Saghai Maroof, E. L. Stromberg, R. M. Biyashev, W.

Zhao, M. R. Chappell, T. H. Pridgen, Y. Dong, and Z. Zeng. 2006. Validation

of two major quantitative trait loci for Fusarium head blight resistance in Chinese wheat line W14. Plant Breed. 125: 99-101

Dubin, H.J., L. Gilchrist, L. Reeves, and A. McNab. 1997. Fusarium head scab: Global status and prospects (CIMMYT, Mexico, DF, Mexico).

Evans, C.K., and J. Pope. 2005. Digital image analysis of primary leaf lesions on wheat seedlings of Frontana and Alsen inoculated with *Fusarium graminearum.* p.27. *In* Proc. Natl. Fusarium head blight (NFHB) Forum, Milwaukee, Wisconsin. 11-13 December 2005. Office Max Print, Okemos, MI.

Frohberg, R.C., M. Mergoum, T. Olson, and R.W. Stack. 2006. Registration of 'Alsen' wheat. Crop Sci. 46:2311-2312.

Fuentes, R. G., H. R. Mickelson, R. H. Busch, R. Dill-Macky, C. K. Evans, W. G. Thompson, J. V. Wiersma, W. Xie, Y. Dong, and J. A. Anderson. 2005. Resource Allocation and Cultivar Stability in Breeding for Fusarium Head Blight Resistance in Spring Wheat. Crop Sci. 45:1965-1972.

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

Gomez, K.A. and A.A. Gomez. 1984. Statistical procedures for agricultural research. 2nd ed. John Wiley & Sons, New York.

Grausgruber, H., M. Lemmens, H. Bürstmayr, and P. Ruckenbauer. 1998. Chromosomal location of Fusarium head blight resistance and *in vitro* toxin tolerance in wheat using the Hobbit 'sib' (*Triticum macha*) chromosome substitution lines. J. Genet. Breed. 52:173-180.

Han, F.P., G. Fedak, T. Ouellet, H. Dan, and D.J. Somers. 2005. Mapping of genes
    expressed in *Fusarium graminearum*-infected heads of wheat cultivar
    'Frontana'. Genome 48:88-96.

Heiner, R.R., and D.R. Johnston. 1967. Registration of 'Chris' wheat. Crop Sci. 7:170

Heyne, E.G. and R.W. Livers 1953. Monosomic analysis of leaf rust reaction,
    awnedness, winter injury and seed color in Pawnee wheat. Agron. J. 45:54-58.

Hilton, A.J., P. Jenkinson, T.W. Hollins, and D.W. Parry. 1999. Relationship between
    cultivar height and severity of Fusarium ear blight in wheat. Plant Pathol. 48:202-
    208.

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

Lanning, S.P., D. Habernicht, W.E. Grey, and L.E. Talbert. 2004. Registration of
    'Choteau' wheat. Crop Sci. 44:2264–2265.

Löffler, M., C. Schön, and T. Miedaner. 2009. Revealing the genetic architecture of
    FHB resistance in hexaploid wheat (*Triticum aestivum* L.) by QTL meta-nalysis.
    Mol. Breeding 23:473-488.

Mardi M., L. Pazouki, H. Delavar, M.B. Kazemi, B. Ghareyazie, B. Steiner, R. Nolz,
    M. Lemmens, and H. Buerstmayr. 2006. QTL analysis of resistance to
    Fusarium head blight in wheat using a 'Frontana' derived population. Plant
    Breed. 125:313–317.

Mergoum, M., R.C. Frohberg, and R.W. Stack. 2007. Breeding hard red spring wheat

    for Fusarium head blight resistance: Successes and challenges *in* Wheat

    production in stressed environments, p. 161-167.

Mergoum, M., R.C. Frohberg, R.W. Stack, T. Olson, T.L. Friesen, and J.B.

    Rasmussen. 2008. Registration of 'Faller' spring wheat. J. Plant Registrations

    2:224-229.

Mergoum, M., R.C. Frohberg, R.W. Stack, T. Olson, T.L. Friesen, and J.B.

    Rasmussen. 2006. Registration of 'Glenn' spring wheat. Crop Sci. 46:473-474.

Mesterhazy, A. 1995. Types and components of resistance to Fusarium head blight of

    wheat. Plant Breeding 114:377-386.

Mesterhazy, A., T. Barto'k, and C. Lamper. 2003. Influence of wheat cultivar, species

    of Fusarium, and isolate aggressiveness on the efficacy of fungicides for control of

    Fusarium head blight. Plant Dis. 87:1107-1115.

Mesterhazy, A., H. Buerstmayr, B. Toth, Sz. Lehoczki-Krsjak, A. Szabo-Hever, and

    M. Lemmens. 2007. An improved strategy for breeding FHB resistant wheat

    must include Type I resistance. Proceedings of the 5th Canadian Workshop on

    Fusarium head blight, Delta, Winnipeg, 51-66.

Mesterhazy, A., B. Toth, T. Bartok, and M. Varga. 2008. Breeding strategies against

    FHB in winter wheat and their relations to Type I resistance. Cereal Research

    Comm., 3rd Int. FHB Symposium, Sept. 12 2008, Szeged, Hungary.

McMullen, M., S. Zhong, and S. Neate. 2008. Fusarium head blight (scab) of small

    grains. Deaprtment of Plant Pathology, North Dakota State University, Fargo

    ND.

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

Miedaner, T., B. Schneider, and H.H. Geiger. 2003. Deoxynivalenol (DON) content and Fusarium head blight resistance in segregating populations of winter rye and winter wheat. Crop Sci. 43:519-526.

Miller, J.D., J.C. Young, and R.D. Sampson. 1985. Deoxynivalenol and Fusarium head blight resistance in spring cereals. Phytopathol. Z. 113:359-367.

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

Pereyra, S.A., R. Dill Macky, and A.L Sims. 2004. Survival and inoculums production of *Gibberella zeae* in wheat residue. Plant Disease 88:724–730.

Schaafsma, A.W., L. Tamburic-Ilincic, J.D. Miller, and D.C. Hooker. 2001. Agronomic considerations for reducing deoxynivalenol in wheat grain. Can. J. Plant Pathol. 23:279-285.

Schlang, N., U. Steriner, H. Dehne, J. Murakami, E. Duveiller, and E. Oerke. 2008. Spatial distribution of Fusarium head blight pathogens and associated mycotoxins in wheat fields. 3rd Int. FHB Symposium, Szeged, Hungary.

Schroeder, H.W., and J.J. Christensen. 1963. Factors affecting the resistance of wheat to scab by *Gibberella zeae*. Phytopathology 53:831–838.

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

Snape, J.W., B.B. Parker, and M.D. Gale. 1983. Use of the backcross reciprocal monosomic method for evaluating chromosomal variation for quantitative

characters: Proceedings of the 6th International Wheat Genetics Symposium, Kyoto Japan 367 – 373. 28 November - 3 December.

Stack, R.W. 1989. Comparison of inoculum potential of ascospores and conidia of *Gibberella zeae*. Can. J. Plant Pathol. 11:137-142

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

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

Tacke, B.K., and H.H. Casper. 1996. Determination of deoxynivalenol in wheat, barley, and malt by column cleanup and gas chromatography with electron capture detection. J. AOAC Int. 79(2):472-5.

Tamburic-Ilincic, L., A.W. Schaafsma, D. Falk, B. Laskar, G. Fedak, and D. Somers. 2006. Registration of winter wheat germplasm line RCATL33 with Fusarium head blight resistance and reduced deoxynivalenol accumulation. Crop Sci. 46:1399-1400.

Van Eeuwijk, E.A., A. Mesterhazy, C.I. Kling, P. Ruckenbauer, L. Saur, H. Burstmayr, M. Lemmens, L.C.P. Keizer, N. Maurin, and C.H.A. Snijders. 1995. Assessing nonspecificity 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.

Waalwijk, C., R. Heide, I. Vries, T. Lee, C. Schoen, C. Corainville, I. Hauser-Hahn, P. Kastelein, J. Kohl, P. Lonnet, T. Demarquet, and G.H.J. Kema. 2004.

Quantitative detection of Fusarium species in wheat using TaqMan. European J. Plant Pathol. 110: 481–494, 2004.

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

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

Wegulo, S. N., and F.E. Dowel. 2008. Near-infrared versus visual sorting of *Fusariium* damaged kernels in winter wheat. Can. J. Plant Sci. 88:1087-1089.

Yang, Z., J. Gilbert, G. Fedak, and D.J. Somers. 2005. Genetic characterization of QTL associated with resistance to Fusarium head blight in a doubled-haploid spring wheat population. Genome 48:187-196.

Yang, Z.P., J. Gilbert, D.J. Somers, G. Fedak, J.D. Procunier, and R. McKenzie. 2003. Marker-assisted selection of Fusarium head blight resistance genes in two doubled-haploid populations of wheat. Mol. Breeding 12:309-317.

Zhou, W.C., F.L. Kolb, G. Bai, G. Shaner, and L.L. Domier. 2002. Genetic analysis of scab resistance QTL in wheat with microsatellite and AFLP markers. Genome 45:719-727.

APPENDIX A. ANOVA TABLES FOR ALL PARAMETERS EVALUATED

Table A1: Combined analysis of variance for Fusarium head blight severity showing source of variation (SOV), degrees of freedom (df), sum of squares (SS), means sum of squares (MS), and F-value.

SOV	df	SS	MS	F Value
Season	3	107638	35879	
Inoculation Type (IT)	1	1867.82	1867.82	
CG†	2	273.65	136.83	0.18
Genotype(CG)	15	106432	7095.44	7.66***
IT*Genotype(CG)	15	4463.28	297.55	0.66
Season*Genotype(CG)	51	47549.0	932.34	2.67***
Residual	322	112283	348.70	

***F-test significant at p=0.001
†Chromosome Group

Table A2: Combined analysis of variance for Fusarium head blight incidence showing source of variation (SOV), degrees of freedom (df), sum of squares (SS), means sum of squares (MS), and F-value.

SOV	df	SS	MS	F Value
Season	3	103310	34437	
Inoculation Type (IT)	1	19585	19585	
CG†	2	242.26	121.13	0.08
Genotype(CG)	15	118694	7912.94	5.47***
IT*Genotype(CG)	15	26815	1787.65	3.03**
Season*Genotype(CG)	51	73855	1448.13	2.45***
Residual	322	190128	590.46	

***F-test significant at p=0.001
**F-test significant at p=0.01
†Chromosome Group

49

Table A3: Seasons 1, 2 and 4 combined analysis of variance for visually assessed Fusarium damaged kernels (VFDK) showing source of variation (SOV), degrees of freedom (df), sum of squares (SS), means sum of squares (MS), and F-value.

SOV	df	SS	MS	F Value
Season	2	23845	11922	
Inoculation Type (IT)	1	6103.09	6103.09	
CG†	2	2920.53	1460.26	2.58
Genotype(CG)	15	27882	1858.77	9.35***
IT*Genotype(CG)	15	1010.06	67.34	0.40
Season*Genotype(CG)	34	12170	357.94	2.10***
Residual	234	39813	170.14	

***F-test significant at p=0.001
†Chromosome Group

Table A4: Analysis of variance for visually assessed Fusarium damaged kernels for season 3 showing source of variation (SOV), degrees of freedom (df), sum of squares (SS), means sum of squares (MS), and F-value.

SOV	df	SS	MS	F Value
Rep	2	17560	8780.03	
CG†	2	798.87	399.43	2.56
Genotype(CG)	15	33997	2266.48	5.21***
IT‡*Genotype(CG)	15	4425.63	295.04	0.68
Residual	60	26114	435.23	

***F-test significant at p=0.001
†Chromosome Group
‡Inoculation Type

50

Table A5: Combined analysis of Fusarium damaged kernels using the single kernel near-infrared (SKNIR) technique showing source of variation (SOV), degrees of freedom (df), sum of squares (SS), means sum of squares (MS), and F-value.

SOV	df	SS	MS	F Value
Season	3	120918	40306	
Inoculation Type (IT)	1	6725.31	6725.31	
CG†	2	3540.54	1770.27	0.94
Genotype(CG)	15	80324	5354.93	4.29***
IT*Genotype(CG)	15	2559.35	170.62	0.34
Season*Genotype(CG)	51	64000	1254.90	2.49***
Residual	322	162334	504.14	

***F-test significant at p=0.001
†Chromosome Group

Table A6: Combined analysis of thousand kernel weight (TKW) showing source of variation (SOV), degrees of freedom (df), sum of squares (SS), means sum of squares (MS), and F-value.

SOV	df	SS	MS	F Value
Season	3	12265	4088.37	
Inoculation Type (IT)	1	1226.07	1226.07	
CG†	2	88.56	44.28	1.31
Genotype(CG)	15	12411	827.39	10.09***
IT*Genotype(CG)	15	493.30	32.89	0.70
Season*Genotype(CG)	51	4184.18	82.04	0.74**
Residual	322	15160	47.08	

***F-test significant at p=0.001
**F-test significant at p=0.01
†Chromosome Group

Table A7: Combined analysis of plant height showing source of variation (SOV), degrees of freedom (df), sum of squares (SS), means sum of squares (MS), and F-value.

SOV	df	SS	MS	F Value
Season	3	75218	25073	
Inoculation Type (IT)	1	11.21	11.21	
CG†	2	183.76	91.89	0.24
Genotype(CG)	15	149577	9971.82	26.30***
IT*Genotype(CG)	15	1351.99	90.13	0.69
Season*Genotype(CG)	51	19342	379.26	2.88***
Residual	322	42336	131.48	

***F-test significant at p=0.001
†Chromosome Group

Table A8: DON analysis for season 1 from gas capillary chromatography technique showing source of variation (SOV), degrees of freedom (df), sum of squares (SS), means sum of squares (MS), and F-value.

SOV	df	SS	MS	F Value
Rep	2	20.13	10.06	
CG†	2	8.32	4.160	4.50
Genotype(CG)	15	29.84	1.99	0.72
IT‡*Genotype(CG)	15	44.30	2.95	1.07
Residual	41	113.08	2.76	

†Chromosome Group
‡Inoculation Type

Table A9: DON analysis for season 2 from gas capillary chromatography technique showing source of variation (SOV), degrees of freedom (df), sum of squares (SS), means sum of squares (MS), and F-value.

SOV	df	SS	MS	F Value
Rep	2	718.16	359.08	3.41
CG†	2	349.02	174.51	1.32
Genotype(CG)	15	4262.14	284.14	2.00*
IT‡*Genotype(CG)	15	3932.27	262.15	1.84
Residual	48	63444	167.40	

*F-test significant at 0.05
†Chromosome Group
‡Inoculation Type

Table A10: DON analysis for season 2 from single kernel technique showing source of variation (SOV), degrees of freedom (df), sum of squares (SS), means sum of squares (MS), and F-value.

SOV	df	SS	MS	F Value
Rep	2	718.16	359.08	
CG†	2	234075	117038	10.55
Genotype(CG)	15	552082	36805	2.29*
IT‡*Genotype(CG)	15	304876	20325	1.26
Residual	60	964382	16073	

*F-test significant at 0.05
†Chromosome Group
‡Inoculation Type

53

Table A11: DON analysis for season 3 from single kernel technique showing source of variation (SOV), degrees of freedom (df), sum of squares (SS), means sum of squares (MS), and F-value.

SOV	df	SS	MS	F Value
Rep	2	718.16	359.08	
CG†	2	2697.19	1348.60	0.41
Genotype(CG)	15	117325	7821.65	2.92**
IT‡*Genotype(CG)	15	31991	2132.72	0.80
Residual	60	160666	2677.77	

**F-test significant at 0.01
†Chromosome Group
‡Inoculation Type

Table A12: DON analysis for season 4 from single kernel technique showing source of variation (SOV), degrees of freedom (df), sum of squares (SS), means sum of squares (MS), and F-value.

SOV	df	SS	MS	F Value
Rep	2	12755	6377.30	
CG†	2	1017.57	508.79	0.69
Genotype(CG)	15	27797	1853.13	2.72**
IT‡*Genotype(CG)	15	12778	851.90	1.25
Residual	58	39540	681.72	

**F-test significant at 0.01
†Chromosome Group
‡Inoculation Type

APPENDIX B. LEAST SQUARE MEAN TABLES FOR BOTH VISUAL AND SKNIR FDK AND TKW UNDER BOTH SPRAY AND SINGLE FLORET INOCULATION

Table B1: Genotypes, means, t-test values, and probabilities of visual Fusarium damaged kernels (VFDK) in three chromosome groups under spray inoculation method for greenhouse seasons 1, 2, and 4.

Genotype	CG†	VFDK (%)	t-test-value	t-Probability.
Alsen	1	4.10	0.48	0.6348
Choteau	1	22.99	2.67	0.0082
Chris	1	12.71	1.47	0.1416
Chris 3A	1	19.58	2.27	0.0239
Frontana 3A	1	7.85	0.91	0.3631
Frontana	1	2.13	0.25	0.8050
Alsen	2	2.08	0.24	0.8091
Choteau	2	30.07	3.49	0.0006
Chris	2	12.92	1.50	0.1349
Chris 6A	2	11.52	1.34	0.1823
Frontana 6A	2	7.77	0.90	0.3681
Frontana	2	2.73	0.32	0.7512
Alsen	3	4.58	0.52	0.6043
Choteau	3	29.30	3.40	0.0008
Chris	3	20.81	2.41	0.0165
Chris 4D	3	15.32	1.78	0.0767
Frontana 4D	3	12.78	1.48	0.1394
Frontana	3	2.49	0.29	0.7724

†Chromosome Group

Table B2: Genotypes, means, t-test values, and probabilities of visual
Fusarium damaged kernels (VFDK) in three chromosome groups under
point inoculation method for greenhouse seasons 1, 2, and 4.

Genotype	CG†	VFDK (%)	t-test-value	t-Probability
Alsen	1	7.03	0.82	0.4151
Choteau	1	30.57	3.55	0.0005
Chris	1	22.79	2.62	0.0090
Chris 3A	1	21.97	2.55	0.0114
Frontana 3A	1	14.88	1.73	0.0855
Frontana	1	5.00	0.58	0.5623
Alsen	2	7.50	0.87	0.3849
Choteau	2	35.56	4.13	<.0001
Chris	2	22.72	2.64	0.0089
Chris 6A	2	15.43	1.79	0.0746
Frontana 6A	2	19.84	2.30	0.0221
Frontana	2	8.47	0.98	0.3265
Alsen	3	10.57	1.23	0.2211
Choteau	3	45.78	5.31	<.0001
Chris	3	31.43	3.65	0.0003
Chris 4D	3	28.58	3.32	0.0011
Frontana 4D	3	33.99	3.94	0.0001
Frontana	3	15.79	1.83	0.0681

†Chromosome Group

56

Table B3: Means, t-test values, and probabilities of visual Fusarium
damaged   kernels (VFDK) of the genotypes and the chromosome under
evaluation using spray inoculation method for greenhouse season 3.

Genotype	CG†	VFDK (%)	t-test-value	t-Probability
Alsen	1	13.87	0.94	0.3500
Choteau	1	42.83	2.91	0.0051
Chris	1	31.87	2.16	0.0344
Chris 3A	1	49.40	3.36	0.0014
Frontana 3A	1	48.47	3.29	0.0017
Frontana	1	0.73	0.05	0.9604
Alsen	2	9.67	0.66	0.5139
Choteau	2	25.80	1.75	0.0848
Chris	2	29.70	2.02	0.481
Chris 6A	2	35.87	2.44	0.0178
Frontana 6A	2	40.37	2.74	0.0080
Frontana	2	0.00	0.00	1.0000
Alsen	3	3.83	0.26	0.7954
Choteau	3	32.57	2.21	0.0308
Chris	3	64.73	4.40	<.0001
Chris 4D	3	48.27	3.28	0.0017
Frontana 4D	3	1.93	0.13	0.8959
Frontana	3	0.17	0.01	0.9910

†Chromosome Group

Table B4: Means, t-test values, and probabilities of visual Fusarium damaged kernels (VFDK) under point inoculation method for greenhouse season 3.

Genotype	CG†	VFDK (%)	t-test-value	t-Probability
Alsen	1	10.70	0.73	0.4701
Choteau	1	56.27	3.82	0.0003
Chris	1	42.13	2.86	0.0058
Chris 3A	1	35.83	2.43	0.0179
Frontana 3A	1	14.23	0.97	0.3375
Frontana	1	0.87	0.06	0.9532
Alsen	2	11.63	0.79	0.4325
Choteau	2	18.77	1.27	0.2073
Chris	2	46.30	3.15	0.0026
Chris 6A	2	31.03	2.11	0.0392
Frontana 6A	2	20.03	1.36	0.1786
Frontana	2	0.30	0.02	0.9838
Alsen	3	6.00	0.41	0.6850
Choteau	3	45.40	3.08	0.0031
Chris	3	44.40	3.02	0.0037
Chris 4D	3	38.27	2.60	0.0117
Frontana 4D	3	6.60	0.45	0.6555
Frontana	3	0.00	0.45	1.0000

†Chromosome Group

Table B5: Means, t-test values, and probabilities of Fusarium damaged kernels (FDK) using the single kernel near-infrared technique (SKNIR) under spray inoculation method.

Genotype	CG†	SKNIR-FDK (%)	t-test-value	t- Probability
Alsen	1	36.65	2.77	0.0060
Choteau	1	57.75	4.36	<.0001
Chris	1	43.15	3.26	0.0012
Chris 3A	1	34.25	2.58	0.0102
Frontana 3A	1	35.05	2.65	0.0086
Frontana	1	16.11	1.22	0.2250
Alsen	2	39.02	2.94	0.0035
Choteau	2	55.46	4.19	<.0001
Chris	2	39.82	3.00	0.0029
Chris 6A	2	28.64	2.16	0.0314
Frontana 6A	2	31.36	2.37	0.0185
Frontana	2	11.56	0.87	0.3837
Alsen	3	45.64	3.40	0.0008
Choteau	3	64.05	4.83	<.0001
Chris	3	46.20	3.49	0.0006
Chris 4D	3	37.98	2.87	0.0044
Frontana 4D	3	37.18	2.81	0.0053
Frontana	3	21.83	1.65	0.1005

†Chromosome Group

Table B6: Means, t-test values, and probabilities of Fusarium damaged kernels (FDK) of the genotypes under evaluation using single kernel near-infrared technique (SKNIR) with point inoculation method.

Genotype	CG†	SKNIR-FDK (%)	t-test-value	t-Probability
Alsen	1	46.04	3.47	0.0006
Choteau	1	64.79	4.89	<.0001
Chris	1	56.73	4.23	<.0001
Chris 3A	1	49.69	3.75	0.0002
Frontana 3A	1	37.63	2.84	0.0048
Frontana	1	15.86	1.20	0.2323
Alsen	2	47.10	3.55	0.0004
Choteau	2	61.52	4.64	<.0001
Chris	2	44.19	3.34	0.0010
Chris 6A	2	39.17	2.96	0.0033
Frontana 6A	2	45.88	3.46	0.0006
Frontana	2	20.49	1.55	0.1230
Alsen	3	47.33	3.57	0.0004
Choteau	3	76.28	5.76	<.0001
Chris	3	58.52	4.42	<.0001
Chris 4D	3	50.97	3.85	0.0001
Frontana 4D	3	39.19	2.96	0.0033
Frontana	3	22.56	1.70	0.0896

†Chromosome Group

Table B7: Means, t-test values, and probabilities of thousand kernel weight (TKW) of the genotypes in the three chromosome groups under point inoculation method.

Genotype	CG†	TKW(g)	t-test-value	t-Probability
Alsen	1	31.09	8.13	<.0001
Choteau	1	21.10	5.52	<.0001
Chris	1	21.95	5.70	<.0001
Chris 3A	1	25.79	6.75	<.0001
Frontana 3A	1	29.78	7.79	<.0001
Frontana	1	37.56	9.83	<.0001
Alsen	2	31.66	8.28	<.0001
Choteau	2	22.56	5.90	<.0001
Chris	2	25.13	6.57	<.0001
Chris 6A	2	27.82	7.28	<.0001
Frontana 6A	2	25.68	6.72	<.0001
Frontana	2	39.17	10.25	<.0001
Alsen	3	29.38	7.68	<.0001
Choteau	3	16.48	4.31	<.0001
Chris	3	22.80	5.96	<.0001
Chris 4D	3	26.80	7.01	<.0001
Frontana 4D	3	29.83	7.80	<.0001
Frontana	3	37.63	9.84	<.0001

†Chromosome Group

Table B8: Means, t-test values, and probabilities of thousand kernel weight (TKW) of the genotypes in the three chromosome groups under spray inoculation method.

Genotype	CG†	TKW(g)	t-test-value	t-Probability
Alsen	1	33.78	8.84	<.0001
Choteau	1	23.14	6.05	<.0001
Chris	1	30.08	7.87	<.0001
Chris 3A	1	31.85	8.33	<.0001
Frontana 3A	1	34.53	9.03	<.0001
Frontana	1	39.10	10.23	<.0001
Alsen	2	32.58	8.52	<.0001
Choteau	2	24.67	6.45	<.0001
Chris	2	28.87	7.55	<.0001
Chris 6A	2	31.83	8.32	<.0001
Frontana 6A	2	30.90	8.08	<.0001
Frontana	2	37.24	9.74	<.0001
Alsen	3	36.21	9.35	<.0001
Choteau	3	21.47	5.62	<.0001
Chris	3	26.34	6.89	<.0001
Chris 4D	3	28.73	7.51	<.0001
Frontana 4D	3	32.00	8.37	<.0001
Frontana	3	39.98	10.46	<.0001

†Chromosome Group

APPENDIX C. LINEAR RELATIONSHIPS FIGURES FOR VARIOUS FHB RELATED PARAMETERS
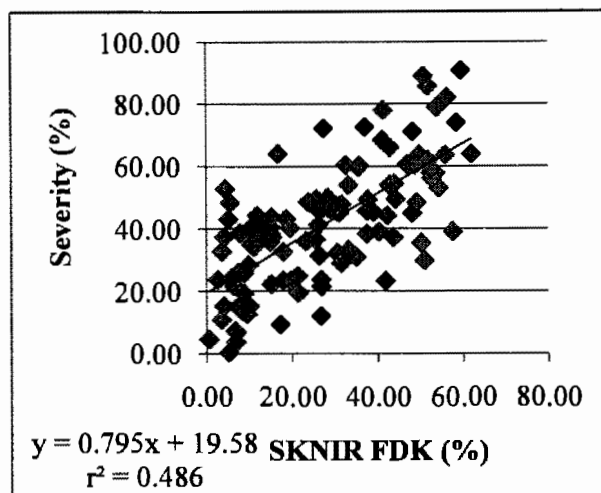
Fig. C1: Linear relationship between FHB severity and FDK using SKNIR technique.
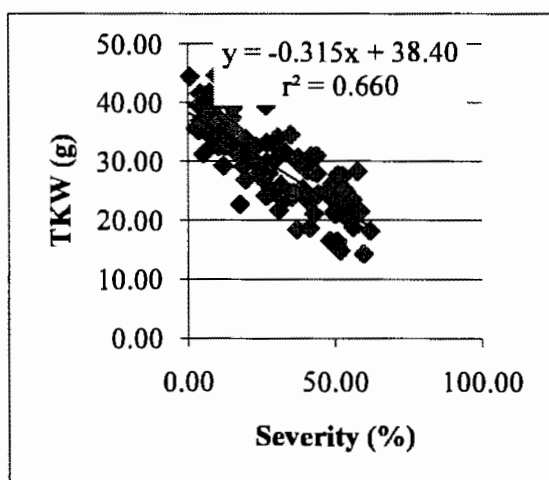
Fig. C2: Linear relationship between TKW and FHB severity.
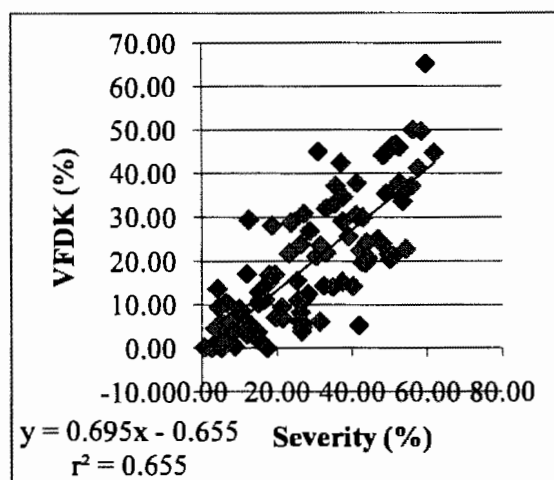
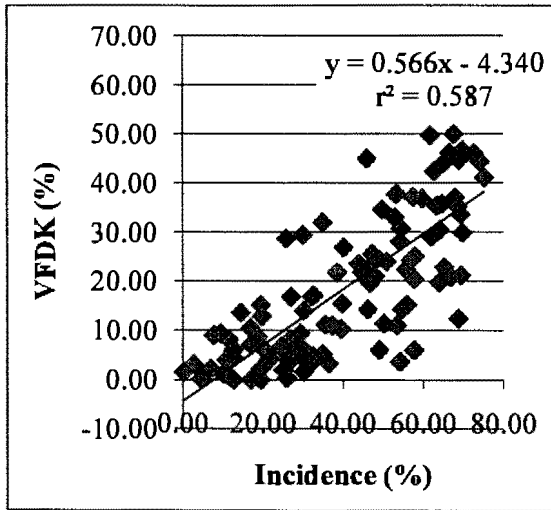Fig. C3: Linear relationship between VFDK and FHB severity.

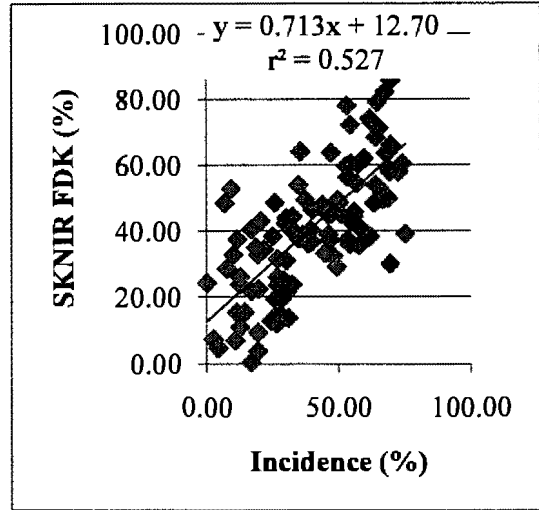Fig. C4: Linear relationship between VFDK and FHB incidence.

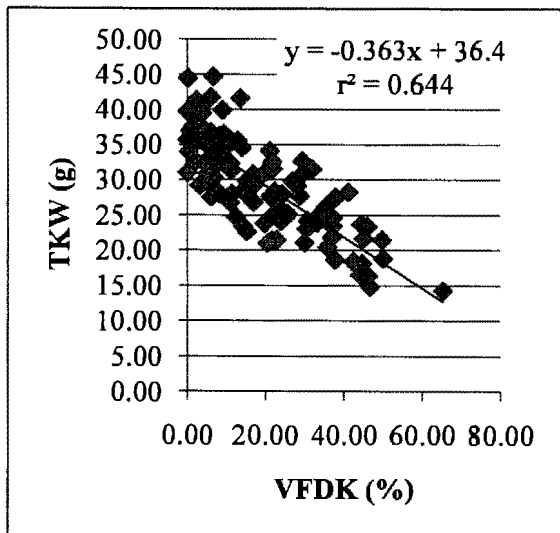Fig. C5: Linear relationship between FDK using SKNIR technique and FHB incidence.

Fig. C6: Linear relationship between TKW and FHB VFDK.
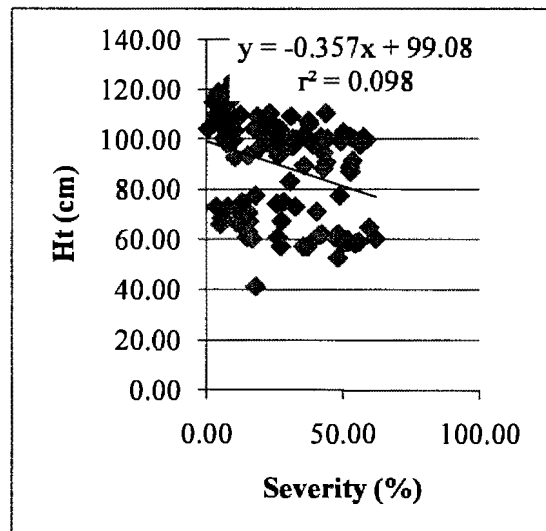
Fig. C7: Linear relationship between plant height (Ht) and FHB severity.
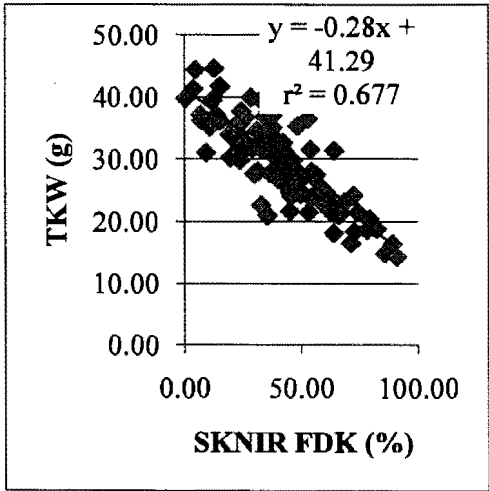
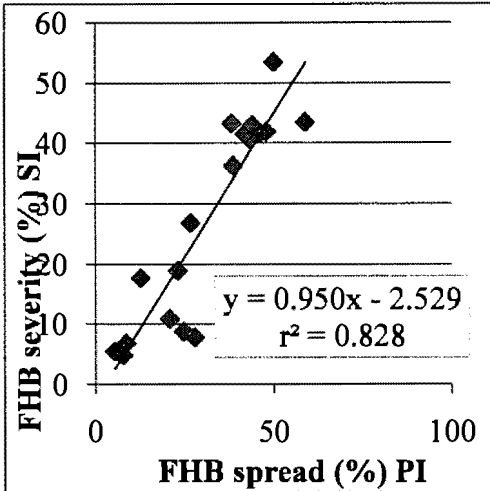Fig. C8: Linear relationship between TKW and FDK using SKNIR technique

Fig. C9: Linear relationship between FHB severity with SI and FHB spread following PI.
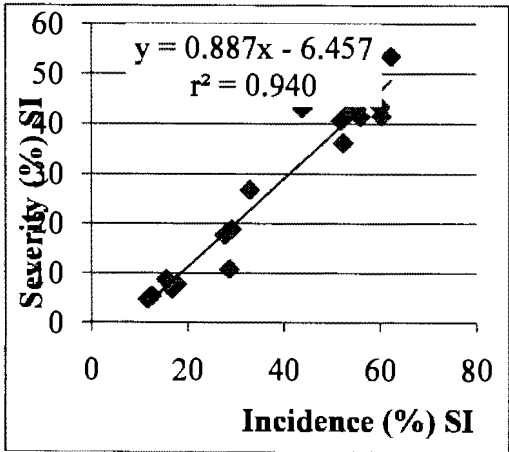

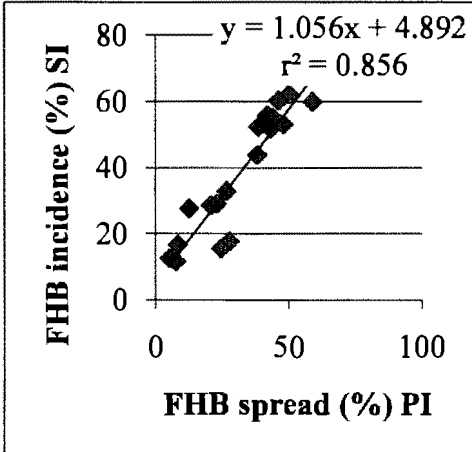Fig. C10: Linear relationship between FHB severity with SI and FHB incidence following SI.

Fig. C11: Linear relationship between FHB incidence with SI and FHB spread following PI.