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FUSARIUM HEAD BLIGHT OF WHEAT: MECHANISMS OF
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FROM A NEW SOURCE OF RESISTANCE

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FUSARIUM HEAD BLIGHT OF WHEAT: MECHANISMS OF RESISTANCE IN NING 7840 AND IDENTIFICATION OF QTL FROM A NEW SOURCE OF RESISTANCE

By

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ABSTRACT

FUSARIUM HEAD BLIGHT OF WHEAT: MECHANISMS OF RESISTANCE IN NING 7840 AND IDENTIFICATION OF QTL FROM A NEW SOURCE OF RESISTANCE

By

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Fusarium head blight (FHB), caused by *Fusarium spp.* and *Microdochium nivale* is a cereal disease threatening the wheat and barley industries in North America as well as Europe, Asia and South America. FHB causes losses in yield as well as reduced grain quality and contamination with mycotoxins that are a serious danger to the health of humans and animals. Host plant resistance to FHB in wheat is quantitative. To date, few sources have been identified that confer a large amount of resistance to FHB. The most widely recognized and used source of resistance is from the Chinese cultivar 'Sumai 3'. The mechanisms underlying Sumai 3 resistance are not understood. Although Sumai 3 provides a large amount of resistance to spread of FHB within infected spikes, this partial resistance is insufficient to prevent the development of FHB under high disease pressure. Identification and verification of other sources of resistance to FHB is critical to enable breeders to combine sources of resistance for a more effective resistance and to prevent the development of genetic uniformity among elite germplasm. The objectives of this research were twofold: 1) to compare progression of disease in a Sumai 3 derived source of resistance ('Ning 7840') versus a susceptible cultivar according to visual and bioassay techniques, and 2) to map potentially novel quantitative trait loci (QTL) controlling

FHB resistance from a wheat line whose parents include a hexaploid wheat artificially synthesized from wheat's progenitor species. The spread of disease in Ning 7840 was compared to a susceptible genotype, 'Norm', for visual symptoms of spread in the spikelets, visual symptoms of spread in the rachis, and the presence of the fungus in the rachis according to a bioassay. For both genotypes, the spread of the fungus in the rachis preceded the development of symptoms in the corresponding spikelets. For Ning 7840, spread of the fungus in the rachis was either greatly restricted or equal to that of the susceptible genotype Norm. Nonetheless, the number of scabby spikelets was smaller in Ning 7840 than Norm. These results reveal that a primary mechanism of Sumai 3 resistance to the spread of scabby spikelets is the prevention of the infection of non-inoculated spikelets from infected rachis tissue. For the identification of QTL for resistance to FHB, a dihaploid (DH) wheat population from a cross of CASS94 and Flycatcher was investigated. One-hundred and eight DH lines were phenotyped in the greenhouse for resistance to spread of FHB, and 130 DH lines were genotyped using simple sequence repeat (SSR) primer pairs. Single marker analysis and composite interval mapping identified the locations of one major QTL for resistance on chromosome arm 2DL, and minor QTL for resistance on chromosome 4A and chromosome arms 5AL and 5DL. These results unequivocally verify reports that a QTL controlling resistance to the spread of scabby spikelets is present on chromosome arm 2DL.

Dedicated to my wonderful parents Frances and Chester Lewis

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

KEY TO SYMBOLS AND ABBREVIATIONS

AUDPC – Area under the disease progress curve

BARC – Beltsville Agricultural Research Center

BIRS – Bioassay infected rachis sections

CIM – Composite interval mapping

CIMMYT – International Center for the Improvement of Maize and Wheat

CMC – Carboxymethyl cellulose

DON – Deoxynivalenol

DH – Dihaploid

DPI – Days post inoculation

FHB – Fusarium head blight

GWM – Glatersleben Wheat Microsatellite

GDM – Glastenben D-genome Microsatellite

LOD – Log of odds ratio

MSU – Michigan State University

PCR – Polymerase chain reaction

PR – Pathogenesis related

QTL – Quantitative trait loci

R^2 – Coefficient of determination

REC – Recombination frequency

SMA – Single marker analysis

SAR – Systemic acquired resistance

SS – Number of scabby spikelets

SSR – Simple sequence repeat

VIRS – Visually infected rachis sections

WMC – Wheat Microsatellite Consortium

CHAPTER 1

FUSARIUM HEAD BLIGHT IN WHEAT: IMPORTANCE, PATHOLOGY AND HOST PLANT RESISTANCE.

1.1 Wheat: Origin and Importance

Wheat domestication is thought to have initiated in the Fertile Crescent over 10,000 years ago (Feldman, 2001). Today's common wheat (*Triticum aestivum* L. ssp. *aestivum*) is a self-pollinating allohexaploid (2N = 6X = 42, AABBDD) consisting of three genomes (2N = 2X = 14), A, B and D. These three genomes (A, B and D) diverged from a common ancestor, and then converged through hybridization followed by endoreduplication to give rise to *Triticum aestivum* (Feldman, 2001). The extant diploid species *Triticum urartu* (AA) and *Aegilops tauschii* are considred direct descendents of the A and D genomes, respectively (Feldman, 2001; Kimber, 1993). The B genome, on the other hand, is no longer found as a diploid species in nature (Kimber, 1993). It is hypothesized that the B genome was derived from Aegilops speltoides (SS), which is found in nature, or an extinct species with a similar genome (Feldman, 2001). However, the B genome is found in nature in combination with the A genome in the tetraploid *Triticum turgidum*, from which *Triticum durum* was domesticated. *T. turgidum* is also the donor of the A and B genomes to *T. aestivum*. The similarity of the three genomes of wheat is great enough that apart from a single locus, *Ph 1* (pairing homoeologous) on chromosome 5B,

homoeologous chromosomes from the three genomes would pair during meiosis (Kimber and Sears, 1987). The *Ph 1* locus on chromosome 5B prevents non-homologous chromosome pairing during meiosis (Kimber and Sears, 1987). Thus, although *T. aestivum* is technically a hexaploid, it exhibits disomic inheritance for individual loci.

The original polyploidization events leading to the tetraploid and hexaploid wheats each represent a bottleneck and a dramatic form of a founder effect in wheat (Ladizinsky, 1985). Interspecific and intergeneric crosses between *T. aestivum* and its progenitors have proven to be valuable tools for introgression of desirable traits into *T. aestivum* (Maan, 1987; Mujeeb-Kazi, 1993). Synthetic wheats are one product of interspecific crosses that have been particularly successful for the introgression of traits (Mujeeb-Kazi, 1995). As described above, hexaploid wheat is composed of the three genomes: A, B and D. Synthetic wheats are hexaploids that are created by combining sources of the A, B and D genomes into a single hexaploid. A common method of creating a synthetic wheat is through hybridizing *Triticum durum* or *Triticum turgidum* (tetraploids containing the A and B genomes) with *Aegilops tauschii* (a diploid of the D genome) (Mujeeb-Kazi, 1993). Chromosome doubling, induced or spontaneous, in the F1 hybrids of these crosses results in a hexaploid wheat (Mujeeb-Kazi, 1995). A powerful application of synthetic wheats is the exploitation of diversity present in *Ae. tauschii* that is not represented in cultivated common wheat due to a founder effect during the natural evolution of *T. aestivum* (Ladizinsky, 1985; Lage et al., 2003b; Lubbers et al., 1991).

Synthetic wheats with desirable traits can be crossed with common wheat for the improvement of elite cultivars. Examples of valuable traits that have been identified in synthetic wheats include agronomically desirable traits such as high yield (Gororo et al., 2002; Haung et al., 2003), pre-harvest sprouting resistance (Gatford et al., 2002; Lan et al., 1997), resistance to waterlogging (Villareal et al., 2001), tolerance to high temperatures (Yang et al., 2002), enhanced photosynthetic performance (Del Blanco et al., 2000), ear emergence time (Haung et al., 2003), plant height (Haung et al., 2003), increased grain weight (Haung et al., 2003), increased grain element concentrations (Calderini and Ortiz-Monasterio, 2003) and components of breadmaking qualities (Pfluger et al., 2001; Wieser et al., 2003). In addition, valuable resistance to biotic factors have been identified in synthetic wheats including resistance to Fusarium head blight (Gilchrist et al., 1999; Mujeeb-Kazi et al., 1999), Karnal bunt (Villareal et al., 1995a; Villareal et al., 1995b), Septoria tritici blotch (Arraiano et al., 2001), leaf and stem rust (Innes and Kerber, 1994) and insects (Lage et al., 2003a; Smith and Starkey, 2003). Desirable traits of have been identified in *Ae. tauschii* accessions through screening them directly before the creation of synthetics, though some traits con not be practically screened in *Ae. tauschii* (Mujeeb-Kazi, 1993). However, it has been observed that many positive traits can be identified in a synthetic without prior screening in *Ae. tauschii*, and that A and B genomes can also affect the expression (positively or negatively) of desirable traits in the synthetic wheat (Mujeeb-Kazi, 1993).

Intergeneric hybridizations have also been used to introgress important traits into *Triticum aestivum*, however intergeneric crosses have a lower success rate and therefore require more time than interspecfic crosses (Mujeeb-Kazi, 1993). Nonetheless, intergeneric hybrids have been successfully made between *Triticum* and several other species including *Secale*, *Agrypyron*, *Hordeum*, *Haynaldia* and *Elymus* (Bommineni and Jauhar, 1997; Maan, 1987; Mujeeb-Kazi, 1993)

Although wide crosses, such as interspecific and intergeneric crosses, can be a good resource for genetic diversity and the identification and introgression of valuable traits, there are disadvantages of using wide crosses between plant species. Some disadvantages of these types of crosses include cross incompatibility, poor vigor in the F1 hybrid, hybrid sterility (often because of a lack of chromosome pairing during meiosis), poor vigor or fertility in the F2 generation (Hadley and Openshaw, 1990), and segregation distortion (Xu et al., 1997). However, wheat synthetics have been observed to behave like common wheat. The high similarity in genome structure between synthetic wheats and domesticated wheats is illustrated by the fact that the wheat genetics community today uses a synthetically derived population (from a synthetic crossed with a common wheat) as its standard reference mapping population and the mapping positions of marker loci are confirmed in other maps (Gupta et al., 2002; Hazen et al., 2002; Mingeot and Jacquemin, 1999; Pestsova et al., 2000; Röder et al., 1998; Waldron et al., 1999).

Since its domestication, wheat has become the most widely adapted cereal crop (Briggle and Curtis, 1987) and a fundamental staple food crop around the world. In the last forty years alone, global wheat production has increased dramatically. In the 1990's (1995-1999) global wheat production (in tones) was more than double that in the 1960s (Marathee and Gomez-Macpherson, 2001). In addition, wheat demands are expected to increase into the future. Projections until 2030 suggest that imports of wheat in developing nations will continue to grow in compensation for an increase in population as well as a shift towards more wheat based products (Marathee and Gomez-Macpherson, 2001). The United States is the largest exporter of wheat in the world (Marathee and Gomez-Macpherson, 2001; Landes et al., 1998) even though the amount of wheat being used domestically in the U.S. (1996 to 2003) has been greater than the amount of wheat exported (Wescott, 2004). Yield per unit area land is required to meet projected demand since additional land for production is limited (Marathee and Gomez-Macpherson, 2001).

1.2 Impact of Fusarium Head Blight

Fusarium head blight (FHB), caused by *Fusarium* spp. and *Microdochium nivale* (Parry et al., 1995), is a disease of wheat and barley currently threatening the wheat and barley industries in North America as well as Europe, Asia and South America (Cook, 1981; McMullen et al., 1997; Snijders, 1990; Stack, 2003). FHB leads to reduction in yield and grain quality. Most critically, contamination of grain with *Fusarium* mycotoxins (especially deoxynivalenol) poses health risks

and diminishes the value of FHB affected grain (McMullen et al., 1997; Snijders, 1990; Wannemacher and Wiener, 1997). Fusarium head blight has been observed in North America since 1884 (Arthur, 1891; Stack 2003), and frequent outbreaks occurred throughout the twentieth century including particularly serious episodes between 1928 and 1937 (McMullen et al., 1997). More recently, the United States has seen an alarming increase in the frequency of FHB epidemics. In the 1990's alone, losses to the wheat and barley industries in the United States due to FHB were estimated to be nearly three billion U.S. dollars (Windels, 2000). Changes in agronomic practices in the United States, including shorter crop rotations and reduced tillage, are considered important factors contributing to the increased frequency of FHB epidemics in recent years (Dill-Macky and Jones, 2000).

1.3 FHB Causal Organisms

Sixteen species of *Fusarium*, and one species of *Microdochium* (*M. nivale*, formerly known as *Fusarium nivale*) have been associated with FHB of small grains around the world (Parry et al., 1995). In North America and Asia, FHB is predominantly caused by *F. graminearum* (Schwabe) (perfect stage *Gibberella zeae* (Schw.) Petch) (Gale et al., 2002; Parry et al., 1995). In Northern Europe, *F. culmorum* is the primary causal agent of FHB (Parry et al., 1995). FHB research has increased recent years, and the majority of this research has focused on *F. graminearum* and *F. culmorum*. The two species, *F. graminearum* and *F. culmorum*, are considered closely related, and resistance to *F.*

graminearum and *F. culmorum* have been similar in both spring and winter wheats (Mesterhazy, 1987; Snijders and Perkowski, 1990; Van Eeuwijk et al., 1995). This review will focus on *F. graminearum* and *F. culmorum*. In addition, although ascospores serve as the primary inoculum in natural field infections, artificial inoculations in the greenhouse and field are often conducted with macroconidia of *Fusarium* spp. Ascospores of *G. zeae* and conidia of *F. graminearum* give similar levels of incidence and severity of disease (Stack, 1989) and both are considered in studies of infection and host plant resistance. In this review, studies using ascospores as inoculum are not distinguished from those using macroconidia.

1.4 Invasion and Colonization of Wheat by *Fusarium*

Initial infection of *Fusarium* on wheat spikes often occurs during warm, wet weather at the time of wheat anthesis (Andersen, 1948; Francl et al., 1999). Ascospores of *G. zeae* are forcibly ejected from perithecia (often on corn or wheat residue in the field) and land on wheat spikes (Andries et al., 2000). The identification of the plant organs most often initially infected by the spores remains unclear. Several studies suggest that anthers extruded during anthesis are the primary target for initial infection, or are important factors in infection (Andersen, 1948; Pugh et al., 1933; Ribichich et al., 2000; Strange and Smith, 1971). Pugh et al. (1933) found that the progression of infection followed the general progression of anthesis, which begins in the center of the spike and proceeds outward towards the tip and base of the spike. Andersen (1948)

confirmed that infection appeared to be dependent upon anthesis. Furthermore, Pugh et al. (1933) found that spikelets that remained closed (did not extrude their anthers) were unlikely to become infected and their histological observations revealed that early stages of colonization frequently began on the anthers and not on the glumes. Similar results were found in a study by Strange and Smith, 1971, where by 48 hours after inoculation anthers were abundantly colonized while other tissue was generally free of infection. In addition, a comparison of the infection of emasculated and non-emasculated heads showed that extruded anthers promote the infection of other parts of the wheat head, and that extracts of anthers stimulated growth of *F. graminearum* more than extracts of other wheat spike tissues (Strange and Smith, 1971). Schroeder and Christensen (1963) found that different host genotypes varied in degree of susceptibility at different stages of maturity (before, during and after anthesis) suggesting that extruded anthers may not always be the only plant tissue to be initially infected since anthers are not exposed before anthesis and are eventually lost after anthesis. In addition, infection of other organs of the spike was observed in several studies. Bennett (1931) induced infection with droplets of inoculum placed on the edges of the glumes and palea. However, the thickness and impenetrability of glumes are thought to impede successful colonization of wheat by *Fusarium* (Pritsch et al., 2000; Pugh et al., 1933). Direct penetration of glumes is rarely reported (Pritsch et al., 2000; Ribichich et al., 2000) although hyphae were observed invading through the stomata of glumes and leaf tissue (Kang and Buchenauer, 2000a; Kang and Buchenauer, 2000c; Pritsch et al.,

1998; Pritsch et al., 2000). *F. culmorum* directly penetrates epidermal cell walls of the lemma and ovary (Kang and Buchenauer, 1999; Kang and Buchenauer, 2000b; Kang and Buchenauer, 2002b). Kang and Buchenauer (2002b) observed infection hyphae develop on the inner surfaces of florets within 36 hours of inoculation. These infection hyphae were outgrowths of hyphae in contact with the inner surface of a floret's lemma, palea and other internal serfaces of a floret. A penetration peg developed from what they described as an infection hyphae (infection hyphae appeared as short hyphae branched off of longer hyphae). The penetration peg did not form from an appressorium, and penetration was direct though not always complete.

From the collection of studies above, it appears that the extrusion of the anthers at anthesis may allow for some initial growth of the fungus, as well as give the fungus easier access to the inner surfaces of the lemma and palea where it can more easily penetrate the host tissues, and that entrance of the fungus through the stomata also occurs. There is, however, a clear and compelling need for additional work to clarify the exacty means by which *Fusarium* infects wheat spikes.

Once the fungus is established in the wheat spike, the fungus colonizes several cell types both inter- and intracellularly (Kang and Buchenauer, 1999; Kang and Buchenauer, 2002b; Pugh et al., 1933; Ribichich et al., 2000; Schroeder and Christensen, 1963). Pugh et al. (1933) found that the mycelia primarily colonize chlorophyllous tissues, while Pritsch et al. (2000) found that when glumes of wheat were inoculated, the fungus tended to colonize the

chlorenchyma and parenchyma. In addition, Pugh et al. (1933) found that although hyphae did not heavily colonize the vascular cells, colonization was greater in of the phloem as opposed to the xylem. In contrast, Schroeder and Christensen (1963) reported that hyphae first colonized vascular bundles, then the surrounding tissues. In a study of *F. culmorum* infection of a susceptible host, the fungus reached the rachis and colonized the xylem vessels and phloem sieve tubes, growing inter- and intracellularly through the parenchyma cells outside the vascular bundles by four to six days post inoculation (Kang and Buchenauer, 1999). From these studies, it is unclear to what extent *F. graminearum* or *F. culmorum* colonizes or the vascular bundles, but it is apparent that the fungus colonizes many different types of host cells.

Cytological investigations of infected wheat kernels have shown that the extent of fungal colonization varies for different sections of the kernel (Pugh et al., 1933; Seitz and Bechtel, 1985). Using serial sections of the kernels, Pugh et al. (1933) observed a difference in colonization of kernels that were infected earlier or later in their maturation. When the ovary was colonized early in seed development, the fungus easily colonized the entire kernel. However, in later infections, hyphae were confined to the pericarp near the embryo and to the embryo itself, possibly because greater resistance of the pericarp to penetration. In contrast, Seitz and Bechtel (1985) found that the fungus colonized throughout the kernel and colonization was most concentrated in the pericarp. Histological studies by Seitz and Bechtel (1985) also found that lightly colonized kernels had hyphae in both the pericarp and the starchy endosperm.

Disease generally progresses from the initially inoculated spikelet, through the rachis to other spikelets on the wheat spike. Some studies have found that the fungus primarily colonizes basipetally from the initial point of inoculation through the rachis (Savard et al., 2000; TeKrony et al., 2000). Other studies have showed substantial colonization both acropetally and basipetally from the point of inoculation (Pritsch et al., 2000; Pugh et al., 1933; Schroeder and Christensen, 1963). In addition, colonization of rachis sections does not always result in infection of spikelets at that point (Pugh et al., 1933; TeKrony et al., 2000).

1.5 Symptoms of FHB

Symptoms of FHB in wheat appear as brownish discoloration, water soaked lesions and loss of chrlorophyll in spikelets and the rachis (Andersen, 1948; Pugh et al., 1933; Savard et al., 2000). In studies of infection with *F. culmorum*, initial symptoms of infection appeared as light brown, water-soaked spots on the glumes (Snijders and Krechting, 1992). Brown discoloration appeared once *F. culmorum* began colonizing intracellularly in another study (Kang and Buchenauer, 1999). However, symptoms, or lack of symptoms, from infections by *F. graminearum* or *F. culmorum*, are not necessarily indicative of the presence or absence of the fungus (Pugh et al., 1933; Schroeder and Christensen, 1963). Several researchers have observed that fungal infection in lower portions of the rachis often results in a loss of chlorophyll, wilting and shriveled kernels in acropetal tissues of the spike (Bai and Shaner, 1996; Kang

and Buchenauer, 1999; Pugh et al., 1933; Savard et al., 2000). Conversely, it was observed that some varieties may have infection in the seed in the absence of visible signs of damage to that tissue (Schroeder and Christensen, 1963). Bushnell et al. (2003) suggests that asymptomatic stage of initial colonization of wheat or barley tissue (Pritsch et al., 2000; Bushnell et al., 2000; Kang and Buchenauer 1999) reflects a biotrophic phase of the pathogen, which is then followed by a necrotrophic phase (killing host cells in advance of colonization) as colonization proceeds (Kang and Buchenauer 2000a).

1.6 *Fusarium* Mycotoxins and Infection

Fusarium mycotoxins have been investigated for their involvement in pathogenicity and virulence of the fungus during infection. Both *F. graminearum* and *F. culmorum* produce trichothecenes (including Deoxynivalenol (DON), 3-acetyldeoxynivalenol (3-ADON), Nivalenol, and T-2 toxin), which are nonvolatile, low molecular weight sesquiterpenoids (Cole and Cox, 1981) that inhibit protein synthesis in plants and many mammals (Wannemacher and Wiender, 1997). To determine if trichothecenes are virulence factors or pathogenicity factors of *F. graminearum* infection of wheat heads, Desjardins et al. (1996) compared the colonizing ability and disease symptoms of a wild type isolate of *G. zeae* and a mutant strain of *G. zeae* that lacked the ability to produce any trichothecene toxins on six different wheat cultivars. The mutant strain was able to infect the wheat heads, but caused fewer disease symptoms. The authors concluded that the trichothecenes are virulence factors and not pathogenicity factors. In a similar

study by Bai et al. (2001) a DON-minus mutant of *F. graminearum* was able to infect wheat but was unable to spread throughout the wheat spike. Wanyoike et al. (2002) tested fifteen different isolates of *F. graminearum* for virulence and mycotoxins production on susceptible ('Agent') and moderately resistant ('Arina') genotypes. DON was the primary trichothecene produced by all fifteen isolates. Isolates differed for virulence on the two genotypes and DON content was significantly correlated with the Area Under the Disease Progress Curve (AUDPC) based on damaged spikelets. Mesterhazy (2002) showed that virulence was associated with the trichothecene production of isolates of *F. culmorum* and *F. graminearum*. Together these results support the hypothesis that trichothecenes are important in the virulence of FHB, but they are not essential for initial infection. Zearalenone, a toxic mycoestrogen is also produced by *F. graminearum*, but no correlation was found with either virulence or pathogenicity in infection of wheat heads (Atanassov et al., 1994; Bagi et al., 2000).

Several studies have examined the localization of trichothecenes with respect to the growth of the fungus. In studies of *F. culmorum* by Kang and Buchenauer (1999 and 2002b) DON and 3-ADON were produced by hyphae in contact with the surface of the host tissue even before penetration of the host. DON was found near penetration pegs before infection, and near hyphae subsequent to infection (Kang and Buchenauer, 1999, 2002b). Toxins were found at lower concentrations in tissue further away from the hyphae (Kang and Buchenauer, 1999, 2002b). Changes in the host cell, such as the breakdown of

the organelles and convolution of the cytoplasm were correlated with the presence of DON and 3-ADON (Kang and Buchenauer, 1999). DON and 3-ADON mycotoxins can be translocated from areas colonized by the fungus either through the xylem and phloem sieve tubes when translocated acropetally or through the phloem sieve tubes when translocated basipetally (Kang and Buchenauer, 2002b). By ten days post inoculation DON and 3-ADON were found in rachis tissue above and below the point initially inoculated, as well as beyond the area colonized by the fungus (Kang and Buchenauer, 1999). Snijders and Krechting (1992) found similar results in a study of *F. culmorum* infection where DON appeared to be transported from the chaff of the spike to the developing kernel prior to the fungus invading the kernel in susceptible genotypes. In a study by Savard et al. (2000) localization and concentration of DON was examined in the internodes of the rachis and the spikelets at regular intervals after inoculation of a susceptible cultivar. DON began to accumulate in high concentrations by 4 days post inoculation and the concentration of DON was much higher in the rachis than in the spikelets. Interestingly, DON amounts in the rachis were much higher below the spikelet initially inoculated as opposed to above.

Toxin localization and concentration has also been examined with respect to general symptoms of the disease. Several studies conclude that symptoms of disease are correlated with the concentration of trichothecenes in the spike (Atanassov et al., 1994; Bai et al., 2001; Lamper et al., 2000; Lemmens et al., 1997; Miedaner et al., 2003; Seitz and Bechtel, 1985; Wong et al., 1995). Seitz

and Bechtel (1985) found that kernels that had the most "scabby" (i.e., shriveled and moldy) appearance had the highest DON and ergosterol content (reflective of fungal mass) and the lowest mass. However, kernels that were moderately affected (normal size but lower mass) still had high ergosterol and DON content, though these concentrations were lower than heavily infected kernels (Seitz and Bechtel, 1985). In another study by Lamper et al., 2000, DON and ergosterol content were positively correlated with each other, and DON and ergosterol content were both positively correlated with the degree of kernel infection. Wong et al. (1995) also found that DON content was highly correlated with the number of shriveled or highly infected kernels. Atanassov et al. (1994) had similar results when thirty strains of seven *Fusarium* species differing in their amount and type of trichothecene produced (either DON, nivalenol and/or T-2 toxin) were used to inoculate seven different wheat genotypes varying in FHB resistance. A significant correlation was found between the reduction of grain weight and the concentration of mycotoxins from infection by the most virulent strains of *Fusarium*. However, for less virulent strains, the correlation between mycotoxin concentration and grain weight reduction was weaker. In contrast, in a field study of four wheat genotypes differing in levels of resistance higher levels of resistance were correlated with reduced DON and visual symptoms of seed damage, however DON levels were not well correlated with infection of the seed (Argyris et al., 2003). The authors concluded that the resistance observed may improve grain quality and reduce yield loss, but may not have a large effect on actual seed infection (Argyris et al., 2003). In addition, infections of forty-five

resistant and susceptible genotypes with a large number of *F. graminearum* and *F. culmorum* isolates revealed that although the most resistant host genotypes consistently accumulated very low concentrations of DON, moderately susceptible and susceptible genotypes with similar visual disease ratings of FHB had varying amounts of DON in grain (Mesterhazy et al., 1999). An *in vitro* study by Seeland and Bushnell (2001) barley leaf segments treated with high concentrations of DON in the light showed a loss of chlorophyll and carotenoids, resulting in the "bleaching" effect, but cell death was delayed as much as five days. Tissues with lower levels of DON exhibited a reddish-brown color (Seeland and Bushnell, 2001). These results support the role of trichothecenes as important factors in the development of disease symptoms.

1.7 FHB and Cell Wall Degrading Enzymes

Kang and Buchenauer (2000b and 2002b) used enzyme and immunogold labeling of cellulose, xylan and pectin to assess the role of cell wall degrading enzymes in wheat. The amounts of cellulose, xylan and pectin were significantly reduced in portions of the floret and rachis colonized by *F. culmorum*. In addition, reductions in cellulose, xylan and pectin were observed in areas not yet colonized by the fungus. Furthermore, the reductions of cellulose, xylan and pectin coincided with morphological changes in cell walls and middle lamella. These results suggest that cell wall degrading enzymes, either produced by the fungus or induced by the host, may facilitate the spread of the fungus in the host plant by weakening cell walls and the middle lamella. However, these data are

from an indirect, correlative measure and more research is required to determine the role of cell wall degrading enzymes are important in pathogenesis.

1.8 Chemical and Biological Controls of FHB

Chemical and biological controls have been investigated for their efficacy in controlling FHB. This literature review will not go into detail about these types of controls of FHB. For reviews of chemical and biological controls of FHB see Mesterhazy (2003) and Da Luz et al. (2003) respectively. In short, many different fungicides and biological controls have been evaluated for efficacy against FHB (McMullen and Milus, 2002; Mesterhazy, 2003). Chemical and biological controls have had limited but significant affects on FHB development. Although chemical applications of fungicides have shown a reduction in FHB, no chemical control has been identified that completely stops or prevents the development of FHB (McMullen and Milus, 2002; Mesterhazy, 2003). In addition, in fungicide trials on artificially inoculated wheat field plots, none of the chemical controls limited the disease (and specifically, DON accumulation) to commercially acceptable levels (Mesterhazy, 2003). Biological controls have also being investigated for efficacy in reducing FHB. Da Luz et al. (2003) lists 12 bacteria, six yeasts and two fungi identified as showing some degree of control of FHB. The mechanisms by which these organisms are effective as biocontrols is not clear, but antibiotic properties, competition, potential inhibition of mycotoxin production by *Fusarium* , and induced host resistance are thought to be some of the factors involved (Da Luz et al., 2003). Apart from the ability of a fungicide or

biocontrol agent to control FHB, there are complications in both method and timing of application that have made the use of chemical and biological controls difficult. A major difficulty has been to identify, or create, equipment that enables effective coating of the wheat head with the fungicide. The majority of equipment available is designed to target the more horizontally oriented leaf tissue rather than the vertically oriented wheat spike (Halley et al., 1999). Researchers have worked to test, modify and create equipment that will spray biological and chemical controls adequately on the wheat head (De Ackermann et al., 2002; Draper et al., 2002; Halley et al., 1999; Halley et al., 2003; Kirk et al., 2003). Another difficulty in applications of chemical and biological controls is timing of applications. Most of the efficacious fungicides identified to date are not translocated well in the plant, thereby requiring application of the control agent after emergence of the spike (Mesterhazy, 2003). However, FHB develops best under wet conditions (Francl et al., 1999), which are not conducive for ground-based applicator equipment. In addition to the timing of application, the use of biological control is also complicated by the ability of the biocontrol to survive in the environment after application (Da Luz et al., 2003).

1.9 Mechanisms of Resistance to FHB

Host resistance to FHB has been categorized according to the specific effect of the resistance (Mesterhazy, 1995; Schroeder and Christensen, 1963; Wang and Miller, 1988). Resistance to initial infection and resistance to spread of infection are the most widely recognized and studied types of resistance and

are referred to as Type I and Type II, respectively (Schroeder and Christensen, 1963). Other types of resistance are postulated to include resistance to kernel infection, tolerance to infection, resistance to mycotoxin accumulation and avoidance (Mesterhazy, 1995; Schroeder and Christensen, 1963; Wang and Miller, 1988).

Type II resistance (resistance to spread) is the most commonly reported type of resistance. Two of the most studied sources of Type II resistance include 'Sumai 3' and 'Frontana' (Singh et al., 1995; Van Ginkel et al., 1996; Waldron et al., 1999). These genotypes also show a low level of Type I resistance (Pritsch et al., 2000; Siranidou et al., 2002).

Schroeder and Christensen (1963) did not identify any gross histological or morphological differences when comparing resistant (Type II) and susceptible genotypes, suggesting that the resistance was due to physiological activities of the host. Kang and Buchenauer (2000c) using electron microscopy to study the differences between resistant varieties (Frontana and 'Arina') and a susceptible variety ('Agent'), the resistant varieties developed a greater number of observable physical barriers during the spread of infection – including a greater degree of lignification, more appositions and papillae, and the accumulation of callose. Further investigations by Kang and Buchenauer (2000c) showed similarities between the resistant (Arina and Frontana) and susceptible (Agent) varieties in the initial infection process (i.e., no difference in resistance to initial infection), but colonization of the pathogen from the rachilla to the rachis was faster in the susceptible cultivar than in the resistant cultivars. In addition, the

resistant cultivars had a lower accumulation of DON in the beginning stages of infection, which the authors postulated may be the result of defense mechanisms that can restrict the translocation of the toxin. The susceptible genotype (Agent) showed alterations of the host cells in contact or in advance of hyphae growing intercellularly in the lemma and palea. An electron dense material was found in the vacuoles, appositions were observed between the host secondary cell wall and the plasmalemma, and the chloroplasts became enlarged subsequent to infection of Agent (Kang and Buchenauer, 2002b). The fungus then began to grow intercellularly. Thionins (which have antimicrobial activity) and hydroxyproline-rich glycoproteins (which are structural proteins in plant cell walls) increased more in the spike tissue of inoculated plants of Arina (resistant) than in Agent (susceptible) (Kang and Buchenauer, 2003), further supporting the supposition that active structural defenses may be induced to a greater degree in resistant genotypes.

In a study by Ribichich et al. (2000) symptom development and rate of hyphae colonization were slower in the resistant cultivar Sumai 3 (Type II resistance) than the susceptible cultivar 'Pro-INTA Oasis', though many of the cellular changes observed in response to disease were similar. However, the authors did find that the abaxial epidermis and hypodermis of the resistant line became thicker than the susceptible line (Ribichich et al., 2000). They concluded that this thickening delayed infection, instead of preventing it.

Pritsch et al. (2000) studied the transcription levels of Pathogenesis Related (PR) proteins in the resistant line Sumai 3 (Type II) and a susceptible

line, 'Wheaton', during the first 48 hours after inoculation of glumes. Although several PR proteins had a similar level of transcription, Sumai 3 showed a greater and earlier accumulation of PR4 and PR5 (thaumatin-like protein). In addition, defense genes in the susceptible genotype began to decline 36 hours after inoculation, whereas they remained stable or continued to increase in the resistant Sumai 3. However, no significant difference was seen in the resistance to fungal colonization between Sumai 3 and Wheaton. The authors suggest that the induction of the PR proteins may reflect a general response to infection, versus a specific resistance to invasion by *F. graminearum*. In a subsequent study the researchers investigated if PR proteins and peroxidase are expressed systemically in the wheat head via Systemic Acquired Resistance (SAR) response after initial infection of the floret (Pritsch et al., 2001). The authors compared the accumulation of defense response gene transcripts with the growth of the fungus on Sumai 3 (resistant), 'Bobwhite' (moderately susceptible) and a Wheaton (susceptible). By forty-eight hours after inoculation they found an accumulation of all four defense response gene transcripts investigated (peroxidase, PR-1, PR-3 – a chitinase, and PR-5 – a thaumatin-like protein) in both colonized and uncolonized regions of infected genotypes, suggesting that these defense responses are expressed systemically. However, this accumulation was not always statistically different from the control heads, nor was it significantly different between resistant and susceptible genotypes. The authors suggested that factors conferring resistance in the resistant genotype must be active later in the infection process. Overall, they concluded that the

four defense genes evaluated are systemically induced in wheat after invasion by the pathogen.

PR protein accumulation was also investigated for Sumai 3 and a Sumai 3-derived susceptible mutant by Li et al. (2001). Two chitinase and two β-1-3 glucanase clones were isolated from a cDNA library constructed from wheat spikelets inoculated with *F. graminearum*. Analysis of transcript accumulation and protein accumulation after inoculation revealed a different pattern of PR protein accumulation in the resistant line, Sumai 3, versus the susceptible Sumai 3-derived mutant (Li et al., 2001). However, both the wild type and mutant lines accumulated these proteins. Further studies of near isogenic lines different in FHB resistance would give a clearer picture into the involvement of these proteins in defense response to FHB.

The prevalence and localization of chitinase and β-1,3-glucanase were also examined in inoculated versus control wheat heads of a resistant line (Arina) and a susceptible line (Agent) (Kang and Buchenauer, 2002a). The resistant line accumulated a greater amount of β-1,3-glucanase and chitinase than the susceptible cultivar after infection. The authors suggest that these antimicrobial enzymes may be involved in resistance (Kang and Buchenauer, 2002a). Together the above studies of PR proteins reveal that defense genes are induced, though their involvement in resistance to FHB remains unclear.

Phenolic compounds have been found to be involved in antimicrobial defense responses in plants in general (Agrios, 1997) and are also components of cell walls in grasses (Wallace et al., 1995). McKeehen et al. (1999) measured

phenolic compound types and concentrations present in six wheat cultivars differing in Type II resistance seven days after anthesis until maturity. The two primary phenolic acids present during grain development were p-coumaric acid and ferulic acid. Both compounds significantly inhibited growth of *F. graminearum* and *F. culmorum*. Accumulation of these compounds was greatest in the resistant varieties, suggesting that these phenolic compounds may be involved in resistance. Siranidou et al. (2002) also examined the role of phenolic compounds along with physically observed defense responses and symptom development in resistant (including Frontana) and susceptible genotypes. They found that there was a greater accumulation of phenolic compounds in Frontana than in the susceptible line Agent. In addition, formation of cell wall appositions was also more pronounced in Frontana than in Agent.

Response of cell wall proteins to *Fusarium* elicitors was investigated *in vitro* by El Gendy et al. (2001). Calli were developed from a resistant line ('DH1015') were treated with extracts from *Fusarium* cultures. Ionically-bound cell surface proteins were then purified and characterized. From their observations of these proteins before and after treatment they concluded that two proteins were deposited into the cell wall matrix after treatment with elicitors. Subsequent exposure to H_2O_2 appeared to render these proteins insoluble. The authors suggest that their data demonstrate the involvement of these proteins in defense response in a manner that may be H_2O_2 dependent pathway. The composition of both putative cell wall structural proteins is high in glycine and serine. Care should be taken in interpreting these results since calli are

undifferentiated cell cultures that do not exactly represent the plant tissue organs that the fungus would infect.

Doohan et al. (2000) examined the constitutive antifungal activity of wheat kernels. Soluble extracts were made from ground kernels of six resistant (including Sumai 3, a Sumai 3-derived line 'CM820036' and Frontana) and five susceptible lines. The extracts were added to fungal growth media and assayed for inhibition of the growth of *F. culmorum*. They found that extracts from five of the six resistant genotypes (including Sumai 3, Frontana, CM820036, 'Arina' and 'WEK0609') significantly inhibited growth of the fungus in comparison with only one of the susceptible genotypes ('Riband'). Heat treatment inactivated this antifungal activity, suggesting that the potentially antifungal compounds could be proteins. Comparisons of fractions of extracts from Arina (resistant) and Riband (susceptible) showed two compounds associated with antifungal activity. Although a correlation has been made between kernel extracts and antifungal activity, it is not know if these compounds are found in other genotypes, either resistant or susceptible. In addition, the compounds were extracted from mature seeds, whereas FHB infection usually takes place during anthesis, therefore these proteins may not be present during early stages of infection.

Peroxidase and polyphenol oxidase production were compared in resistant (Sumai 3 and 'Wangshuibai') and susceptible ('Falat' and 'Golestan') wheat lines at different stages of maturity after single floret inoculation with *F. graminearum* (Mohammadi and Kazemi, 2002). Peroxidase activity was induced after inoculation, but did not show a consistent trend with respect to resistance

(Sumai 3 and Wangshuibai) or susceptibility (Falat and Golestan). Polyphenol oxidase activity, on the other hand, was two times greater in resistant versus susceptible lines, suggesting that polyphenoloxidase activity could be a defense response against FHB.

Resistance to mycotoxin accumulation was attributed to DON degradation in a study by Miller and Arnison (1986). Callus cultures of both a resistant line (Frontana) and a susceptible line ('Casavant') were exposed to C14 labeled DON. Cells from the resistant line showed an 18% decrease in DON, which had been converted to three other compounds. Callus cells from the susceptible line showed only a 5% decrease in DON, which had been converted to just one compound (Miller and Arnison, 1986). However, this study should be interpreted with caution because the callus cells may not directly reflect the reaction of specific plant organs. In another study of DON by Snijders and Krechting (1992), investigation of 22 genotypes varying in degrees of resistance showed that DON translocation into developing kernels is inhibited in resistant lines inoculated with *F. culmorum*, and fungal colonization of the kernels was less than in susceptible genotypes. The authors suggested that resistant lines had a membrane-based tolerance to trichotehecenes.

Although tolerance to infection is considered a mechanism of resistance to FHB (Mesterhazy, 1995), no research has examined the molecular mechanisms responsible for this resistance. Tolerance to infection would allow for wheat plants to be heavily infected, but not show a significant impact of disease on the plant as with shriveled grain. Sound grain that is heavily colonized by the fungus

is not in fact desirable for the wheat industry unless DON production is suppressed.

Type I resistance, resistance to initial infection, is dependent on genetic factors as well as host stage of development (see "Invasion and Colonization of *Fusarium*" above). Although a moderate level of Type I resistance has been identified in some genotypes, the mechanisms underlying genetic resistance have not been closely examined. However, histological studies by Schroeder and Christensen (1963) suggested that the genetic component of Type I resistance was due to physiological activities and not anatomical differences present in resistant hosts prior to inoculation.

1.10 Artificial Inoculation Methods for FHB Induction

Three different general methods are used for aritificial inoculation of wheat spikes with *Fusarium* spp. to induce FHB: 1) single floret inoculation, 2) spray inoculation of the head with a liquid spore suspension and 3) distribution of infected grain (grain spawn) or other plant material. Single floret inoculation is a controlled method of inoculating the wheat spike so the initial inoculation point is restricted to a single floret within one spike. This is typically done through using a pipette or hypodermic needle to place a small quantity (e.g. 10µl) of liquid fungal spore suspension between the lemma and palea of a floret (Rudd et al., 2001; Schroeder and Christensen, 1963). A variation of this method is to place a piece of cotton saturated with liquid inoculum inside the floret (Gilchrist et al., 1997). Single floret inoculation is used in both the greenhouse and the field for

observing resistance to the spread of the disease. Spray inoculation involves using spraying equipment to coat the wheat spike with a liquid spore suspension (Schroeder and Christensen, 1963). This type of inoculation is often used in both the greenhouse and the field for assessing resistance to initial infection of the disease (Type I resistance) (Rudd et al., 2001). Grain spawn inoculum is generally conducted in field conditions. For grain inoculum, sterilized cereal grains are inoculated with the pathogen and then spread over the surface of the soil before anthesis (Rudd et al., 2001). This type of inoculation may best mimic a natural type of inoculation, since natural primary inoculum develops on colonized surface plant residue (Champeil et al., 2004).

With any of these inoculation methods, environmental conditions are usually altered after inoculation to promote the development of disease. A high relative humidity following inoculation (in the case of single floret or spray inoculation) or both before and after anthesis (in the case of grain inoculum) promotes disease development (Francl et al., 1999). Many researchers have used overhead misting systems in growth chambers/greenhouses or in the field to provide a high relative humidity (Anderson et al., 2001; Rudd et al., 2001; Snijders and Perkowski, 1990). Another method of providing a high relative humidity is to cover wheat spikes with a plastic bag (Anderson et al., 2001; Bai et al., 2000; Bourdoncle and Ohm, 2003; Mohammadi and Kazemi, 2002; Shen et al., 2003a).

Miedaner et al. (2003) compared the effects of spray and point inoculation for 20 wheat genotypes across seven field environments. They found that

although repeatability of host response was high within a method, there was not a significant relationship between the two methods. These results probably reflect the ability of the different inoculation methods to reveal the action of different types of resistance.

1.11 Genetics of Resistance to FHB

Van Eeuwijk et al. (1995) investigated FHB in 25 wheat genotypes in six field locations over three different years using 17 strains of *F. graminearum*, *F. culmorum* and F. nivale. One strain of *F. culmorum* was used as a check at all locations. Their results indicated that although different strains varied for their level of aggressiveness, very little variation among genotypes could be attributed to differences between *Fusarium* strains. In a study by Mesterhazy et al. (1999), seven isolates of *F. graminearum* and eight isolates of *F. culmorum* were used to inoculate 20-25 wheat genotypes with different degrees of resistance. Their results showed that within a genotype, similar responses were obtained irrespective of isolate. However, in a study by Gilchrist et al. (2000) eight isolates of *F. graminearum* inoculated across four resistant and one susceptible cultivar showed significant differences between isolates in addition to cultivar x isolate interactions.

In many studies, resistance has been confirmed to be quantitative, and no complete resistance to infection has been identified for FHB (Anderson et al., 2001; Bai et al., 1999; Bai et al., 2000; Ban, 2000; Bourdoncle and Ohm, 2003; Buerstmayr et al., 1999a; Ittu et al., 2000; Rudd et al., 2001; Shen et al., 2003b;

Snijders, 1990; Steiner et al., 2004; Waldron et al., 1999). Many Quantitative Trait Loci (QTL) for FHB resistance have been identified from different genetic sources. *Qfhs.ndsu-3BS* on chromosome arm 3BS is the most widely studied and verified FHB resistance QTL in wheat, and explains as much as 60% of the FHB phenotypic variation in mapping populations (Anderson et al., 2001; Buerstmayr et al., 2002; Buerstmayr et al., 2003; Chen et al., 2003; Del Blanco et al., 2003; Zhou et al., 2003; Zhou et al., 2002a). The resistant allele of *Qfhs.ndsu-3BS* is from the genotype Sumai 3 (Anderson et al., 2001; Bai and Shaner, 1994; Waldron et al., 1999). Among a diverse group of 66 cultivars from several countries with different levels of resistance, AFLP and SSR analyses revealed that the Sumai 3 haplotype in the *Qfhs.ndsu-3BS* region was present in almost all the Sumai 3 derived lines, as well as a few other genotypes (Bai et al., 2003). However, most other sources of resistance showed non-Sumai 3 haplotypes at the *Qfhs.ndsu-3BS* region (Bai et al., 2003). In another study of haplotype diversity of a collection of 79 genotypes from around the world (most of which exhibit resistance or moderate resistance), the Sumai 3 haplotype at in the *Qfhs.ndsu-3BS* region occurred in seven lines, one of which is known to be a Sumai 3 - derived line (McCartney et al., 2004). These studies suggest that resistance like that of Sumai 3 at *Qfhs.ndsu-3BS* may exist in many other genotypes, but there is also a large amount of diversity in the *Qfhs.ndsu-3BS* region.

Other QTL with smaller effects for FHB resistance have been identified through mapping studies on sixteen chromosomes of wheat: 2A (Gervais et al.,

2003; Paillard et al., 2004; Steiner et al., 2004; Waldron et al., 1999; Zhou et al., 2002b), 3A (Anderson et al., 2001; Gervais et al., 2003; Paillard et al., 2004; Shen et al., 2003b; Steiner et al., 2004), 4A (Paillard et al., 2004), 5A (Buerstmayr et al., 2002; Buerstmayr et al., 2003; Gervais et al., 2003; Paillard et al., 2004; Shen et al., 2003b; Somers et al., 2003; Steiner et al., 2004), 6A (Anderson et al., 2001), 1B (Buerstmayr et al., 2002; Ittu et al., 2000; Shen et al., 2003b; Steiner et al., 2004), 2B (Gervais et al., 2003; Steiner et al., 2004; Zhou et al., 2002b), 3B (Gervais et al., 2003; Paillard et al., 2004; Shen et al., 2003a; Somers et al., 2003), 4B (Somers et al., 2003; Steiner et al., 2004), 5B (Paillard et al., 2004), 6B (Anderson et al., 2001; Shen et al., 2003a; Steiner et al., 2004), 1D (Ittu et al, 2000), 2D (Shen et al., 2003a; Somers et al., 2003), 3D (Paillard et al., 2004; Shen et al., 2003b), 5D (Gervais et al., 2003) and 6D (Gervais et al., 2003; Paillard et al., 2004). Chromosome substitution lines and monosomic analyses have also implicated chromosomes with potential QTL influencing FHB resistance, identifying chromosomes 2A, 3A, 4A, 5A, 7A, 1B, 2B, 3B, 4B, 5B, 6B, 7B, 2D, 4D, 5D, 6D and 7D in FHB resistance (Buerstmayr et al., 1999b; Liao and Yu, 1985 – see Bai and Shaner, 1994; Yu et al., 1986 – see Buerstmayr et al., 1999b; Yu, 1982; Yu, 1991 – see Bai and Shaner, 1994 and Buerstmayr et al., 1999b; Zhou et al., 2002a).

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CHAPTER 2

BIOASSAY VS. CONVENTIONAL CHARACTERIZATION OF FUSARIUM HEAD BLIGHT RESISTANCE IN NING 7840.

2.1 Abstract

Fusarium head blight (FHB), caused by *Fusarium* spp. is a devastating disease of wheat and barley. Type II resistance (resistance to spread) to FHB is the form of resistance most commonly assessed by wheat breeding programs. Type II resistance is usually rated by observing visually diseased (scabby) spikelets on a spike. However, visually scabby spikelets may not be an accurate reflection of the spread of disease overall. Two wheat genotypes, 'Ning 7840' (resistant) and 'Norm' (susceptible), were evaluated at seven and fourteen days post inoculation (dpi) for the number of 1) visually scabby spikelets (SS), 2) visually infected rachis sections (VIRS) and 3) infected rachis sections according to a bioassay (BIRS). The number of visually scabby spikelets was significantly fewer than the number of infected rachis sections (either visual or bioassay) for both genotypes at both 7 and 14 dpi. Ning 7840 had significantly lower mean values than Norm for each measurement (SS, VIRS and BIRS) at both 7 and 14 dpi. Measurements (SS, VIRS or BIRS) above (apical) the point of inoculation were lower than the respective measurements below (basal) the point of inoculation for both genotypes at both 7 and 14 dpi, though this difference was not always significant. In addition, Ning 7840 was significantly lower than Norm for each corresponding measurement (SS, VIRS and BIRS) up from (towards the

terminal spikelet) and down from (toward the peduncle) the inoculated spikelet at both 7 and 14 dpi except for SS spread down from the inoculated spikelet at 7 dpi, for which the difference was not significant. BIRS data of Ning 7840 revealed a bimodal trend in the spread of the *Fusarium graminearum*, suggesting that the point of greatest resistance to spread in Ning 7840 may be the rachis node.

2.2 Introduction

Fusarium head blight (FHB) caused by *Fusarium* spp. is a devastating disease of wheat (*Triticum aestivum* and *T. durum*) and barley (*Hordeum vulgare*) resulting in yield loss, reduced grain quality, and reduced test weight (McMullen et al., 1997). More important is the contamination of the grain with mycotoxins that are a serious health threat to humans and animals (Snijders, 1990). FHB is caused by several *Fusarium* species including *F. graminearum* and *F. culmorum* (Parry et al., 1995). In North America FHB of wheat is caused predominantly by *F. graminearum* (Schwabe) (perfect stage *Gibberella zeae* Schw. and Petch) (Parry et al., 1995). Recently, FHB epidemics have dramatically impacted the wheat and barley industries in the United States and in have resulted in losses of nearly three billion U.S. dollars to farmers since the 1990's (Windels, 2000). In 1993, Minnesota, North Dakota, South Dakota and Manitoba (Canada) combined suffered approximately $1 billion in losses in the wheat and barley industries (McMullen et al., 1997). From 1998 through 2000, U.S. economic losses due to FHB in soft red winter wheat and hard red spring

wheat alone were estimated at $333 million and $330 million, respectively (Nganje et al., 2001).

FHB is caused by the infection of wheat spikes by *Fusarium* ascospores or conidia (Markell and Francl, 2001; Stack, 1989). Initial *Fusarium* infection on wheat spikes occurs primarily at anthesis (Andersen, 1948; Pugh et al., 1933; Strange and Smith, 1971) and is influenced by temperature and moisture (Snijders, 1990). Many studies have been conducted on either *F. graminearum* or *F. culmorum*, and histological investigation reveals that the two species are similar for their infection and colonization processes of wheat spikes (Wanjiru et al., 2002). Several routes of initial penetration by *Fusarium* spp. of the host floret tissue have been observed including colonization of extruded anther, direct penetration of the interior surfaces of the lemma, palea and ovary and stigma, and penetration through stomates (Andersen, 1948; Bushnell et al., 2003; Kang and Buchenauer, 2000a; Kang and Buchenauer, 2000c; Kang and Buchenauer, 2002b; Lewandowski and Bushnell, 2001; Pritsch et al., 2000; Ribichich et al., 2000; Strange and Smith, 1971; Tu, 1950). After initial infection, some studies have found that the fungus grows primarily basipetally in the rachis from the point of inoculation (Savard et al., 2000; TeKrony et al., 2000). Other studies have seen a large amount of movement both acropetally and basipetally in susceptible cultivars (Kang and Buchenauer, 2000a; Schroeder and Christensen, 1963), while in highly resistant cultivars the fungal colonization may be restricted to the inoculated spikelet (Wang et al., 1982). Colonization of the rachis has been suggested to cause bleaching (loss of pigment) and shriveled kernels in

uncolonized, upper portions of the spike or in adjoining spikelets (Bai and Shaner, 1996; Kang and Buchenauer, 1999; Pugh et al., 1933; Savard et al., 2000). In addition, some studies have shown that infection in the rachis is more wide-spread than that in the floral organs (Edge et al., 2001; TeKrony et al., 2000; TeKrony et al., 2001).

Breeding for disease resistance is considered to be the best method to combat FHB (Ruckenbauer et al., 2001; Schroeder and Christensen, 1963). Several types of resistance have been described for FHB (Mesterhazy, 1995; Schroeder and Christensen, 1963; Wang and Miller, 1988). Type I resistance, (resistance to initial infection) and Type II resistance (resistance to spread of infection from a point of initial infection), as described by Schroeder and Christensen, 1963, are the most widely accepted and researched types of resistance. Other types of resistance have also been described: resistance to kernel infection, tolerance to infection, and resistance to mycotoxin accumulation (Mesterhazy, 1995). In addition, avoidance of infection via plant height or flowering in the boot is considered a form of passive resistance (Mesterhazy, 1995). No complete resistance to the disease has been found, but several sources of partial resistance are known. At this time, the genes conferring FHB resistance in the Chinese cultivar 'Sumai 3' and its derivatives are used more than any other source (Kolb et al., 2001; Rudd et al., 2001). Sumai 3 has strong Type II resistance (resistance to spread), as well as some Type I resistance (resistance to initial infection) (Ban and Suenaga, 2000; Rudd et al., 2001; Wilcoxson et al., 1992). Inheritance of this resistance is quantitative and exhibits

low heritability. A QTL explaining 25-60% of the variation for Type II FHB resistance in Sumai 3 is present on chromosome arm 3BS, (Anderson et al., 2001; Bai et al., 1999; Waldron et al., 1999). That same QTL is present in 'Ning 7840', a Sumai 3 derivative (Bai et al., 2003; Bai et al., 1999; Sourdille et al., 2000; Zhou et al., 2002).

The level of Type II resistance is usually determined by visual assessment of the number of diseased ("scabby") spikelets after inoculation of a single spikelet under greenhouse or field conditions (Schroeder and Christensen, 1963). Visual symptoms of FHB appear as water soaked lesions, reddish discoloration, chlorosis, necrosis and bleaching (complete loss of pigment) of spikelet and rachis components (Andersen, 1948; Kang and Buchenauer, 2000a; Pugh et al., 1933; Savard et al., 2000; Snijders and Krechting, 1992). Although the number or proportion of scabby spikelets is a convenient method for scoring genotypes for resistance, bioassay and histological studies of the infection process show that visual ratings of disease may not be indicative of the presence or absence of the fungus (Edge et al., 2001; Pugh et al., 1933; Schroeder and Christensen, 1963; TeKrony et al., 2000; TeKrony et al., 2001).

Rachis measurements have also been used in a few breeding programs when observing Type II resistance. Wang et al. (1982) used a four-grade level rating system in which the highest level of resistance showed no visual symptoms of disease, while the second highest level showed symptoms in the rachis but no symptoms in other adjoining spikelets. Jiang et al. (2001) reported

a similar scoring system using visual symptoms in the rachis, in addition to spread to adjoining florets from the initially inoculated floret.

Despite the wide-spread focus by plant breeders on Type II resistance, little is known of the actual mechanism by which the rate of disease development is retarded. This study was conducted to shed light on the mechanism of one source of Type II resistance through a comparative analysis of the progression of FHB in Ning 7840, a resistant cultivar, and Norm, a susceptible cultivar.

2.3 Materials and Methods

2.3.1 Plant Materials

Seeds of the cultivars Ning 7840 and Norm were hydrated at room temperature in the dark for 24 hours in petri dishes containing Steel Blue Seed Germination Blotter Paper (Anchor Paper, St Paul, MN) saturated with deionized water. Petri dishes were then transferred to an unlighted cold room maintained at ~2°C for 5 weeks. Deionized water was added to petri dishes as necessary. After vernalization, petri dishes were kept at room temperature in the dark for 48 hours before transplanting.

Seeds were then transplanted into 4-inch plastic pots at a density of four plants per pot (one genotype per pot). Pots were placed on greenhouse benches in a manner that reduced the effects of environmental gradients within the greenhouse. This included alternating rows according to genotype.

2.3.2 Inoculum Production

F. graminearum (Schwabe) isolate PH-1 (NRRL 31084) was isolated by Dr. L. Patrick Hart, East Lansing, MI, from a Michigan wheat field during a FHB epidemic in 1996. Inoculum was produced in Carboxymethyl Cellulose (CMC) liquid medium (Cappellini and Peterson, 1965) as follows. The CMC liquid medium was divided into 250 ml flasks each containing approximately 100ml of CMC liquid, then autoclaved. Soil stock of PH-1, or a small block (~0.5cm^3) of potato dextrose agar colonized by PH-1, was then added to a flask of CMC liquid medium that was cool or at room temperature. The flask was shaken at 180-250 rpm at room temperature for 3 days to induce the production of macroconidia. The macroconidia suspension was centrifuged to produce a pellet, from which the CMC liquid medium was decanted and sterile water was added. Macroconidia concentration was adjusted to 1.4×10^6 spores/ml.

2.3.3 Inoculation of Plants

Forty-four plants of each genotype with main stem spikes in growth stage Feekes 10.5 (i.e., immediately prior to anthesis with anthers slightly yellow to very yellow) were identified for use in the experiment. Only main stem spikes were used, and preference was given to pots having more than one plant at Feekes 10.5 to minimize space usage. Four plants of each genotype were randomly selected as controls and were not inoculated. The remaining forty spikes of each genotype were inoculated by pipetting 7µl of spore suspension (equivalent to 10,000 macroconidia) into one of the two most basal florets of a

central spikelet. The inoculated spikelet was marked with black permanent black ink for later identification.

2.3.4 Post-Inoculation Growth Conditions

Immediately after inoculation, pots were randomly positioned in a growth chamber set to maintain a constant temperature of approximately 22°C. Plants were subjected to overhead misting for 5 seconds every 5 minutes for 72 hours after inoculation.

2.3.5 Evaluation of Fusarium Head Blight on Spikes

Seven and 14 days post inoculation (dpi), 20 inoculated and two control spikes of each genotype were randomly chosen for harvest and processing for disease evaluation. Each spike was sequentially evaluated for three measures of disease as described below and illustrated in Fig. 2-1.

1. Number of Scabby Spikelets (SS): A spikelet was considered scabby if there was visibly damaged tissue on any of its glumes, lemmas or paleas. Counts of total SS were recorded for each spike. Recorded data included the number of SS above, below, and at the inoculated spikelet. (Fig. 2-1A)

2. Visual Infected Rachis Sections (VIRS): After the SS data was collected, spikelets were removed from the spike (by twisting spikelets off the rachis by hand) and each section of the rachis (as

defined below) was classified as symptomatic or asymptomatic of disease. The composition of a rachis section depended on its position relative to the inoculated spikelet. The node to which the inoculated spikelet was attached was designated the 'inoculated node', and was treated as a distinct rachis section. Rachis sections above the inoculated node included a single node and the subtending internode. Rachis sections below the inoculated node were classic phytomers and included a single node and the internode above it. Recorded data included the number of VIRS above, below, and at the inoculated node rachis section. (Fig. 2-1B)

3. Bioassay Infected Rachis Sections (BIRS): After VIRS data was collected, the rachis of each spike was surface sterilized by soaking in 20% bleach + 0.1% Tween 20 for 2 minutes followed by a rinse with sterile water. The rachis was then cut into sections (as defined in VIRS) using a sterile scalpel that was sterilized between each cut. Rachis sections from a single spike were plated sequentially in a circular pattern on a large petri dish (150 x 15mm) containing potato dextrose agar (PDA). Petri dishes were placed (PDA side down) on shelves under constant illumination (lamps placed approximately 1 to 1 ½ feet above cultures) at room temperature (~ 68-72°F). Petri dishes were checked regularly for growth of *F. graminearum* for many days following plating until it was clear

which rachis sections were and were not infected. Rachis sections were classified as infected or not infected on the basis of the presence or absence of the growth of *F. graminearum* from each section. Recorded data included the number of BIRS above, below, and at the inoculated node rachis section. (Fig. 2-1C)

2.3.6 Experimental Design

The experiment was replicated three times over a period of several months. A group of forty inoculated and four control plants (both resistant and susceptible) was considered a replication of the experiment. A random number generator was used to determine pot placement in the growth chamber after inoculation and to determine which spikes to harvest at 7 and 14 dpi.

2.3.7 Missing Data

Twenty-eight of the 240 inoculated spikes were discarded because of escape, injury, missing data, suspected second infection points other than the originally inoculated spikelet, or symptoms inconsistent with FHB. Surface sterilization of spikes was ineffective in replication one at 7 and 14 dpi, and replication two at 7 dpi. The resulting contamination precluded BIRS data acquisition and those data were accordingly excluded from the analysis.

2.3.8 Data Analysis

The raw count data were transformed using a square root + 1 transformation. All analyses were performed with SAS® Version 8.2 (SAS Institute 2000). Least square means of SS, VIRS and BIRS were calculated with the Proc Mixed LSMeans procedure. Back transformation was used to report the estimated means. The transformed data was tested for normality using Proc Univariate. Analysis of Variance was performed using Proc Mixed "repeated" command since multiple response variables were determined on a single experimental unit (i.e. SS, VIRS and BIRS data from a single spike). Pairwise comparisons of means were determined using Proc Mixed LSMeans "pdiff" command.

2.4 Results

2.4.1 SAS analysis

Plots of the residuals showed a near-normal distribution.

2.4.2 Comparisons among Measures of Disease Development within a Genotype

The number of Scabby Spikelets (SS) was significantly fewer ($P < 0.05$) than the number of infected rachis sections (determined either visually, VIRS, or with bioassay, BIRS) for both genotypes at 7 and 14 days post inoculation (dpi) (Fig. 2-2A). In contrast, there was no significant difference between VIRS and BIRS for either genotype at either 7 or 14 dpi.

For a large proportion of Ning 7840 spikes, spread of the fungus and symptoms was limited to the inoculated spikelet and associated node at both 7

and 14 dpi (Fig. 2-3). On the other hand, the BIRS values at 14 dpi for Ning 7840 showed a striking bimodal distribution (Fig. 2-3B) Forty-four percent of the Ning 7840 BIRS values were between 0 and 2 (referenced below as "mode 1"), while the remainder were between 9 and 14 (referenced below as "mode 2"). To investigate this further, each Ning 7840 spike was classified on the basis of the aforementioned modes. Re-analysis of the Ning 7840 data for 14 dpi with "mode" as a classification factor showed that the SS, VIRS and BIRS means for mode 1 spikes were all significantly smaller than the respective measurements for the mode 2 spikes (data not shown). Within mode 1 none of the measurements (SS, VIRS and BIRS) were significantly different from each other ($P > 0.14$). However, within mode 2 SS was significantly less than VIRS and BIRS ($P < 0.0001$), while VIRS was not significantly different from BIRS ($P = 0.15$).

The number of VIRS and BIRS was significantly greater at 14 dpi than at 7 dpi for both genotypes. The number of SS for Norm was also significantly larger at 14 dpi than at 7 dpi. In contrast, the numbers of SS at 7 and 14 dpi for Ning 7840 were not significantly different (see Fig. 2-2A and 2-2B).

The numbers of SS, VIRS and BIRS above the inoculated spikelet were smaller than corresponding values for spikelets or rachis sections below the inoculated node, though the differences were not always significant (Fig. 2-2C). Overall, the number of SS, VIRS and BIRS above the inoculated node represented 30-49% of the total spread from the inoculated node.

When comparing SS, VIRS and BIRS, SS up was significantly less than VIRS or BIRS up ($P < 0.01$) in all cases except for Ning 7840 BIRS at 7 dpi (for which the difference was not significant). Similarly, SS down was significantly less ($P < 0.01$) than VIRS or BIRS down for both genotypes at both 7 and 14 dpi. In contrast, VIRS up was never significantly different from BIRS up, and VIRS down was never significantly different from BIRS down (in all cases, $P > 0.4$ except Norm VIRS and BIRS up at 14 dpi, for which $P > 0.05$)

2.4.3 SS, VIRS and BIRS between Genotypes

The mean SS, VIRS and BIRS of Ning 7840 were significantly lower than the corresponding means for Norm at both 7 and 14 dpi ($P < 0.05$) (Fig. 2-2B). However, as reported above, BIRS revealed a bimodal trend for Ning 7840 at 14 dpi in which 44% of spikes have BIRS values between 0 and 2 (mode 1), while 56% of spikes have BIRS values between 9 and 14 (mode 2) (Fig. 2-3). When analyzed separately, Ning 7840 mode 1 SS, VIRS and BIRS were all significantly less than the respective measurements for Norm ($P < 0.0001$). In contrast, for Ning 7840 mode 2, although SS was significantly less than Norm ($P < 0.0001$), VIRS and BIRS were not significantly different than the respective measurements for Norm ($P > 0.24$).

In all cases except one, the mean number of SS, VIRS and BIRS above and below the inoculated node was significantly lower ($p<0.05$) for Ning 7840 than Norm at both 7 and 14 dpi (Fig. 2-2D). The one exception was for SS below

the inoculated node at 7 dpi, where Ning 7840 had a lower mean, but the difference with Norm was only significant at $p = 0.07$.

2.4.4 Bleached versus Not Bleached Tissue

Bleached tissue was considered to be tissues that had a loss of pigmentation, without an expectation of the accompanying presence of the fungus in those specific tissues. Bleached tissues were not clearly observed in Ning 7840 and were only observed in Norm above the node inoculated. Data including and excluding bleached tissues were compared for differences in results. The two data sets, one excluding bleached tissue and one including bleached, showed the same trends ($p < 0.05$) for the analyses of SS, VIRS and BIRS within the genotypes, as well as SS, VIRS and BIRS between the genotypes. Since it is expected that most research programs do not distinguish between bleached and colonized tissues, the results reported here include bleached tissues.

2.4.5 Limitations of SS, VIRS and BIRS

The ability to measure spread using SS, VIRS and BIRS was limited by the morphology of the wheat spike. The spread of SS, VIRS and BIRS can not be measured beyond the spike itself. The average node inoculated was the eighth node from the bottom of the spike for Ning 7840 and Norm. The average number of spikelets on a spike was 17 in Ning 7840 and 15 in Norm. Therefore, on average, the ability to measure the spread of SS, VIRS or BIRS was limited to

seven sections below the node inoculated, the node inoculated itself, and nine (for Ning 7840) and seven (for Norm) sections above the node inoculated. The data for each spike was inspected to determine the number of spikes in which SS, VIRS or BIRS spread to the top or bottom of a spike, and therefore to the extent of the measuring abilities. Table 2-1 shows the number and percentage of observations of Ning 7840 and Norm spikes at 7 and 14 dpi with SS, VIRS and BIRS values that reached the extremes of the spike (at tip or peduncle) by 7 or 14 dpi. Care should be taken in interpreting the data because not all spikes represented in SS and VIRS are also represented in BIRS (BIRS at 7 dpi is only representative of replication 3, while BIRS at 14 dpi includes replications 2 and 3). Overall, many spikes showed symptoms spreading to the top or bottom of the spike, and more spikes showed symptoms spreading to the bottom than to the top. No spikes had BIRS values extending to the top of the spike (in either genotype at either 7 or 14 dpi), though some spikes had SS or VIRS data to the top of the spike – revealing that visible symptoms at the top of the spike did not always indicate of the presence of the fungus at that position.

2.5 Discussion

2.5.1 SS, VIRS and BIRS within a Genotype

The results for SS, VIRS and BIRS clearly demonstrate that in both genotypes the fungus had spread internally through the rachis further than was evident by visual symptoms in the spikelets. These data are consistent with other studies that have shown that the spread of the fungus within the rachis is

more extensive than the spread of the fungus among the spikelets (Edge et al., 2001; Pugh et al., 1933; TeKrony et al., 2000). Histological studies by Pugh et al. (1933) showed that the fungus would advance from the point of infection through the rachis, but would not always infect adjoining spikelets, and that the visual symptoms of infection were not always indicative of the presence of the fungus. Similarly, TeKrony et al. (2001) showed that fungal infection in the rachis was more widespread than in the floral organs overall. However, in TeKrony et al.'s study (2001) the bioassay results were poorly related to the visual estimation of disease in the spikelets. In this experiment, BIRS was not significantly different from VIRS for either genotype, revealing that visual estimations of the disease in the rachis were reliable indicators of the presence of the fungus in the rachis for Ning 7840 and Norm at 7 and 14 dpi. In addition, as a general rule the development of scabby spikelets progressed sequentially from the point of inoculation outward (i.e. an asymptomatic spikelet between two scabby spikelets was almost never observed - data not shown).

These results showed a lower rate of spread up (towards the terminal spikelet) as opposed to down (towards the peduncle) from the inoculated spikelet in both Ning 7840 and Norm, which is in agreement with other studies (Savard et al., 2000; TeKrony et al., 2000). However although the spread upward was less than down, the data presented in this study still showed a substantial amount of spread up from the inoculated spikelet, as is consistent with other studies (Kang and Buchenauer, 2000a; Schroeder and Christensen, 1963). Furthermore, the

bioassay provided solid evidence that in this study, symptoms in the rachis above the node inoculated were accompanied by the presence of the fungus.

From my knowledge, this is the first study to report a bimodal trend in the disease symptoms of a resistant genotype, as observed for BIRS data of Ning 7840 at 14 dpi. In addition, the greater spread of symptoms in the rachis versus the spikelets in both Ning 7840 and Norm, and the constancy of Ning 7840's SS compared with an increase in Ning 7840's VIRS and BIRS between 7 and 14 dpi suggests that different mechanisms may be responsible for these two measurements, as has been proposed by Yu, 1990 (as referenced by Bai and Shaner, 1996).

Many studies have investigated the biochemical differences between inoculated and un-inoculated spikes, and between FHB resistant and susceptible genotype (Kang and Buchenauer, 2000a; Kang and Buchenauer, 2000b; Kang and Buchenauer, 2002a; Kang and Buchenauer, 2002b; Kang and Buchenauer, 2003; Mohammadi and Kazemi, 2002; Pritsch et al., 2000; Pritsch et al., 2001; Savard et al., 2000; Siranidou et al., 2002). Correlations are reported between degree of host plant resistance and the accumulation of several defense related substances such as thionins, polyphenol oxidases, hydroxyproline-rich glycoproteins, β-1,3 glucanase, chitinase and thaumatin-like proteins (Kang and Buchenauer, 2002a; Kang and Buchenauer, 2003; Mohammadi and Kazemi, 2002; Pritsch et al., 2000). In addition, structural differences have been observed between inoculated and un-inoculated spikes, and between FHB resistant and susceptible genotypes. Histopathological examinations of Sumai 3

(from which Ning 7840 was derived) shows occlusions forming in some xylem vessels after infection, which were eventually overcome by the fungus, and deposition of an amorphous substance in the phloem (Ribichich et al., 2000). Similarly, Kang and Buchenauer (2000c) observed the formation of wall appositions and large papillae after infection, which were more pronounced in the resistant than the susceptible cultivar, and an increase in lignin content in the host cell walls, which was observed in the infected lemma and infected xylem cells. Kang and Buchenauer (2000c) suggest that these physical barriers may restrict the fungus as well as the movement of the trichothecene mycotoxins (including DON and 3-ADON). Other studies show that *Fusarium* produced trichothecene mycotoxins are translocated to uncolonized tissues through phloem sieve tubes and xylem vessels (Kang and Buchenauer, 1999; Kang and Buchenauer, 2002b). *Fusarium* mycotoxins are thought to be important in the virulence and spread of the disease (Bai et al., 2002; Desjardins et al., 1996; Eudes et al., 1997; Mesterhazy, 2002; Mirocha et al., 1997; Proctor et al., 2002). Resistant genotypes of wheat, including Sumai 3, exhibit a lower accumulation of DON (Kang and Buchenauer, 2000c; Mesterhazy, 2002), and a study of tissue callus formation on DON containing media suggests that Sumai 3 may possess some tolerance to DON (Yang et al., 1998).

Kang and Buchenauer (2000a) present a diagram of the intersection of the spikelet and the rachis, showing what they call a vascular tissue "plexus" in the rachis node, which consists of a cluster of vascular bundles in the rachis node joining the rachis and the spikelet tissues. Although researchers have found

differing results with respect to the extent of fungal growth in the host's vascular tissues (Kang and Buchenauer, 2000a; Pugh et al., 1933; Ribichich et al., 2000; Tu, 1950), the high concentration of vascular bundles in the plexus in combination with an increased lignification of xylem cells (Kang and Buchenauer, 2000c) transient physical barriers (Ribichich et al., 2000) and other biochemical defense responses together may contribute to the hindered spread of the fungus into the rachis from the infected spikelet, as well as out of the rachis to adjoining spikelets. These types of obstacles to spread, when present at the rachis node may provide some explanation for the bimodal results in Ning 7840 in which the fungus didn't spread beyond the initially inoculated node in several spikes, while in others it moved extensively in the rachis but did not invade many of the adjoining spikelets.

A surprising outcome of this study is that the fungus is equally able to colonize the Ning 7840 and Norm rachis tissues in roughly half of the spikes. Barriers in the rachis node, along with the other general biochemical defense responses and a greater resistance/tolerance to DON may be sufficient to restrict the fungus near the inoculated spikelet for about half of the Ning 7840 spikes. However, for the other half of the Ning 7840 spikes the fungus was able to spread past barriers in the rachis node early in disease and spread successfully in the rachis, but barriers at the rachis node prevented colonization of additional adjoining spikelets. The idea that the rachis node may present a greater barrier for the fungus than other spike tissue could also explain the tendency for the scabby spikelets symptoms to be less extensive than scabby rachis symptoms in

both resistant and susceptible genotypes, as was observed also in this and other studies (Pugh et al., 1933; TeKrony et al., 2001). In susceptible genotypes, the rachis node may not provide as great a barrier as in resistant genotypes such as Ning 7840, resulting in a greater number of scabby spikelets.

2.5.2 SS, VIRS and BIRS between Genotypes

The comparison of the three measurements (SS, VIRS and BIRS) between the Ning 7840 and Norm revealed that the total SS, VIRS and BIRS were all effective at distinguishing between the two genotypes ($p < 0.05$) at 7 and 14 dpi. Similarly, the spread up or down for SS, VIRS and BIRS also distinguished between the two genotypes, except for SS down at 7 dpi. However, although the total Ning 7840 data showed significant differences between Ning 7840 and Norm for all three measurements (SS, VIRS and BIRS), the bimodal trend in Ning 7840's BIRS data at 14 dpi gave surprising results when the two modes were analyzed separately. Separate analysis of the upper and lower modes revealed that approximately half of the time the spread in the rachis of Ning 7840 was no different from Norm, though this similarity was not reflected in the number of scabby spikelets. Therefore, the earlier results of this study, showing that SS, VIRS and BIRS were all effective in distinguishing between the two genotypes, should be qualified by considering that this was due to the average of the upper and lower modes of Ning 7840 at 14 dpi, and not because symptoms in the rachis of Ning 7840 were always less than those of Norm.

2.5.3 Bleached versus Not Bleached Tissue

Many researchers have observed bleached tissues (tissues with a loss of pigment, appearing whitened as if drying out/wilting) above the point of inoculation and have attributed this to colonization in the lower parts of the spike, resulting in a lack of water and/or nutrients to the upper portion of the spike, rather than to the presence of the fungus at the bleached portion itself (Bai and Shaner, 1996; Kang and Buchenauer, 1999; Pugh et al., 1933; Savard et al., 2000). Visual estimation of the presence of the fungus in a tissue, versus bleaching in a tissue is highly subjective. In this study, bleached tissues suspected of being free from pathogen colonization, and tissues having disease symptoms suggesting of the presence of the fungus (i.e. darker brownish/reddish pigmentation accompanied by chlorosis and necrosis) were distinguish from each other in the visual scoring of SS and VIRS. The purpose in distinguishing bleached and not-bleached tissues was to determine if the values for SS and VIRS would be significantly different when bleached tissues were included or excluded from the data sets. In this studies, bleaching was only clearly observed in the susceptible genotype, Norm, and was not widespread. Data including bleached tissues showed the same trends as data excluding bleached. Although the data sets with and without bleached sections were not effectually different, it is expected that more bleaching may have been observed if the plants were harvested at later days post inoculation. In this study statistics from the data set including bleached tissues are reported because it is expected that few

laboratories distinguish between bleached and not bleached tissues (since it is laborious to make this distinction).

A possible explanation for the presence of bleaching in Norm and the apparent absence of it in Ning 7840 is the interaction of each genotype with mycotoxins produced by the fungus. A study by Seeland and Bushnell (2001) indicated that application of DON on detached barley could alter leaf pigmentation dramatically and could result in bleaching depending on the concentration of DON applied and the presence or absence of light. Other studies have shown that the mycotoxins produced by *Fusarium* can be translocated in the wheat head from the area colonized by the fungus to uninfected tissues (Kang and Buchenauer, 1999; Savard et al., 2000; Snijders and Krechting, 1992). It has also been suggested that bleaching above infected spikelets may be caused by toxin and/or fungal accumulation blocking movement of water and nutrients (Bai and Shaner, 1996; Kang and Buchenauer, 1999; Pugh et al., 1933). Considering resistant and susceptible genotypes, in a study of *F. culmorum* infection of wheat, DON accumulation was significantly greater in the susceptible cultivar examined than in the two resistant cultivars, though the toxin was present in the same cellular structures in both resistant and susceptible types (Kang and Buchenauer, 2000c). Furthermore, resistant lines, including Sumai 3, were found to have lower accumulation of DON and other *Fusarium* produced toxins relative to more susceptible genotypes (Bai et al., 1998; Mirocha et al., 1994). A study of tissue callus formation on DON containing media suggests that Sumai 3 cells may possess some tolerance to DON (Yang et al.,

1998), and greater DON degradation appeared to be present in a resistant genotype, 'Frontana', in comparison with a susceptible genotype, 'Casavant' (Miller and Arnison, 1986). Overall, the presence of bleaching in Norm may be due to an accumulation of toxin, resulting in the blockage of the movement of water and nutrients to apical portions of the spike and/or the presence of the toxin itself in those tissues may be contributing to the bleaching. The absence of bleaching in Ning 7840, on the other hand, may be due not only to a lower accumulation of this toxin and a restriction of the spread of mycotoxins (as discussed above), but perhaps to a higher tolerance to the toxin itself.

2.5.4 Limitations of Scoring Methods

There are some limitations to the phenotypic disease scoring methods used in this study. The spread of SS, VIRS or BIRS to the extremes of many spikes (either to the terminal spikelet or the peduncle) of Ning 7840 and Norm results in an underestimation of SS, VIRS and BIRS at both 7 and 14 dpi. In addition, the presence of spikes with SS or VIRS values to the terminal spikelet, in contrast to the lack of spikes with BIRS values up to the terminal spikelet, indicates that visual symptoms did not always correspond exactly with the presence of the pathogen. However, VIRS and BIRS were not significantly different overall, indicating that overall visual symptoms in the rachis reflected the presence of the fungus for Ning 7840 and Norm at 7 and 14 dpi. In addition, since VIRS and BIRS were significantly greater than the SS data, the inability to track the spread of the fungus beyond the extremes of the spike is not expected

to affect the overall trend between SS, VIRS and BIRS because SS rarely reached the terminal and basal spikelets (except for Norm at 14 dpi where it still did not reach the ends of the spike the majority of the time).

Another limitation is that no differentiation was made between the two bottommost florets on the inoculated spikelets during inoculations. Although the two florets are not equidistant from the rachis node they were not distinguished from one another since visual estimation of the positioning of the two florets was not obvious from external examination. It is possible that the rate at which the fungus reaches the rachis would be slightly different according to which floret was inoculated. In addition, for VIRS and BIRS, the rachis was sectioned according to the position of the nodes. This method of scoring does not consider if the internode length is constant within a genotype, or whether the two genotypes have a similar distance between nodes. However, since the grain is of primary interest in the spike for production practices, the scoring system used in this study may be more relevant to the effect on the grain than would absolute distance of spread in the head.

An additional limitation to the scoring methods of this study is that it is not know if the visual symptoms of the spikelets (SS) correlate well with the presence of the fungus or DON in the spikelets. TeKrony et al. (2000 and 2001) reported that visual ratings of disease in the spikelets may not correlate well with the presence of the fungus in those spikelets. In addition, studies have found that the toxin can be translocated beyond areas of fungal colonization (Kang and Buchenauer, 1999; Savard et al., 2000; Snijders and Krechting, 1992). However,

other studies have indicated that visual ratings of FHB are well correlated with seed infection (Argyris et al., 2003) and/or DON contamination (Lamper et al., 2000; Mesterhazy, 2002). This study showed good correlation between visual symptoms and the presence of the fungus in the rachis, suggesting that visual symptom in the spikelets in this study are likely well correlated with the presence of the fungus.

2.5.5 Conclusions

The fungus spreads internally through the rachis more rapidly than is exhibited by scabby spikelets, showing that there is a great amount of fungus in the rachis that is not present in adjoining spikelets. The vast majority of the spikelets were infected as a result of spread through the rachis, rather than through contacting adjacent infected spikelets. As a general rule, the spread of scabby spikelets was sequential from the point of initial infection, having very few instances of spikelets being skipped in this progression of disease. A specific mechanism appears to be responsible for preventing infection of non-inoculated spikelets adjoining infected rachis sections. Further study needs to be conducted to determine if this trend is reflected when fungal colonization and DON contamination of spikelets (versus visual symptoms) is investigated, and what mechanisms are responsible for preventing initial entry into the rachis, or later entry into the spikelets from an infected rachis. In addition, additional studies should be conducted to determine if similar results are obtained using different isolates of *F. graminearum* and different species of *Fusarium*. This study

illustrates the need for researchers working with FHB to look for alternative mechanisms of resistance.

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Fig. 2-1. Diagram of three methods of disease evaluation. A) Scabby Spikelets (SS). Spikelets examined for visual symptoms of infection. Spikelet inoculated shown in black at arrow. B) Visually Infected Rachis Sections (VIRS). Rachis sections examined for visual symptoms of infection. Borders of rachis sections indicated by horizontal lines. Node inoculated indicated in black at arrow. C) Bioassay Infected Rachis Sections (BIRS). Rachis sectioned according to horizontal lines and plated in a circular pattern on petri dish containing PDA. Sections from which Fusarium graminearum grown are considered infected. Tissues having no visual symptoms of disease are white, while those having visual symptoms of disease are either checked or black.

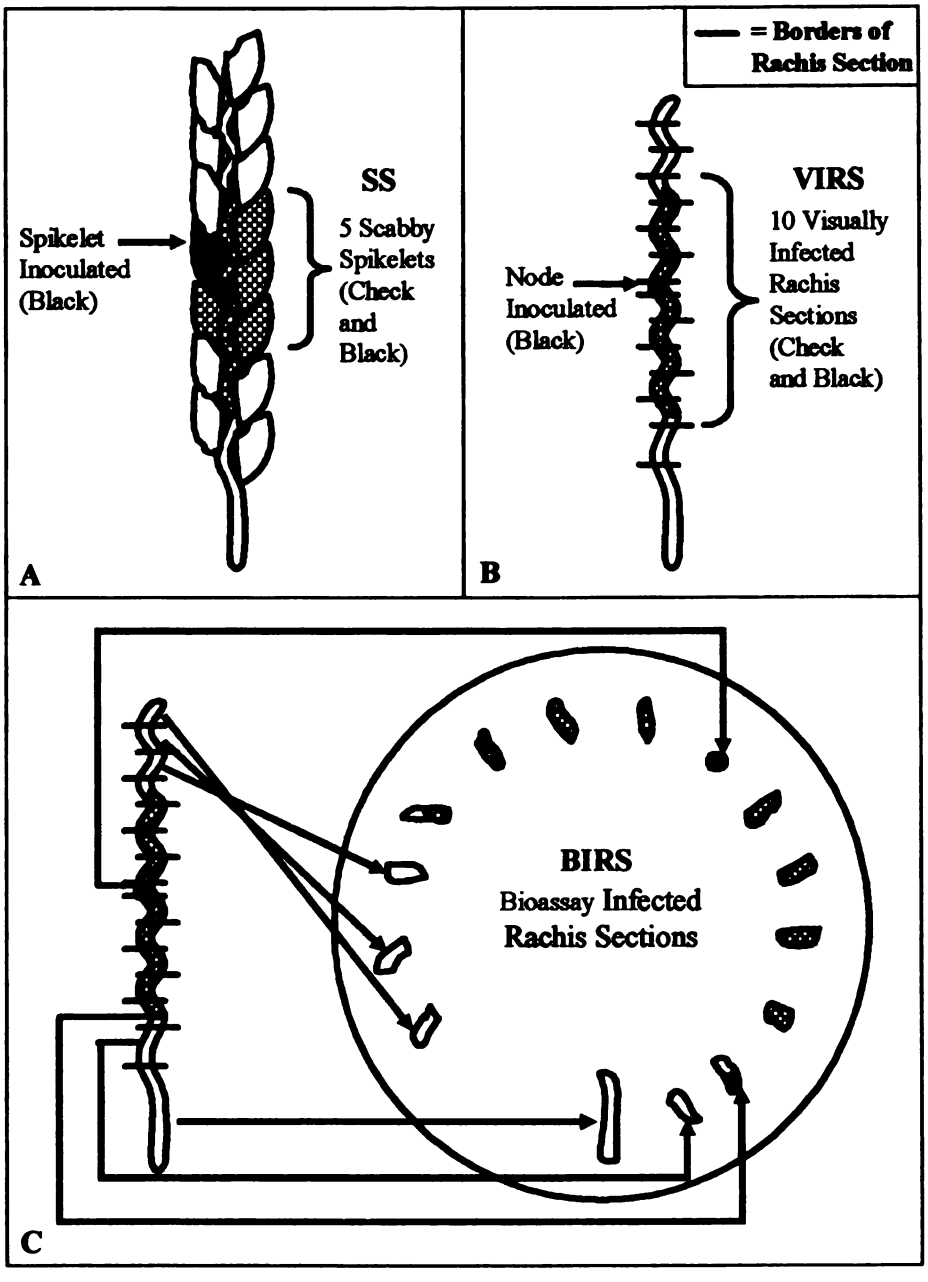
= Borders of Rachis Section
SS
5 Scabby Spikelets (Check and Black)
Spikelet Inoculated (Black)
A
VIRS
10 Visually Infected Rachis Sections (Check and Black)
Node Inoculated (Black)
B
BIRS
Bioassay Infected Rachis Sections
C

Fig. 2-1

Fig. 2-2. Effect of Genotype, time of assay, and method of assay on the number of infected spikelets or rachis sections in a resistant (Ning 7840) and susceptible (Norm) wheat cultivar. A. Comparisons between the number of scabby spikelets (SS) and the number of infected rachis sections determined either visually (VIRS) or by bioassay (BIRS) within genotypes at 7 and 14 dpi. Asterisks above a column indicate a significant difference between that column's mean and the # of scabby spikelets for that genotype/dpi combination. **B.** Comparisons between Ning 7840 and Norm for three measures of disease spread at 7 and 14 dpi. Asterisks above a column indicate a significant difference between Ning7840 and Norm for that measurement/dpi combination. **C.** Comparisons between upward and downward spread from the inoculated node for three measures of disease spread in Ning 7840 and Norm. Asterisks above a column indicate a significant difference between the upward and downward spread for that measurement/genotype/dpi combination. The "0" on the Y axis represents the inoculated node. Spread up from the inoculated node is represented by numbers >0, and down from the node inoculated is represented by numbers <0. **D.** Comparisons between Ning 7840 and Norm for upward and downward spread from the inoculated node for three measures of disease. Asterisks indicate a significant difference between Ning7840 and Norm for the upward (asterisks above upper bars), or downward (asterisks below lower bars) spread for that measurement/dpi combination. The "0" on the Y axis represents the inoculated node. Spread up from the inoculated node is represented by numbers >0, and down from the inoculated node is represented by numbers <0. *p< 0.05, ** p < 0.01, ***p< 0.001. 1. SS = Number of Scabby Spikelets. 2. VIRS = Visually Infected Rachis Sections. 3. BIRS = Bioassay Infected Rachis Sections.

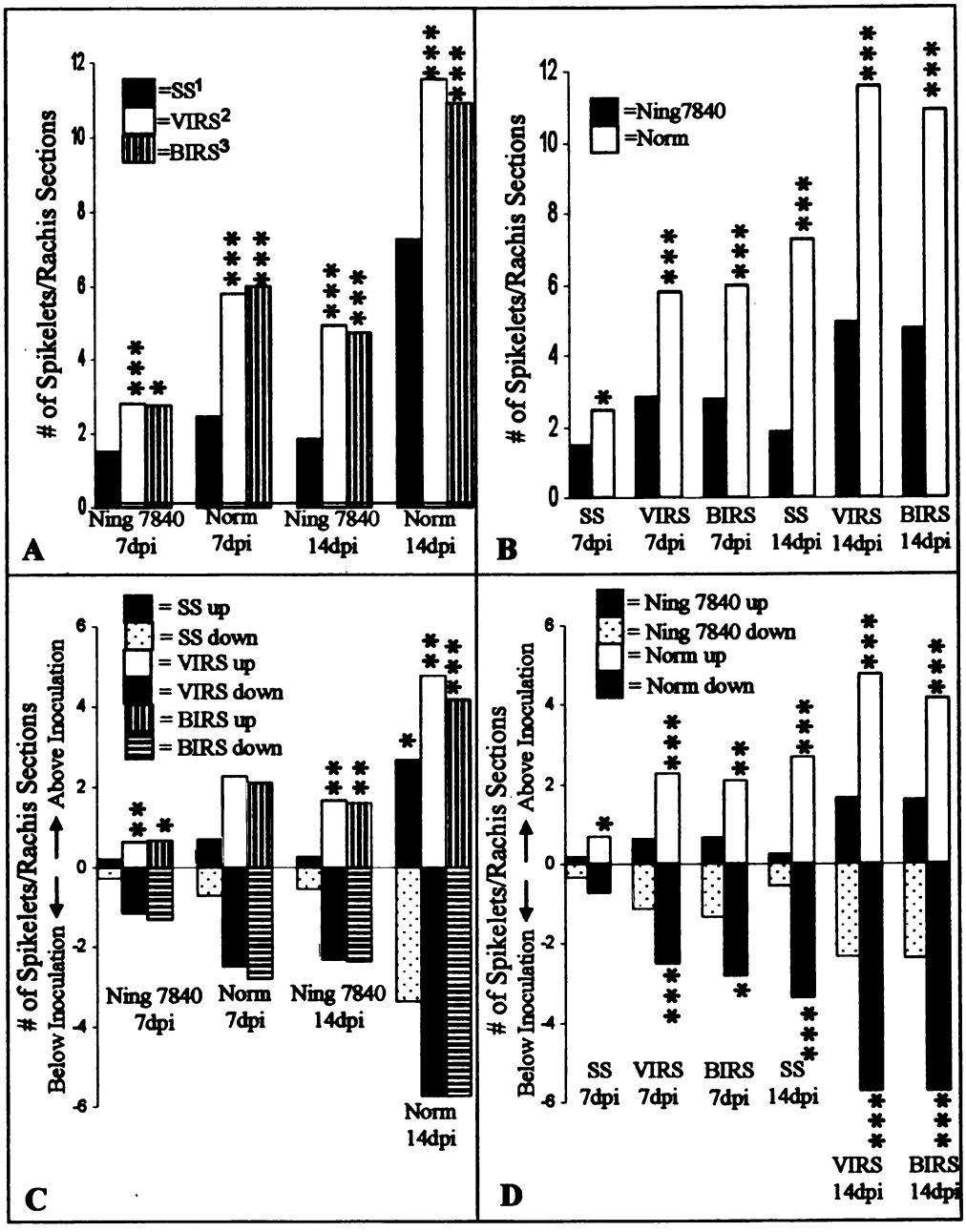
A
# of Spikelets/Rachis Sections
=SS1
=VIRS2
=BIRS3
Ning 7840 7dpi
Norm 7dpi
Ning 7840 14dpi
Norm 14dpi
B
=Ning7840
=Norm
SS 7dpi
VIRS 7dpi
BIRS 7dpi
SS 14dpi
VIRS 14dpi
BIRS 14dpi
C
= SS up
= SS down
= VIRS up
= VIRS down
= BIRS up
= BIRS down
Below Inoculation ← → Above Inoculation
D
= Ning 7840 up
= Ning 7840 down
= Norm up
= Norm down

Fig. 2-2

Fig. 2-3. Relative frequency distributions of disease as determined by three measures in Ning 7840 and Norm at 7 dpi (A) and 14 dpi (B). The Y axis represents the percent of observed spikes with a given X axis category. Note that scaling of the Y axis values varies with the measure of disease, but is consistent between the two dpi. '>' indicates values of the X axis greater than 17. Black bands = Ning 7840, White bands = Norm.

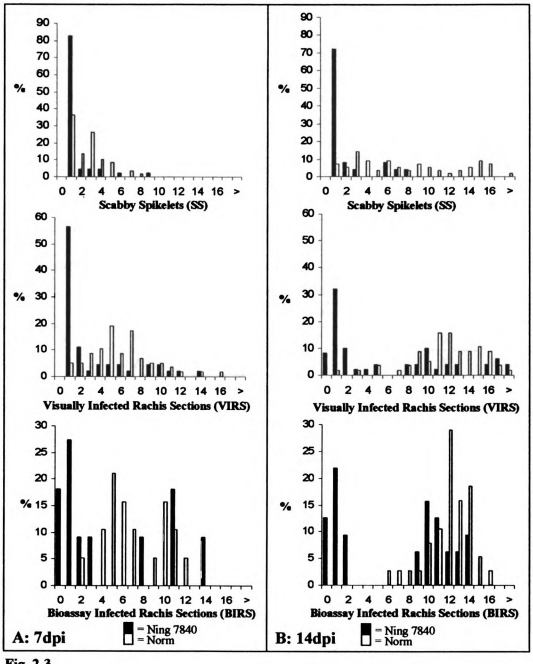
%
Scabby Spikelets (SS)
Visually Infected Rachis Sections (VIRS)
Bioassay Infected Rachis Sections (BIRS)
0 2 4 6 8 10 12 14 16 >
A: 7dpi
B: 14dpi
= Ning 7840
= Norm

Fig. 2-3.

Table 2-1: the number and percentage of observations of Ning 7840 and Norm spikes at 7 and 14dpi with SS, VIRS and BIRS values that reached the extremes of the spike (top = terminal spike, bottom = peduncle) by 7 or 14dpi. Obs. = Observations.

Genotype/dpi	Meas-urement	# Top	% Top	# Bottom	% Bottom	Total # Obs.
Ning7840 7dpi	SS	0	0	1	2	46
	VIRS	0	0	3	7	46
	BIRS	0	0	3	27	11
Norm 7dpi	SS	0	0	0	0	58
	VIRS	1	2	4	7	58
	BIRS	0	0	4	21	19
Ning7840 14dpi	SS	0	0	1	2	50
	VIRS	6	12	20	40	50
	BIRS	0	0	18	56	32
Norm 14dpi	SS	15	26	17	30	57
	VIRS	16	28	38	67	57
	BIRS	0	0	27	71	38

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CHAPTER 3

IDENTIFICATION AND MAPPING OF A QTL FOR RESISTANCE TO FUSARIUM HEAD BLIGHT ON CHROMOSOME ARM 2DL OF WHEAT

3.1 Abstract

Fusarium head blight caused by *Fusarium* spp. and *Michrodochium nivale* is a devastating disease of wheat and barley resulting in major economic losses in the wheat and barley industries in North America. To date, few sources of strong resistance have been identified and verified. The most recognized and utilized source of resistance is from the Chinese cultivar 'Sumai 3'. The identification and verification of other sources of resistance to FHB is critical to enable breeders to combine sources of resistance for a more effective resistance and to prevent the development of genetic uniformity among elite germplasm. The objectives of this research were to identify and map quantitative trait loci (QTL) for resistance from a synthetically derived wheat line. One hundred and thirty dihaploid lines developed from a cross between 'CASS94' (a synthetic derivative) and 'Flycatcher' were genotyped with 180 SSR primer pairs. One hundred and eight of the dihaploid lines were phenotyped for Type II resistance to FHB in the greenhouse using what may be a new procedure for phenotyping mapping populations for FHB resistance in the greenhouse. The strength of this phenotyping procedure is reflected in the low LSD values relative to the frequency distribution of the phenotypic means for each genotype. QTL mapping by composite interval mapping revealed a major QTL on 2DL, unequivocally

confirming the existing of an important QTL for Type II on 2DL. Additional QTL were identified on chromosome 4A and chromosome arms 5AL and 5DL.

3.2 Introduction

Fusarium head blight (FHB) caused by *Fusarium* spp. and *Michrodochium nivale* has caused major losses to the wheat and barley industries in North America (McMullen et al., 1997; Stack, 2003). FHB infection of wheat and barley not only causes losses in yield, but reduced grain quality and contamination with mycotoxins that are a serious threat to both humans and animals (McMullen et al., 1997; Snijders, 1990; Wannemacher and Wiener, 1997). Economic losses due to FHB in wheat and barley in the United States were estimated to be nearly three billion dollars during the 1990's alone (Windels, 2000).

Resistance to FHB has been categorized according to the effect of the resistance (Mesterhazy, 1995; Schroeder and Christensen, 1963; Wang and Miller, 1988) with Type I, resistance to initial infection, and Type II, resistance to spread being the most frequently studied forms of resistance (Schroeder and Christensen, 1963). Known resistance to FHB is partial and not highly heritable with the phenotypic response to infection being greatly influenced by the environment (Rudd et al., 2001). To date, no source of complete resistance to FHB has been identified, and strong resistance (i.e. resistance resulting in a large reduction in disease severity) to FHB in wheat has been confirmed for a relatively small number of resistance sources (Kolb et al., 2001; Rudd et al., 2001). The most widely recognized source of resistance is from the Chinese

wheat line 'Sumai 3', which has a major QTL (i.e. explaining a large proportion of phenotypic variance), *Qfhs.ndsu-3BS*, for Type II resistance to FHB mapped to chromosome 3BS, which explains as much as 60% of the phenotypic variation (Anderson et al., 2001; Buerstmayr et al., 2002; Buerstmayr et al., 2003; Chen et al., 2003; del Blanco et al., 2003; Zhou et al., 2003; Zhou et al., 2002). Sumai 3 resistance is being actively employed in wheat breeding programs throughout the world as wheat breeders work to develop cultivars with improved resistance to FHB (Rudd et al., 2001).

Although Sumai 3 offers a source of strong Type II resistance, the identification of other sources of resistance is important to enable breeders to avoid genetic uniformity for resistance to FHB, as well as allow breeders to pyramid resistance genes to develop cultivars with more effective and more durable resistance (Ruckenbauer et al., 2001).

Wide crosses have been used to identify and introgress important traits into cultivated crop species (Bommineni and Jauhar, 1997; Tanksley et al., 1996). Synthetic wheats, created via the hybridization of *Triticum turgidum* L. (AABB) with *Aegilops tauschii* Coss. (DD), have proven to be a powerful tool for introgression of genes from the diverse accessions of *Ae. tauschii* into the fairly narrow germplasm pool of common wheat (*Triticum aestivum*, AABBDD) (Ladizinsky, 1985; Lage et al., 2003; Lubbers et al., 1991; Maan, 1987; Mujeeb-Kazi, 1993; Mujeeb-Kazi, 1995). The objective of this study was to map QTL for Type II resistance to FHB using a dihaploid population of wheat developed from

a cross between a synthetic derivative ('CASS94') and a susceptible genotype ('Flycatcher').

3.3 Materials and Methods

3.3.1 Plant Materials:

A population of 171 putatively distinct dihaploid (DH) lines developed by Dr. A. Mujeeb-Kazi was employed in this study. The parents of the pop were 'Flycatcher', an FHB susceptible common wheat (*Triticum aestivum* L. ssp. *aestivum*), and CASS94Y00009S-51PR-4B-0M-1Y-0M (designated 'CASS94' from this point onward) (Mujeeb-Kazi, 1995). CASS94 had been identified as an FHB resistant F6 line derived from a cross between 'Mayoor', a moderately resistant common wheat, and the synthetic hexaploid TK SN1081/*Ae. tauschii* (222) (Mujeeb-Kazi et al., 1999). The pop was developed using wheat x maize pollination (Laurie and Bennett, 1988) followed by doubling of the chromosomes (induced with cholchicine or spontaneous).

One hundred and thirty distinct DH lines were used for construction of a molecular marker map (see DNA Marker Analysis below). Of those 130 DH lines, 108 were evaluated for resistance to FHB.

3.3.2 Greenhouse Planting:

One hundred and eight DH lines, CASS94, Flycatcher, 'W14' (an FHB resistant genotype - Chen et al., 2003; Jiang, 1997) and 'Norm' (an FHB susceptible genotype) were evaluated for FHB resistance in sixteen replications

planted over a period of seven and a half weeks in the greenhouse at Michigan State University in 2003. The experiment included 36 additional DH lines, which were subsequently identified as likely duplicates of other DH lines (see DNA Marker Analysis below). In total, the experiment included 148 treatments. One to three seeds were planted per pot. Six to seven days after planting, seedlings were vernalized (average temp of 2.5°C) for two weeks to accelerate the rate of maturation and reduce the range in flowering times among the different genotypes. After vernalization, seedlings were placed in a chamber at 15.6°C for three days to prevent de-vernalization. Pots were returned to the greenhouse for the remainder of the experiment. Thinning and transplanting was conducted so that each pot had only one plant. Pots were arranged in rows according to genotype, so that a single row would include the sequential planting dates for a single genotype. Each DH line was arranged in a single row, while parent lines (CASS94 and Flycatcher) and check lines (W14 and Norm) were arranged in more than one row for better representations of different microenvironments in the greenhouse (all within a single greenhouse room). An average of two plants per DH line, 4 plants per parental line (CASS94 and Flycatcher) and an average of 12 plants per resistant and susceptible check (W14 and Flycatcher, respectively) were used from each planting date. For the first two planting dates, seeds were sown in 3.5-inch square plastic pots, but these seedlings were transplanted in the greenhouse after vernalization into D40 Deepots™ cells, which were used for all other planting replications. Plants were maintained in the

greenhouse at an average temperature of 19.3°C (min 7.8°C and max 33.2°C) with natural sun and artificial lighting providing a total daylength of 20hrs.

3.3.3 Inoculum Production

Two isolates of *Fusarium graminearum* were mixed to generate inoculum, isolates PH-1 (NRRL 31084) and an isolate collected by Dr. Jianrong Shi from a Michigan wheat field in 2002. Macroconidial spores of both isolates were produced in mung bean liquid media (Bai and Shaner, 1996 modified). The procedure was as follows. Mung beans were boiled in water until approximately 5-10% had burst. The liquid was then strained to remove the mung beans and the liquid was aliquoted into flasks which were then autoclaved. After being cooled, the liquid media was inoculated with a small amount of *F. graminearum* mycelia and shaken at room temperature at 250-350rpm for approximately 10 days to induce sporulation. Macroconidia were counted using a hemacytometer and the two isolates were mixed for a 1:1 ratio for a final concentration of 50,000 spores/ml (25,000 spores/ml per isolate).

3.3.4 Inoculation

Plants were inoculated by single floret inoculation (Schroeder and Christensen, 1963) using a micropipette to dispense 10µl of inoculum into a single floret at anthesis. Florets were selected as close to the onset of anthesis as possible, preferring florets just before dehiscence. Florets in the center of the spike were targeted, but florets in spikelets towards the top or bottom of the spike

were also used as necessary, excluding the terminal and basal spikelets. Immediately after inoculation, plants were misted twenty seconds every six minutes for 72 hours in a greenhouse misting chamber under natural light conditions at an average temperature of 18.6°C (min 9.0°C, max 31.0°C). During and after misting, plants were arranged on the benches using a scheme devised to avoid plants from the same genotype being clustered together during misting and during evaluations of the plants later. This scheme was not a formal statistical randomization of the genotypes, as it was impractical to do a formal randomization with spikes selected on-the-spot. After misting, plants were placed on benches in the greenhouse under natural light conditions (ranged from 12.27 to 15.11h) at an average temperature of 21.8°C (min 9.7°C, max 30.7°C).

All plants inoculated on a single day were considered a single replication of the experiment. In total, twelve replications were conducted over a 10-week period. Because different genotypes matured at different rates, a single replication included plants from more than one of the sixteen planting dates. This approach resulted in all spikes in a given replication being inoculated on the same day, but not necessarily from the same planting date. In addition, not all genotypes were represented in all replications, and different genotypes were represented to a greater or lesser extent in different replications. Considering all replications, in total 21 to 42 spikes were inoculated per DH line. In addition, 67 spikes of CASS94, 62 spikes of Flycatcher, 184 spikes of W14, and 193 spikes of Norm were inoculated in total. At least one spike each of W14 and Norm was inoculated in each replication. The largest replication included 620 spikes total,

while the smallest replication included 88 spikes. The average number spikes per replication was 395.

3.3.5 Evaluation of Disease

The number of visually scabby spikelets on a spike was recorded at 7, 10, 14, 17 and 21 days post inoculation (dpi) (plus or minus approximately 12h) for all replications except replication three, for which most spikes were not evaluated at 14 dpi, and replication four, for which symptoms at 10 dpi were not evaluated and symptoms were evaluated at 18 dpi, instead of 17 dpi. For all spikes, in addition to the number of visually scabby spikelets the total number of spikelets per spike was also recorded.

3.3.6 Data Analysis

Of the 4,737 spikes evaluated in the entire experiment, eighty-eight (<2%) did not show any symptoms by 21 dpi and were not included in the analysis based on the assumption that these spikes were not inoculated. In addition, spikelets within an inoculated spike that appeared to be infected only due to outside contact from an adjacent spikelet (i.e., not from spread through the rachis) were not counted in the total number of spikelets considered infected because it did not represent internal spread through the plant tissue, but instead represented another initial infection point. Overall, infection from contact on the outside of the spike occurred very infrequently. Bleaching (a general loss of pigment) and/or wilting symptoms (Bushnell et al., 2003) of spikelets apical of

colonized portions of a spike were not distinguished from symptoms physically coinciding with colonization of the fungus in a spike.

The number of scabby spikelets and the total number of spikelets per spike were used to determine three other measurements of disease: percentage of scabby spikelets, area under the disease progress curve (AUDPC) for the number of scabby spikelets, and AUDPC for the percentage of scabby spikelets. The AUDPC was separately calculated as described in Shaner and Finney, 1977, for each of the observation dates after 7 dpi (i.e. 10, 14, 17 and 21 dpi). Specifically:

$$AUDPC = \sum_{i=1}^{n} [(Y_{i+1} + Y_i)/2] [X_{i+1}-X_i]$$

where Y_i = either the number or percent of scabby spikelets, and X_i = the day post inoculation of the i'th observation, and n = the total number of observations (Shaner and Finney, 1977). Replication 4 was not included in the AUDPC analyses because symptoms were not observed at 10 dpi. In addition, much of replication 3 was not included in the analyses of AUDPC after 10 dpi because symptoms for most spikes were not observed at 14 dpi.

Estimations of genotypic means and experiment-wise LSDs for all measures of disease were determined with the Proc Mixed LSmeans procedure of SAS® 8.2 (SAS Institute, 2000) with replication designated as a random factor in the model. This analysis included all 148 treatments. For the number of scabby spikelets and the percent of scabby spikelets, the observations at 18 dpi

in the fourth replication were evaluated with the observations at 17 dpi for the other replications (though they were not included in the AUDPC analyses, as mentioned above).

3.3.7 Independent Phenotyping of CASS94

Investigation of the phenotypic data of CASS94 revealed that it did not exhibit a high level of resistance (Fig. 3-2), in contrast to preliminary field data obtained at CIMMYT (data not shown). A second shipment of CASS94 was sent from CIMMYT to MSU. A small-scale phenotypic study was conducted to compare the two different sources of CASS94, using Flycatcher, W14 and Norm as checks. Seeds (treated with pentachloronitrobenzene) of each genotype were sown at a rate of approximately 10 seeds per pot. Seven days after planting seedlings were vernalized for two weeks at approximately 4.3°C (39.7°F). Vernalized seedlings were then moved to an air conditioned room for two to three days to prevent de-vernalization. These seedlings were then transplanted in the greenhouse at a rate of 4 seeds per pot (4-inch square plastic pots). Genotypes were arranged in rows according to genotype and planting date, but were re-arranged into rows only according to genotype (i.e., combine all planting dates for a single genotype) once the first group of inoculations were begun. Inoculum production, inoculations and misting were performed as described above, except that carboxymethylcellulose media (Cappellini and Peterson, 1971) was used for inoculum production instead of mung bean media, and no specific method was used for pot placement during or after misting. Plants inoculated on a single day

were considered a single replication and four replications were conducted total. Terraguard ® 50W, a fungicide having the active ingredient triflumizole, was accidentally applied to three of the four replications: two of which were exposed at least 3 days after inoculation, and the third replication being exposed two days before inoculation. Triflumizole is not listed as having efficacy on *Fusarium* spp., and disease progression appeared to be normal in all replications according to the expected responses of the check varieties (W14, Norm and Flycatcher) as well as the first source of CASS94. Plants were evaluated for the number of scabby spikelets at either 14 or 17 dpi, depending on when a wide range of disease symptoms within the replication was apparent. The total number of spikelets on each spike was also recorded and the percentage of scabby spikelets was calculated. An estimate of the means of each genotype, for the number of scabby spikelets and the percent of scabby spikelets, as well as a means separation test was conducted using the Proc Mixed procedure of SAS® 8.2. (SAS Institute, 2000), with replication as a random factor in the model.

3.3.8 DNA Marker Analysis

A cetyltrimethylammonium bromide procedure modified from Edwards et al., 1991 (Edwards et al., 1991) was used for DNA extraction.

The population and parents were genotyped using microsatellite (SSR) primer pairs. Four-hundred and fifty-seven BARC microsatellite primer pairs (Song et al., 2002) were screened for polymorphism between CASS94 and Flycatcher. One hundred and sixty-nine (37%) of primer pairs screened were

polymorphic and were used to genotype the population, the majority of which had already been mapped genetically or physically (Shi et al., 2003). Eighteen GDM, thirty-two GWM (Pestsova et al., 2000; Röder et al., 1998a; Röder et al., 1998b) and three WMC primers (Wheat Microsatellite Consortium, 2004) that had been identified by Dr. Kazuhiro Suenaga as being polymorphic between the parent lines and some DH lines of the population were also used for genotyping the population. In addition to the parent lines (CASS94, both sources analyzed separately, and Flycatcher) and the 171 DH lines, Mayoor (the *T. aestivum* L. parent of CASS94), the original synthetic (TKSN1081/*Ae. tauschii* (222), cross id #CIGM88.1217-0B) as well as the *T. turgidum* parent (TKSN1081, *Triticum turgidum* ssp. *durum*) of the synthetic hexaploid were genotyped.

PCR amplification was conducted with a total volume mixture of 15µl containing 37.5ng DNA, 0.15µM of each primer, 1X PCR buffer (Promega), 1.5mM MgCl2 (Promega), 0.15 of each dNTP and approximately 1.8 units of Taq. It should be noted that the Taq was not formally quantified, but the concentration needed was empirically determined. PCR reactions were performed on an MJ Research PTC-225 Thermocycler and an MJ-Research PTC-0220 DNA DYAD Thermocycler using a touchdown PCR program (Sambrook and Russel, 2001) with the following parameters: three minutes at 95°C, then 10 cycles of: 95°C for 25s, annealing temperature + 10°C for 25s (decreasing by 1°C each cycle) 72°C for 45s, followed by 35 cycles of: 94°C for 25s, annealing temperature for 25s, 72°C for 45s, followed by 72°C for 10 minutes. PCR products were visualized on a C.B.S. Scientific "Mega-Gel High

Throughput Vertical Unit" (model C-DASG-400-50) according to the method detailed in Wang et al., 2003.

Inspection of the haplotypes of all DH lines revealed a number of suspected duplicates. One DH per group of duplicates was arbitrarily selected for use in linkage and QTL analysis. No phenotypic data was used to decide which of the genotypes remained in the population. In total, 41 of the original 171 DH lines were excluded. Therefore a total of 130 DH lines were used for linkage map and QTL analysis, of which 108 lines had been phenotyped.

Thirty four loci, amplified by thirty-two primer pairs (22 BARC, 3 GDM and 7 GWM), showed alleles of unknown origin (i.e., not matching those of either parent) in the dihaploid population and/or polymorphism between the DNA of the two different sources of CASS94. These primer pairs were not used in linkage or QTL analyses, but were examined for their putative mapping positions according to previously reported maps (Pestsova et al., 2000; Röder et al., 1998b; Shi et al., 2003; Somers, Isaac and Edwards, *pers. comm.*) (Table 3-1).

3.3.9 Linkage Map Construction

Linkage map construction was performed in JoinMap® 3.0 (Van Oijen and Voorrips, 2001) using SSR data for 130 DH lines. Segregation ratios of loci alleles were investigated and loci having extreme segregation ratio distortion ($p < 0.001$ or less, 8 markers total) were excluded at the onset of linkage map construction. Unordered linkage groups with a LOD≥ 4.0 were selected for map creation from the JoinMap "LOD groupings (tree)" generated with a LOD ranges

set at 3.0 to 10.0. Ordered line maps were generated using the following criteria: LOD threshold of 3.0, goodness- of-fit "jump score" threshold of 5.0 and a recombination frequency threshold of 0.30 except for three linkage groups (3D, the linkage groups closer to the short arm telomere on 5D and 1A) for which a 0.35 recombination frequency threshold was used. In many cases a single ordered linkage map could not be created from a single unordered linkage group. In these instances, more than one ordered linkage map was generated using subsets of loci from the original unordered linkage group. Upon investigation, these subsets of loci from a single unordered linkage group created non-overlapping linkage maps for a single chromosome, as revealed by comparison with a previously generated linkage map (Pestsova et al., 2000; Roder et al., 1998b, Shi et al., 2003; Somers, Isaac and Edwards *pers comm.*).

3.3.10 QTL Analysis

QTL analysis was performed in Windows QTL Cartographer V. 2.0 (Wang et al., 2001-2004). The trait data used in the analysis included the number of scabby spikelets and the percentage of scabby spikelets at all five days of evaluation (7, 10, 14, 17/18 and 21 dpi), plus the AUDPC calculated for the number of scabby spikelets and the percentage of scabby spikelets at 10, 14, 17 and 21 dpi. Single Marker Analysis (SMA) was performed and markers showing significance ($p<0.05$) were identified. Composite Interval Mapping (CIM) was performed with forward and backward regression using a walking speed of 2cM and a window size of 10cM. The LOD threshold value of each trait was

empirically derived for a 0.01 experiment-wise error rate using 1000 permutations, and this LOD threshold was used to declare significance using CIM (Table 3-2). For putative QTL regions consistently identified using SMA but not identified using CIM, the CIM p-values corresponding to these putative QTL were determined and those with p-values<0.05 are reported. Epistatic interactions of QTL were tested using Multiple Interval Mapping function in Windows QTL Cartographer V. 2.0.

QTL were graphically displayed with line maps using MapChart (Voorrips, 2002). Support intervals were determined in MapChart for each QTL and displayed graphically. Support intervals are determined relative to the highest LOD score in the QTL region. A 1-LOD support interval shows the region of the QTL whose LOD scores do not differ by more than 1 from the highest LOD score in the QTL. A 2-LOD support interval shows the region of the QTL whose LOD scores do not differ by more than 2 from the highest LOD score in the QTL. These support intervals were determined to give a more accurate picture of the region of the QTL of greatest significance.

3.4 Results

3.4.1 SSR Data

The banding patterns for the two sources of CASS94 were the same in 91% of the primers evaluated. The banding patterns of Mayoor (the *T. aestivum* parent of CASS94) were similar to CASS94 (from either source) for approximately 84% of the primer pairs investigated. The synthetic wheat parent

of CASS94, however, had banding patterns matching that of CASS94 in only approximately 15% of the primer pairs investigated. In addition, the *T. turgidum* parent of CASS94 had banding patterns matching that of CASS94 in approximately 16% of the primer pairs investigated, and matched that of the synthetic wheat (for which it is a parent) for only approximately 47% of the primer pairs for which the durum parent was amplified (i.e., excluding primers for which the durum did not amplify, which would be expected for D genome primers since the durum wheat only possesses the A and B genomes).

Of the BARC primers screened, 37% were polymorphic, 49% percent were monomorphic and 14% were indeterminable (inadequate amplification) between CASS94 and Flycatcher. A total of 180 primer pairs (117 BARC primers pairs, and 63 GWM/GDM or WMC primer pairs) were used to genotype the population, amplifying a total of 191 putative loci. However, of these 191 putative loci, 33 (17.3%) showed inconsistencies in banding patterns between the two different sources of CASS94 (received in 2002 and 2003), and/or the population displayed multiple allele (more than two) banding patterns (Table 3-1), primarily due either to differences between the two sources of CASS94 or a second allele in Mayoor, a parent of CASS94. These problematic loci were not used in mapping or QTL analysis.

3.4.2 Linkage Map Construction

Of the 158 putative loci (amplified by 149 primer pairs) investigated 85.4% fit a 1:1 segregation ratio at $p<0.05$, an additional 7% fit a 1:1 segregation ratio at

$p<0.01$, 2.5% fit a 1:1 segregation ratio at $p<0.001$, and the remaining 5% did not fit a 1:1 segregation ratio at $p<0.001$. Those that did not fit a 1:1 segregation ratio at $p<0.001$ were removed from linkage analysis, and the majority of the other putative loci mapped to expected positions on the basis of previous reports (Pestsova et al., 2000; Röder et al., 1998b; Shi et al., 2002; Somers, Isaac and Edwards pers comm.). Nine BARC SSR primer pairs were mapped that had not been previously mapped (to my knowledge) (Table 3-3). Overall, one hundred and twenty –nine loci, amplified by 123 primer pairs were mapped to 26 linkage groups on 16 chromosomes, covering a total of 700.8 cM (Fig. 3-1).

The putative locations of markers showing inconsistencies in banding patterns between the two sources of CASS94 and/or multiple allelic banding patterns was also investigated. Nine of these problematic primers have putative mapping locations on chromosomes 2A and 3A. Furthermore, all the SSRs identified for chromosomes 2A and 3A were problematic, and hence neither of these chromosomes is included in the final linkage map. Four additional problematic primers were identified with putative mapping positions flanking one of the linkage groups associated with chromosomes 2D (*Xbarc168* to *Xgdm6*), and a number of other problematic markers scattered across the remainder of the genome.

3.4.3 Phenotypic Data

There were significant differences among progeny for all disease scoring methods at all dpi. LSDs ($p<0.05$) and frequency distributions of means are

presented in Fig. 3-2. The LSD ($p<0.05$) and frequency distribution of the average spike size for each genotype is shown in Fig. 3-3.

Neither CASS94 nor Flycatcher exhibited a high level of resistance and both appear to be susceptible by 21 dpi according to the percentage of scabby spikelets. In addition, although CASS94 had consistently lower means than Flycatcher for each type of disease measure, the means were not significantly different ($p< 0.05$) for the number of scabby spikelets and the AUDPC of the number of scabby spikelets at 17 and 21 dpi, or for the percent of scabby spikelets and the AUDPC of the percent of scabby spikelets at 21 dpi. Furthermore, CASS94 was not significantly different from the susceptible check, Norm for the number of scabby spikelets and the AUDPC of the number of scabby spikelets at 14, 17 and 21 dpi, or for the AUDPC of the percent of scabby spikelets at 21 dpi. With respect to the resistant check, W14, the mean of CASS94 was significantly greater for all types of disease measurements at all dpi.

No significant difference was detected between the phenotypic means for the two sources of CASS94, suggesting that the initial source of CASS94 provided an accurate measure of the CASS94 parent of the population. Thus, the population appears to exhibit a high level of transgressive segregation, revealed through the wide distribution around the means of the parent genotypes for most of the dpi for the different disease measurement methods.

For all days post inoculation and for all disease measures, except for the percentage of scabby spikelets at 21 dpi and the AUDPC of the percentage of

scabby spikelets at 21 dpi, there were genotypes of the population that had disease rating scores that were not significantly different from W14 ($p<0.05$), but no genotypes were identified that had a significantly less spread of disease than W14. For the number of scabby spikelets at 21 dpi, two genotypes were identified that were not significantly different from W14, and for the AUDPC of the number of scabby spikelets there was an additional third genotype that was not significantly different from W14. The haplotypes of these three very resistant genotypes showed that they all carried the CASS94 allele at *Xgwm539*, but they differed for alleles at *Xgwm30* and *Xwmc41/Xbarc228*. For these three genotypes, the total number of spikelets on a spike (i.e. the overall spike size) was significantly less than W14.

3.4.4 QTL Analysis

Single Marker Analysis (SMA) and Composite Interval Mapping (CIM) both revealed a prominent QTL for FHB resistance on chromosome arm 2DL, having LOD scores as high as 25.1 and R^2 values (i.e., the proportion of the phenotypic variance explained by the QTL) ranging between 0.29-0.60 (Fig. 3-4, Table 3-4). This QTL was identified in every type of analysis that was used for every dpi of the disease evaluation. The QTL analysis reveals that this QTL is most closely linked to *Xgwm539* and resistance is from CASS94. The average phenotypic mean for 99 of the 108 DH lines (data for this locus was unavailable for 9 lines) with the CASS94 allele versus the Flycatcher allele at *Xgwm539* is shown in Table 3-4. The average percent reduction in phenotypic means for genotypes

with the CASS94 versus Flycatcher allele at *Xgwm539* was between 24 and 60% (Table 3-4). The frequency distribution of the 99 DH lines with and without the CASS94 allele for *Xgwm539* was determined for the number of scabby spikelets at 10 dpi (Fig. 3-6) and the percent of scabby spikelets at 21 dpi (Fig. 3-7). As an additional confirmation of these results, phenotypic data that were excluded from QTL analysis due to duplication were averaged according to duplication group (i.e. genotypes suspected to be duplications of one another were averaged together). These averages were treated as single individuals in an average of the phenotypic data for progeny having the CASS94 or the Flycatcher alleles at *Xgwm539*. These new averages also showed a reduction in disease of 24 – 60% with the CASS94 allele, supporting the suspicion that these genotypes are duplications, and supporting the evidence of the QTL on chromosome arm 2DL.

Other regions with putative QTL were identified (Table 3-5). A QTL was detected (with both SMA and CIM) spanning the centromere on chromosome 4A at 14 dpi for the AUDPC of the percent of scabby spikelets where the resistance allele came from Flycatcher [SMA A-value (i.e., the effect of substituting one CASS94 allele with a Flycatcher allele) = -18.24 area units and CIM R^2 = 0.07] (Fig. 3-5). However, although this QTL was only detected for a single measure, all loci on the linkage group spanning the centromere on chromosome 4A were identified with SMA for every disease measure at all dpi. A smaller linkage group on 4AL (consisting of only *Xbarc1047* and *Xbarc343*) was identified with SMA as significant for every dpi for the percent of scabby spikelets and the AUDPC of the percent of scabby spikelets (CIM $p<0.016$ for the AUDCP of the percent of

scabby spikelets at 10dpi, SMA A-value -12.59 area unites, CIM R^2 = 0.15). QTL were also identified (with both SMA and CIM) on chromosome arms 5AL (SMA A-value = -1.06 spikelets, CIM R^2 = 0.12) and 5DL (SMA A-value = 1.12 spikelets, CIM R^2 = 0.11), for the number of scabby spikelets at 21 dpi (Fig. 3-5). Flycatcher provides the resistant allele for the 5AL QTL at 21dpi, whereas CASS94 provides the resistant allele for the 5DL QTL at 21 dpi. For chromosome arm 5AL, a different QTL was identified with CIM at 10 dpi for the percent of scabby spikelets (SMA p <0.21, SMA A-value = 2.58%, CIM R^2 value = 0.06) and 14 dpi for the AUDPC of the percent of scabby spikelets (SMA p<0.20, A-value = 7.44 area units, CIM R^2 value = 0.06) for which resistance is provided by the CASS94 allele (Fig. 3-5). With SMA, although one locus (*Xgwm271*) was detected as significant for this QTL region for the percent of scabby spikelets at 7dpi, the corresponding CIM value was not significant (CIM p< 0.28). Chromosome arm 3BS was identified by SMA for the number and percent of scabby spikelets as well as the AUDPC of both the number and percent of scabby spikelets at 14, 17 and 21 dpi (CIM p<0.034 for the percent of scabby spikelets at 14dpi, SMA A-value = -5.47 percent of scabby spikelets, CIM R^2 value = 0.04; CIM p<0.039 for the AUDPC of the percent of scabby spikelets at 17dpi, SMA A-value = -10.08 area units, CIM R^2 value = 0.05). Chromosome arm 1BL was regularly identified as significant using SMA (CIM p<0.032 for the AUDPC of the percent of scabby spikelets at 10dpi).

Tests for epistasis were conducted using Multiple Interval Mapping function of QTL Cartographer V 2.0 for the QTL being significant with CIM ($p<0.01$) or significant with SMA and also having a CIM $p<0.05$. No epistatic interactions were identified between any of these QTL.

3.5 Discussion

3.5.1 QTL on 2DL

These results provide extremely strong confirmation of the presence of a QTL on chromosome arm 2DL, as first reported by Somers et al., 2003. This QTL has been named *Qfhs.crc-2D* and this name will be used to refer to this QTL for the remainder of this article.

In this study, *Qfhs.crc-2D* was identified with CIM LOD scores between 10.5 and 25.1 for all four disease measurement methods and each dpi, including 7 dpi which had a frequency distribution that was dramatically skewed towards zero. The greatest R^2 values (as high as 0.60) occurred at 10 dpi for all types of disease scoring methods except for the percent of scabby spikelets, for which the R^2 value at 14 dpi was the highest. This trend of high R^2 values at 10 and 14 dpi might reflect a greater precision in identifying the means of the genotypes at 10 and 14 dpi, or could indicate that this resistance is more pronounced at 10 and 14 dpi in this study. In either case, the effect of *Qfhs.crc-2D* was consistently identified throughout the development of the disease between 7 and 21 dpi.

Somers et al., 2003, investigated FHB resistance in a dihaploid population developed from a cross between 'Wuhan-1' and 'Maringa'. Their study also

reported this QTL as being associated with Type II resistance (resistance to spread). In their population, resistance was associated with the genotype 'Wuhan-1', which is a Chinese accession of unknown pedigree (Daryl Somers pers comm.). Wuhan-1 is not part of the pedigree of CASS94, from which resistance is derived in the population reported here. Somers et al., 2003 detected this QTL with a LOD between 2.5 and 3.0 and an R^2 value of 0.09, revealing that this QTL explained a relatively small amount of the phenotypic variance observed. However, when the mean percent of scabby spikelets at 21 dpi was compared for genotypes having the Wuhan-1 versus the Maringa allele at *Xgwm539*, the average of the phenotypic means was 23.2% and 33.1% for the Wuhan-1 and Maringa alleles, respectively, revealing an overall reduction of 29.9%. The percent reduction observed by Somers et al., 2003 is comparable to the percent reduction observed in this study at 21 dpi (26.1%, see Table 3-4) at *Qfhs.crc-2D*.

3.5.2 Potential for Marker Assisted Selection

One critical requirement for marker assisted selection is that the markers used in selection are polymorphic between the parents of a cross (Koorneef and Stam, 2001). *Xgwm539* has been identified as having an especially high level of polymorphism in two different studies across diverse collections of germplasm (McCartney et al., 2004; Quarrie et al., 2003). Quarrie et al., 2003, used 37 SSR primer pairs amplifying loci across the genome to genotype a diverse collection of 96 genotypes from different countries. On average, each primer pair detected

8 alleles per locus, while four primer pairs, including *Xgwm539*, detected over 15 alleles per locus. Furthermore, *Xgwm539* only amplified a single locus across the 96 genotypes. McCartney et al., 2004, used 41 SSR primer pairs (amplifying loci in regions of known FHB QTL) to genotype a diverse collection of 79 wheat cultivars, most of which have been identified as resistant or moderately resistant to FHB. In their study, the *Xgwm539* locus revealed the highest number of alleles (20) of all loci examined. These studies indicate that the *Xgwm539* locus is highly polymorphic across a broad range of genotypes and is likely to only amplifying a single locus. Such a locus is ideal for marker assisted selection because it is tightly linked to a major QTL for resistance, it is likely to be polymorphic between parents of a cross and it is likely to amplify a single locus (therefore eliminating confusion that can occur when more than one locus is amplified by a single primer pair).

3.5.3 Combining Resistance

Many minor QTL have been identified for resistance to FHB (Anderson et al., 2001; Bourdoncle and Ohm, 2003; Gervais et al., 2003; Shen et al., 2003; Waldron et al., 1999). To date the *Qfhs.ndsu-3BS* QTL confers the greatest proportion of resistance to FHB of QTL that have been identified and verified. Previous QTL analyses of Type II resistance conferred by the Sumai 3 and or Sumai 3-derived line alleles at *Qfhs.ndsu-3BS* has suggested that these alleles contribute as much as a 60% reduction in disease severity (Anderson et al., 2001; Bai et al., 1999; Buerstmayr et al., 2002; Waldron et al., 1999). Even with

a large reduction of disease, this source of resistance is still not considered adequate to prevent FHB during years with severe epidemics (Buerstmayr et al., 1999; Hall and Van Sanford, 2003). Furthermore, dependence of resistance from Sumai 3 and Sumai-3 derived lines can lead to genetic vulnerability that could be overcome by the fungus (Ruckenbauer et al., 2001).

Studies of the Sumai 3 resistance report QTL analyses conducted using AUDPC data at 21/22dpi, percent scabby spikelets at 21 dpi, or the number of scabby spikelets at 22dpi (Anderson et al., 2001; Bai et al., 1999; Buerstmayr et al., 2002; del Blanco et al., 2003; Waldron et al., 1999). In this study, however, *Qfhs.crc-2D* alone contributes the greatest proportion of resistance at 10-14 dpi, according to the R^2 values. It is unknown if a similar trend is present with *Qfhs.ndsu-3BS* resistance, such that an earlier stage of resistance would detect a greater proportion of the resistance being explained by *Qfhs.ndsu-3BS*, or if these two different QTL confer resistance at different stages of FHB development. If *Qfhs.ndsu-3BS* and *Qfhs.crc-2D* both contribute a greater proportion of resistance in earlier stages of disease (versus later) then it suggests that an earlier dpi could be used for phenotyping populations for FHB. However, if the Sumai 3/Sumai-3 derived *Qfhs.ndsu-3BS* alleles contribute the greatest proportion of resistance in later stages of disease (in contrast with *Qfhs.crc-2D*) then a combination of these two sources of resistance could confer a combined resistance that is greater than either QTL alone.

An advantage of pyramiding *Qfhs.crc-2D* and *Qfhs.ndsu-3BS* is that in addition to the *Xgwm539* being highly polymorphic, the *Qfhs.ndsu-3BS* region

has been densely populated with markers (Liu and Anderson, 2003), the Sumai 3 haplotype is relatively rare throughout the world (Liu and Anderson, 2003), and marker assisted selection has already shown success with a Sumai-3 derived line (Zhou et al., 2003). A potential difficulty that could be encountered with pyramiding resistance alleles between *Qfhs.crc-2D* and *Qfhs.ndsu-3BS* is that although additive gene action has been identified as being the most prevalent and important in FHB resistance (Anderson et al., 2001; Bai et al., 2000), the type of gene-action of *Qfhs.crc-2D* is unknown and the type of interaction that will be observed through pyramiding these QTL is also unknown. However, Somers et al., 2003 found that genotypes having both a resistant allele at *Qfhs.crc-2D* in and a resistant allele at a QTL that appears to coincide with *Qfhs.ndsu-3BS* have a greater resistance than genotypes with either of the two QTL alone.

3.5.4 W14 Resistant Check

The presence of three transgressive segregants that were not significantly different from W14 for the percent and/or AUDPC of scabby spikelets at 21 dpi supports the fact that *Qfhs.crc-2D* provides a source of strong resistance. The significant difference between these three genotypes and W14 for the percent and the AUDPC of the percent of scabby spikelets at 21 dpi can be explained by the fact that the W14 has a significantly larger spike than any of these genotypes, and therefore the percent of infection is reduced. Recombination events detected between *Xgwm539* and neighboring loci for these three transgressive segregants further supports the strength of the linkage of *Xgwm539* to *Qfhs.crc-*

2D and the evidence that *Xgwm539* is the closest molecular marker (of those examined in this study) linked to *Qfhs.crc-2D*.

3.5.6 Transgressive Segregation

Transgressive segregation is frequently observed for FHB resistance (Anderson et al., 2001; Ban, 2000; Singh et al., 1995; Somers et al., 2003; Van Ginkel et al., 1996; Waldron et al., 1999), and resistant progeny have previously been identified in dihapliod lines developed from crosses between moderately susceptible and a very susceptible parents (Ban, 2000). The presence of the transgressive segregation in the CASS94/Flycatcher population could be caused by a number of factors such as epistasis or parent lines being fixed for alleles with opposite effects at loci where effects are mutually independent (Lynch and Walsh, 1998). Genome coverage in this study was only partial, and in some cases entire chromosomes were excluded. The limited coverage of the genetic map in this study leaves many areas of the genome unexplored for potential QTL. Therefore, transgressive segregation due to epistatic QTL cannot be ruled out. In addition, several of the minor QTL and putative QTL identified had positive alleles from Flycatcher, revealing that QTL existed in opposing relationships in the parents.

3.5.7 Disease Measurement Methods and QTL Detection Overall

In this study, four different measurements of disease (number of scabby spikelets, percent of scabby spikelets, and the AUDPC of the number or percent

of scabby spikelets) and five different dpi were investigated to determine whether or not different disease measurements and/or different stages of disease would be more effective for QTL identification, and whether or not these different measurement/dpi combinations would enable the identification of different QTL. Although the analyses were not independent of one another (i.e., different experiments were not conducted for each measurement method and dpi), all disease measurements and all dpi identified *Qfhs.crc-2D* as a major QTL, even though the disease had not manifested strongly by 7 dpi (as reflected by the skewed distribution of the phenotypic means) and disease was skewed towards 100% for the percent of scabby spikelets at 21 dpi. These results reflect the importance of this QTL in resistance and suggest that major QTL for Type II resistance can be adequately identified using any one of these disease measurement methods and dpi.

In contrast to *Qfhs.crc-2D*, other QTL (and putative QTL) identified were not detected in every measurement method and every dpi. The detection of significant loci on 4A (spanning the centromeric region) with all disease measurement methods and dpi using SMA, compared with the detection of a QTL in that region only at 14 dpi for the AUDPC of the percent of scabby spikelets using CIM, suggests that there is a QTL in that region and that it is most influential at 14 dpi. Sparse linkage map coverage of chromosome 4A may be hindering the detection of this QTL using other disease measurement methods and at different dpi using CIM. Paillard et al., 2004, also identified a QTL on chromosome 4A for FHB resistance according to the AUDPC for the percent of

scabby spikelets after spray inoculation in the field, but the QTL was identified in a different region of chromosome 4A.

The detection of QTL on 5AL and 5DL (SMA and CIM) at 21 dpi for the number of scabby spikelets, but not for the percent of scabby spikelets at the same dpi, may reflect the influence of spike size on the ability to detect this QTL. At 21 dpi, the distribution was skewed towards 100% for the percent of scabby spikelets, for which over twenty percent of the genotypes had spread an average of between 90-100% of the spike. Comparison of the frequency distribution for the percent of scabby spikelets versus the number of scabby spikelets at 21 dpi reveals that variation exists in spike size between genotypes since a similar skewedness is not apparent for the number of scabby spikelets at 21 dpi. The distribution of the AUDPC for the number and percent of scabby spikelets, however, were more similar to each other at 21 dpi, revealing that the overall progression of disease development was not very different between the number and percent of scabby spikelets when considering all dpi. Although the AUDPC were more reflective of the total spread of disease, the QTL on 5AL and 5DL were not detected with either AUDPC measurement at 21 dpi. The results suggest that the QTL on 5AL and 5DL are most influential at 21 dpi and the confounding effects of spike size hinder detection of this QTL using the percent of scabby spikelets. An alternate explanation is that the QTL identified on 5AL and 5DL for the number of scabby spikelets at 21 dpi are artifacts of the confounding effects of spike size for the number of scabby spikelets. Two other studies reported the identification of a QTL in the same region of chromosome

arm 5AL based on scabby spikelets observed in the field after spray inoculation, having R^2 values of 0.087 and 0.108 (Gervais et al., 2003; Paillard et al., 2004). Regarding 5DL, Gervais et al., 2003, also identified a QTL in the same region according to the proportion of scabby spikelets after spray inoculation in the field. Gervais et al., 2003 reported an R^2 value of 0.07 for the QTL on 5DL. This study confirms the presence of a minor QTL for FHB resistance on 5AL and 5DL.

The identification of a putative QTL on 4AL for the percent and AUDPC of the percent of scabby spikelets for all dpi for SMA (and CIM $p<0.016$ for the percent of scabby spikelets at 10dpi) also reflects the influence and confounding effect of spike size on QTL detection. To my knowledge, no other QTL mapping study to date has reported a QTL in this region of 4AL.

The identification of a putative QTL on 5AL (CIM) for the percent of scabby spikelets at 10 dpi and the AUDPC of the percent of scabby spikelets at 14 dpi, in the absence of SMA identification of significant loci for these two dpi/disease measuring methods, may be the result of relatively large gaps (>10cM) between loci present on these linkage groups and the weak contribution of the putative QTL to resistance. This QTL does not appear to correspond with other reported QTL according to the LOD graph of the QTL illustrated in Fig. 3-5, which suggests that the QTL is most closely linked to *Xbarc151.*

The putative QTL identified on 3BS by SMA for all disease measurement methods at 14, 17 and 21 dpi (CIM $p<0.034$ and 0.039 for the percent of scabby spikelets at 14dpi and AUDPC of the percent of scabby spikelets at 17dpi, respectively) suggests that the alleles for resistance in this region may have

some small influence in later stages of disease. The 3BS region identified here corresponds with *Qfhs.ndsu-3BS* detected in many studies as having a major QTL for Type II resistance to FHB (Anderson et al., 2001; Bai et al., 1999; Buerstmayr et al., 2003; Chen et al., 2003; Somers et al., 2003; Waldron et al., 1999).

The putative QTL identified on 1BL by SMA (CIM $p<0.032$ for the AUDPC of the percent of scabby spikelets at 10dpi) for several disease measurement methods at various dpi suggests that a minor QTL may also reside on this chromosome. Buerstmayr et al., 2002, and Shen et al., 2003 also reported the detection of a QTL for Type II resistance in the same region of 1BL having R^2 values between 0.072 and 0.16.

Overall, the use of different disease measurement methods and different dpi has enabled the identification of different QTL, including minor and putative QTL that have also been detected in other studies. The QTL explaining the largest proportion of phenotypic variance, *Qfhs.crc-2D*, was identified using any measurement method at any dpi.

3.5.8 Greenhouse Screening Design

The phenotyping scheme described here is different from those reported by other researchers for screening FHB resistance in the greenhouse. It is accepted that error variance is large in experiments characterizing FHB resistance. The approach described here was devised in an attempt to reduce the confounding effect of environment on the spread of disease for each

genotype. To accomplish this, a short period of vernalization was applied to condense the flowering period between different genotypes (as it was known that the population did exhibit some response to vernalization) and each genotype was planted a total of sixteen different times over a period of several weeks. The result was that the plants for a replication flowered and were inoculated on the same day. This means that the inoculum and post inoculation environment were the same for all plants in a give replication. This approach is effective for reducing the error variance and thus increases the proportion of phenotypic variance attributed to genotypic variance.

3.5.9 Marker Data

The population showed the expected 1:1 segregation ratio for the majority of loci. The presence of additional alleles detected with some primer pairs, in addition to occasional polymorphism detected between different sources of CASS94, suggests that CASS94 is likely not completely homogeneous. The putative clustering of some problematic loci on chromosomes 2A and 3A may reflect the fact that the heterogeneity is more centralized to certain areas of the genome. Similar phenomena were encountered by Kammohlz et al., 1998 and 2001, where segregation ratios in wheat x maize derived dihaploid populations conformed to those expected overall, but segregation distortion could usually be attributed to heterogeneity in one of the parents or alien introgression.

The high percentage of CASS94 alleles with amplicons matching those in its common wheat parent Mayoor, as opposed to its synthetic wheat parent, may

reflect selection for agronomic and quality traits after the initial cross. Analysis of the banding patterns of CASS94, its immediate parents and the *T. turgidum* parent of the synthetic wheat parent suggest the positive allele for *Qfhs.crc-2D* arises from Mayoor. It cannot be ruled out, however, that *Ae. tauschii* may be the source since that ancestor's DNA was not evaluated.

3.5.10 Consistency of CASS94 Resistance

Although the sources of CASS94 tested at MSU did not show a high level of resistance, as had been identified previously at CIMMYT (Toluca, Mexico) the conditions under which the resistance was screened were very different from those used here. At CIMMYT single floret inoculation is conducted in the field at a high elevation, low latitude research station in Mexico (Toluca) using a cotton inoculation method (i.e., soaking a cotton wad in inoculum and placing this wad in a floret) followed by covering the spike with a glassine bag (Gilchrist et al., 1997). The difference of environmental conditions, in addition to heterogeneity in CASS94 as discussed earlier and the use of different isolates of *F. graminearum* could greatly contribute to the different responses of CASS94 in the two locations. However, even though CASS94 did not show strong resistance in the greenhouse at MSU, the CASS94/Flycatcher population showed dramatic, repeatable variation for resistance and many highly resistant progeny were observed.

3.5.11 Conclusions

This work unequivocally confirms that presence of a major QTL for FHB Type II resistance at *Qfhs.crc-2D*. Currently, the *Qfhs.crc-2D* is mapped on this population in a 23.4 cM region between *Xgwm30* and *Xwmc41/Xbarc228*, with *Xgwm539* approximately in the center of that region. The density of the genetic map in the region of *Qfhs.crc-2D* needs to be increased. Either this QTL has an even greater effect than indicated here, or *Xgwm539* is extremely closely linked to the QTL. A higher density of markers would potentially enable the identification of flanking markers closer to the QTL. This, in turn, would be a very powerful tool for marker-assisted selection.

Exploitation and further study of this source of resistance at *Qfhs.crc-2D* is likely to be most effective using highly resistant progeny of this population as opposed to using CASS94 directly. In this study, the lack of strong resistance in CASS94 and the large amount of transgressive segregation observed in the population suggests that this source of resistance may be affected greatly by the genetic background with which it is associated. The Type II resistance contributed by CASS94 at *Qfhs.crc-2D* needs to be verified in additional genetic backgrounds as well as other environments. Furthermore, this study was conducted in a controlled greenhouse environment, but resistance observed in the greenhouse may not be an accurate reflection of what will be observed in a field environment, as may be suggested by the difference in the ratings of CASS94 in the greenhouse at MSU versus the field at CIMMYT, as well as from the results of other researchers (Hall and Van Sanford, 2003).

In addition to Type II resistance, the CASS94 source of *Qfhs.crc-2D* should be examined for its contribution to other types of resistance (i.e., Type I - resistance to initial infection, resistance to DON accumulation, resistance to kernel infection and the ability to degrade DON). Somers et al., 2003, tested DON accumulation and field resistance in the Wuhan-1 x Maringa population. In their study, Type II resistance conferred by Wuhan-1 at *Qfhs.crc-2D* was independent of QTL identified for DON accumulation and field resistance. However, the percent of phenotypic variance explained by the genotypic variance (i.e. R^2) was much lower in their population than in the CASS94/Flycatcher population reported here. Therefore, although they were unable to detect an effect on DON accumulation and field resistance coinciding with *Qfhs.crc-2D*, CASS94 may have a different allele than Wuhan-1 with more effective resistance at *Qfhs.crc-2D*, which can be useful for determining, with greater certainty, the involvement of *Qfhs.crc-2D* in other types of resistance. A number of other researchers have investigated the correlations of phenotypic data of DON accumulation and visual symptoms of disease, showing that visual symptoms of disease are often correlated highly with DON accumulation (Bai et al., 2001; Lemmens et al., 1997; Mesterhazy, 2002; Miller and Arnison, 1986), as well as kernel infection (Bai et al., 2001; Snijders and Perkowski, 1990) though not always (Lemmens et al., 2004). Determining if *Qfhs.crc-2D* has an effect on DON accumulation is critical, since high DON levels in grain are currently the greatest threat FHB presents to the wheat industry.

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Table 3-1. Problematic primer pairs. Primer pairs amplifying loci with alleles of unknown origin (indicated with an "A") and/or polymorphism between the two sources of CASS94 (indicated with a "P"). Mapping locations of these loci, according to previously published maps, are shown.

Primer Pair	Putative Mapping Locations	Problem?
BARC4	5BS	A
BARC12	3AS	A
BARC45	3AS	P, A
BARC57	3AS/3AL	P
BARC67	3AS	P
BARC70	4AL	P
BARC134	6BS/6BL	A
BARC136	Unknown	A
BARC164	3BL	P, A
BARC173	6DS	P, A
BARC177	5DL	P
BARC203	3BL	P
BARC212	2AS	P, A
BARC253	Unknown	P, A
BARC273	6DL	P, A
BARC278	7BL	P
BARC294	3AS	P, A
BARC301	Unknown	P, A
BARC310	3AS	A
BARC321	3AS/3DS	P, A
BARC1169	6BS	P
BARC1138	2AS	P, A
GDM93	2DL	A
GDM132	6DS	P, A
GDM136	1AS/1BL/5DL	P
GWM2	3AS	A
GWM111	7DS	A
GWM261	2DS	P
GWM296	2AS/2DS	P
GWM349	2DL	A
GWM469	5DL/6DS	P, A
GWM583	5DL	A

Table 3-2. Empirically determined LOD threshold values for each disease measure/dpi combination for $p<0.01$.

Disease Measure	DPI	LOD Threshold
Number of Scabby Spikelets	7	3.8
	10	3.5
	14	3.4
	17	3.5
	21	3.4
Percent Scabby Spikelets	7	3.5
	10	3.6
	14	3.5
	17	3.6
	21	3.6
AUDPC of the Number of Scabby Spikelets	10	3.4
	14	3.7
	17	3.6
	21	3.5
AUDPC of the Percent of Scabby Spikelets	10	3.4
	14	3.5
	17	3.6
	21	3.8

Table 3-3. Mapping locations of previously unmapped BARC primer pairs.

Xbarc Loci	Chromosome
Xbarc1155	2B
Xbarc132	3D
Xbarc266	5B
Xbarc365	6D
Xbarc357	6D
Xbarc281	7A
Xbarc338	7B
Xbarc224	7D
Xbarc336	7D

Table 3-4: Average of phenotypic means and the percent reduction for 99 DH lines with either the CASS94 or Flycatcher allele for *Xgwm539*. SS = number of scabby spikelets. P = Percent of scabby spikelets. AUDPC:SS = AUDPC of the number of scabby spikelets. AUDPC:% = AUDPC of the percent of scabby spikelets. The A-value (the value of replacing a single CASS94 allele with a Flycatcher allele) at *Xgwm539* according to Single Marker Analysis (SMA) is given. The percent reduction is calculated as 100*[1 – (average CASS94 allele phenotypic means)/(average Flycatcher allele phenotypic means)].

dpi / scoring method	CASS94 allele	Flycatcher allele	A – Value	Percent reduction
7 dpi SS	1.6 SS	3.6 SS	1.0 SS	55.6 %
10 dpi SS	3.4 SS	8.5 SS	2.6 SS	60.0 %
14 dpi SS	6.0 SS	12.3 SS	3.3 SS	51.2 %
17 dpi SS	8.5 SS	13.7 SS	2.7 SS	38.0 %
21 dpi SS	11.0 SS	14.5 SS	2.0 SS	24.1 %
7 dpi %	9.9 %	22.3 %	6.0 %	55.6 %
10 dpi %	20.7 %	51.9 %	15.4 %	60.1 %
14 dpi %	36.3 %	74.4 %	19.0 %	51.2 %
17 dpi %	50.2 %	82.3 %	16.1 %	39.0 %
21 dpi %	64.7 %	87.5 %	11.6 %	26.1 %
10 dpi AUDPC:SS	8.3 units area	20.1 units area	5.9 units area	58.7 %
14 dpi AUDPC:SS	13.6 units area	27.6 units area	7.2 units area	50.7 %
17 dpi AUDPC:SS	17.0 units area	29.5 units area	6.5 units area	42.4 %
21 dpi AUDPC:SS	22.0 units area	31.1 units area	4.9 units area	29.3 %
10 dpi AUDPC:%	51.3 units area	122.9 units area	35.1 units area	58.3 %
14 dpi AUDPC:%	83.5 units area	168.5 units area	42.0 units area	50.4 %
17 dpi AUDPC:%	103.1 units area	179.4 units area	37.9 units area	42.5 %
21 dpi AUDPC:%	132.0 units area	189.0 units area	28.6 units area	30.2 %

Table 3-5: QTL and putative QTL identified by Single Marker Analysis (SMA) ($p<0.05$) and/or Composite Interval Mapping (CIM) ($p<0.01$). For QTL identified by CIM ($p<0.01$), the dpi/disease measure listed is the dpi/disease measure with the highest CIM R^2 value if more than one dpi/disease measure was identified for that QTL. For putative QTL not identified by CIM ($p<0.01$), the dpi/disease measure having the lowest CIM p-value is indicated. SS = Number of Scabby Spikelets. P = Percent of Scabby Spikelets. AP = AUDPC of the Percent of Scabby Spikelets. Loci mapping to the same position in JoinMap are listed in the same row. The A-value is the affect the replacement of a single CASS94 allele with a Flycatcher allele at the locus. Positive A-values reveal that the Flycatcher allele increases the amount of disease, while negative A-values reveal that the Flycatcher allele decreases the amount of disease. LOD threshold values (CIM $p<0.01$) and corresponding p-values are given for each dpi/disease measure combination. The locus closest to the peak CIM LOD score of the QTL region is indicated with an *.

Chromosome Region	Dpi / disease measure	Locus	SMA		CIM		
			A-value	p-value	LOD Threshold	LOD (p-value)	R^2 value
2DL	10dpi-SS	*Xbarc168*	0.84 SS	0.0125	3.54	24.09 ($p<0.01$)	0.60
		Xgwm30	1.93 SS	0.0000			
		*Xgwm539**	2.61 SS	0.0000			
		Xwmc41 / Xbarc 228	2.13 SS	0.0000			
		Xgdm6	1.97 SS	0.0000			
5AL	21dpi-SS	*Xbarc151*	-1.14 SS	0.0013	3.42	4.84 ($p<0.01$)	0.12
		*Xgwm271**	-1.06 SS	0.0030			
		Xbarc319	-1.11 SS	0.0017			
		Xgdm63-5A	-0.87 SS	0.0155			
5AL	14dpi-AP	*Xbarc151**	7.44 area units	0.1905	3.47	3.73 ($p<0.01$)	0.06
		Xgwm271	9.25 area units	0.1037			
		Xbarc319	2.59 area units	0.6503			
		Xgdm63-5A	1.77 area units	0.7556			

Table 3-5 (Cont'd)

Chromosome Region	Dpi / disease measure	Locus	SMA		CIM		
			A-value	p-value	LOD Threshold	LOD (p-value)	R^2 value
5DL	21dpi-SS	*Xgwm292**	1.12 SS	0.0011	3.42	4.09 (p<0.01)	0.11
		Xbarc322	0.91 SS	0.0106			
		Xwmc97	0.91 SS	0.0106			
		Xgdm116-5D	0.83 SS	0.0202			
		Xgdm133	0.71 SS	0.0478			
		Xgdm63-5D	0.68 SS	0.0602			
4AS-L	14dpi-AP	*Xgwm192-4A*	-17.45 area units	0.0017	3.47	3.51 (p<0.01)	0.07
		Xgwm165	-17.45 area units	0.0017			
		Xbarc106 / Xbarc224	-18.24 area units	0.0010			
		*Xbarc233**	-18.24 area units	0.0010			
		Xbarc170	-20.89 area units	0.0001			
4AL	10dpi-AP	*Xbarc1047*	-11.13 area units	0.0189	3.41	3.22 (p<0.016)	0.05
		*Xbarc343**	-12.59 area units	0.0074			
3BS	14dpi-P	*Xbarc75*	-4.45 P	0.0725	3.42	2.91 (p<0.034)	0.04
		Xbarc133	-5.47 P	0.0263			
		Xbarc147	-5.47 P	0.0263			
		*Xgwm493**	-5.47 P	0.0258			
		Xbarc131	-3.96 P	0.1085			

Table 3-5 (Cont'd)

Chromosome Region	Dpi / disease measure	Locus	SMA		CIM		
			A-value	p-value	LOD Threshold	LOD (p-value)	R^2 value
1BL	10dpi-AP	*Xbarc137*	-8.76 area units	0.0646	3.41	2.87 (p<0.031)	0.04
		Xbarc1160 / Xbarc128	-8.77 area units	0.0643			
		Xbarc240	-9.66 area units	0.0413			
		Xbarc181	-9.19 area units	0.0517			
		Xbarc1131	-8.96 area units	0.0580			
		*Xbarc61**	-8.23 area units	0.0817			
		Xbarc81	-6.50 area units	0.1704			
		Xbarc188	-8.05 area units	0.0898			

Fig 3-1. Linkage maps for 130 DH lines. Positions are indicated according to cM distance from the 0.0cM position on a given linkage group. The 0.0cM positions represent the locus of the linkage group that is closest to the telomere of the short arm of that chromosome. In cases where two or more distinct linkage groups were known to be part of a single chromosome, all were displayed as one chromosomes delineated with parallel zig-zag lines. Linkage groups assigned to a single chromosome are arranged so that the linkage groups above are closer to the telomere of the short arm of the chromosome than linkage groups below on the same chromosome.

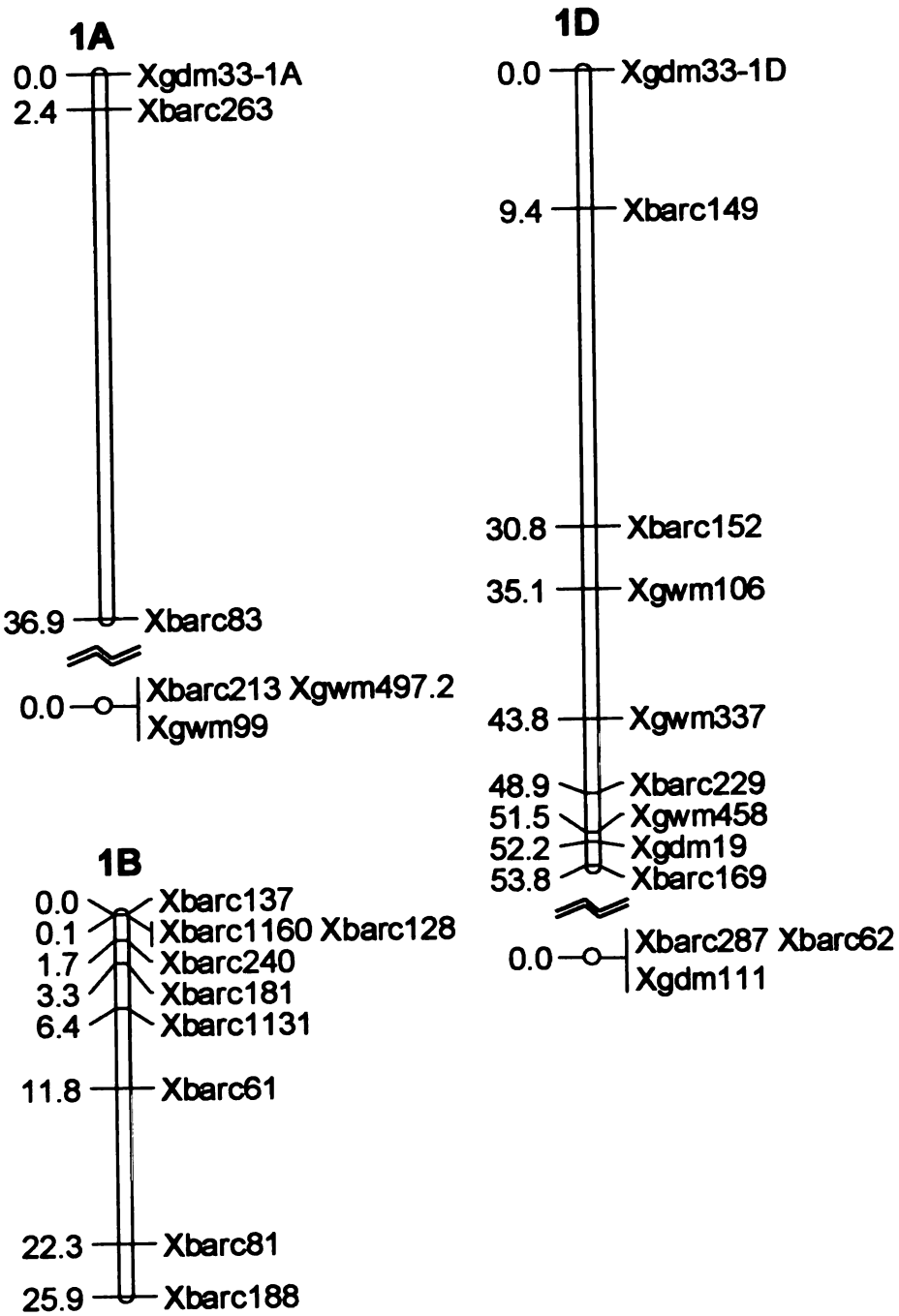
1A
0.0 Xgdm33-1A
2.4 Xbarc263
36.9 Xbarc83
0.0 Xbarc213 Xgwm497.2
Xgwm99
1B
0.0 Xbarc137
0.1 Xbarc1160 Xbarc128
1.7 Xbarc240
3.3 Xbarc181
6.4 Xbarc1131
11.8 Xbarc61
22.3 Xbarc81
25.9 Xbarc188
1D
0.0 Xgdm33-1D
9.4 Xbarc149
30.8 Xbarc152
35.1 Xgwm106
43.8 Xgwm337
48.9 Xbarc229
51.5 Xgwm458
52.2 Xgdm19
53.8 Xbarc169
0.0 Xbarc287 Xbarc62
Xgdm111

Fig 3-1 (cont'd)

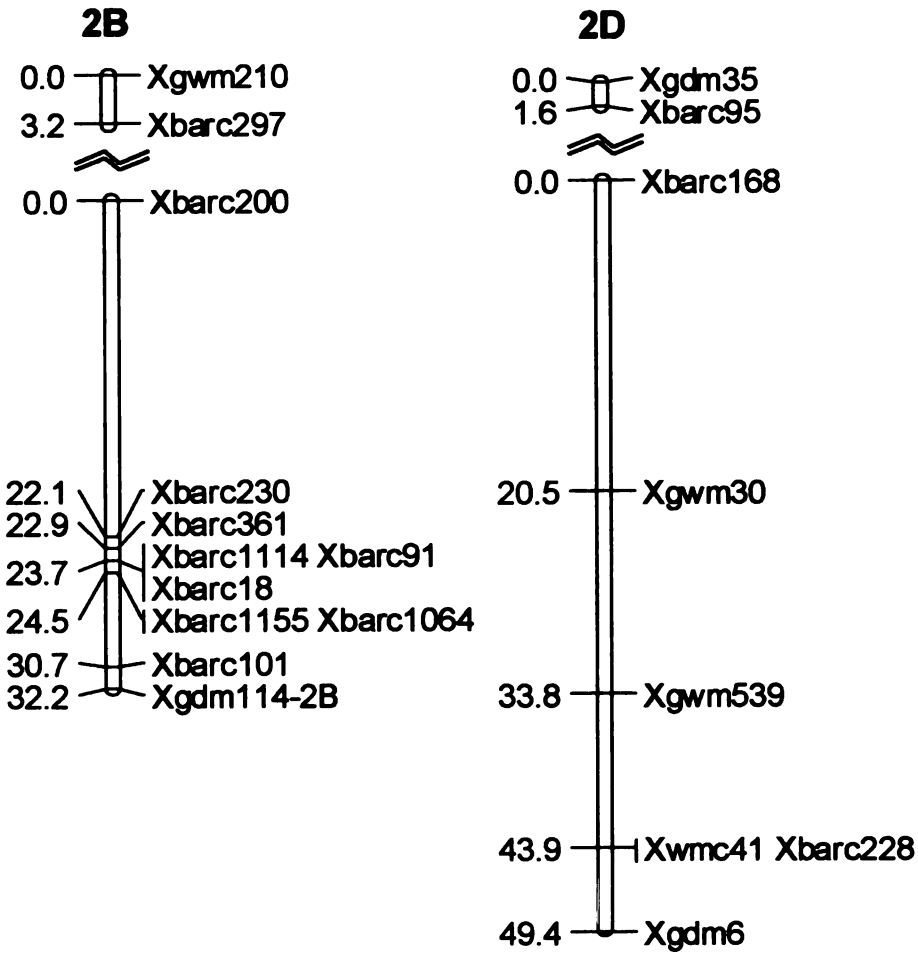
2B
0.0
Xgwm210
3.2
Xbarc297
0.0
Xbarc200
22.1
Xbarc230
22.9
Xbarc361
23.7
Xbarc1114 Xbarc91
Xbarc18
24.5
Xbarc1155 Xbarc1064
30.7
Xbarc101
32.2
Xgdm114-2B
2D
0.0
Xgdm35
1.6
Xbarc95
0.0
Xbarc168
20.5
Xgwm30
33.8
Xgwm539
43.9
Xwmc41 Xbarc228
49.4
Xgdm6

Fig 3-1 (cont'd)

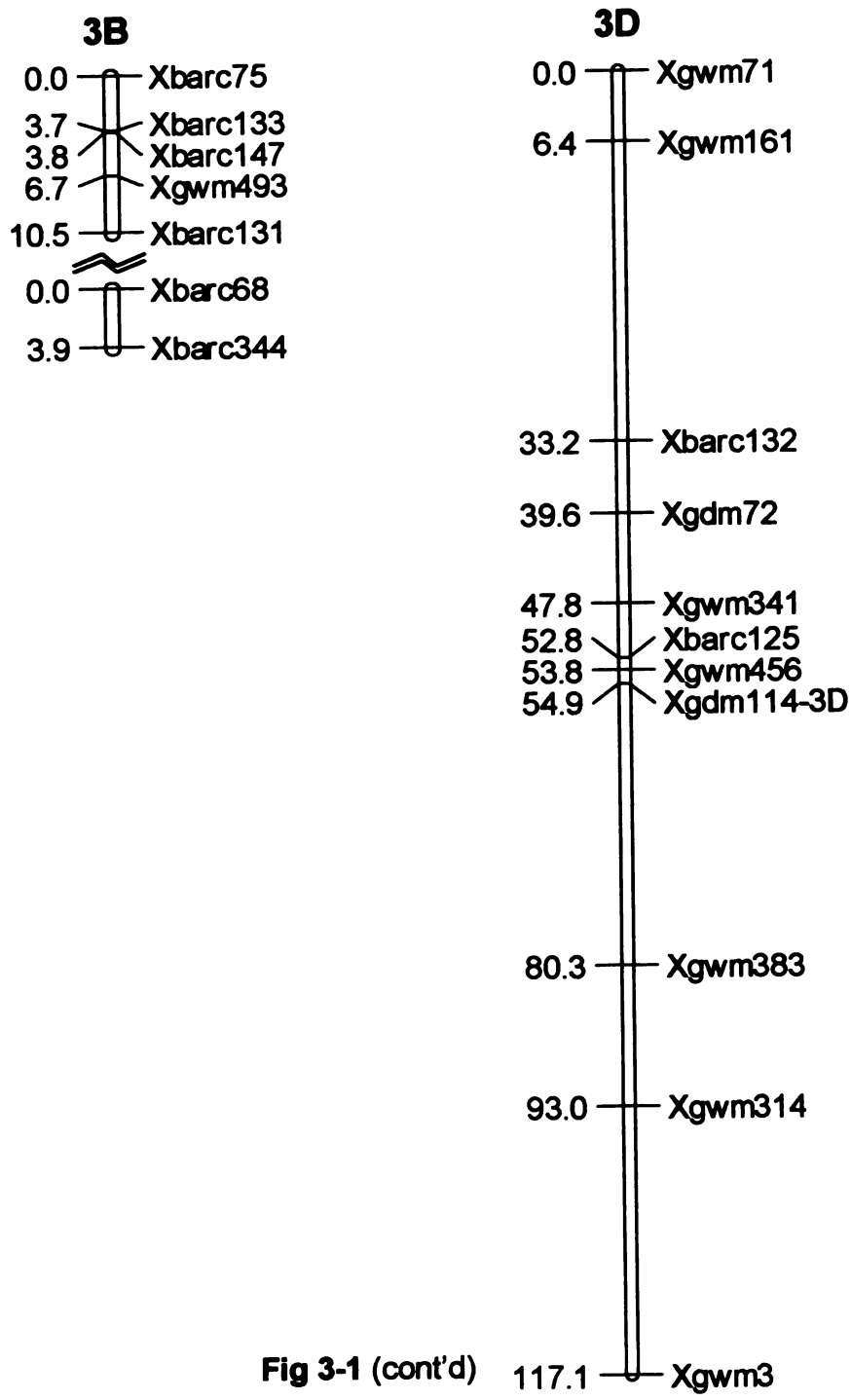
3B
0.0
Xbarc75
3.7
Xbarc133
3.8
Xbarc147
6.7
Xgwm493
10.5
Xbarc131
0.0
Xbarc68
3.9
Xbarc344
3D
0.0
Xgwm71
6.4
Xgwm161
33.2
Xbarc132
39.6
Xgdm72
47.8
Xgwm341
52.8
Xbarc125
53.8
Xgwm456
54.9
Xgdm114-3D
80.3
Xgwm383
93.0
Xgwm314
117.1
Xgwm3

Fig 3-1 (cont'd)

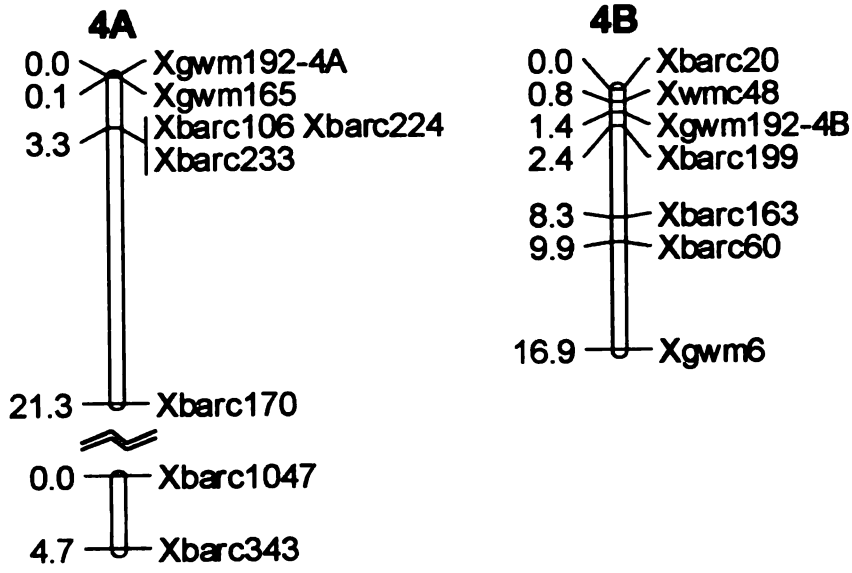
4A
0.0
0.1
3.3
21.3
0.0
4.7
Xgwm192-4A
Xgwm165
Xbarc106 Xbarc224
Xbarc233
Xbarc170
Xbarc1047
Xbarc343
4B
0.0
0.8
1.4
2.4
8.3
9.9
16.9
Xbarc20
Xwmc48
Xgwm192-4B
Xbarc199
Xbarc163
Xbarc60
Xgwm6

Fig 3-1 (cont'd)

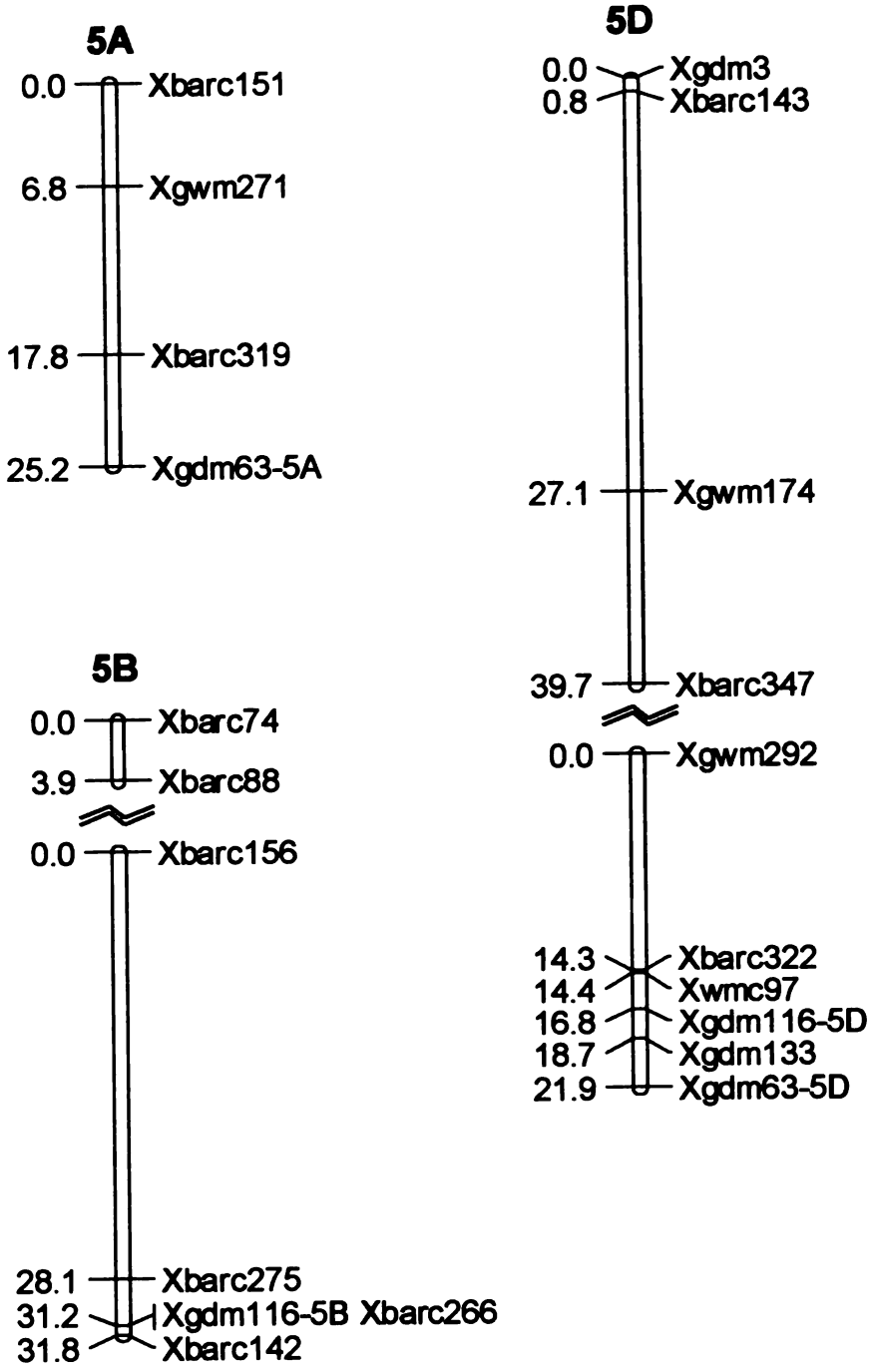
5A
0.0 Xbarc151
6.8 Xgwm271
17.8 Xbarc319
25.2 Xgdm63-5A
5B
0.0 Xbarc74
3.9 Xbarc88
0.0 Xbarc156
28.1 Xbarc275
31.2 Xgdm116-5B Xbarc266
31.8 Xbarc142
5D
0.0 Xgdm3
0.8 Xbarc143
27.1 Xgwm174
39.7 Xbarc347
0.0 Xgwm292
14.3 Xbarc322
14.4 Xwmc97
16.8 Xgdm116-5D
18.7 Xgdm133
21.9 Xgdm63-5D

Fig 3-1 (cont'd)

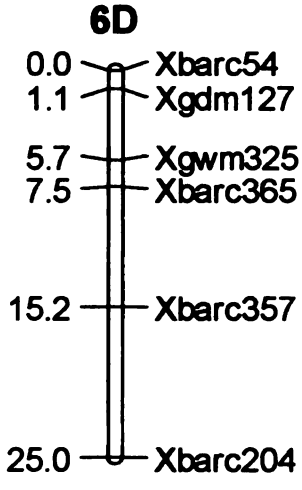
6D
0.0
Xbarc54
1.1
Xgdm127
5.7
Xgwm325
7.5
Xbarc365
15.2
Xbarc357
25.0
Xbarc204

Fig 3-1 (cont'd)

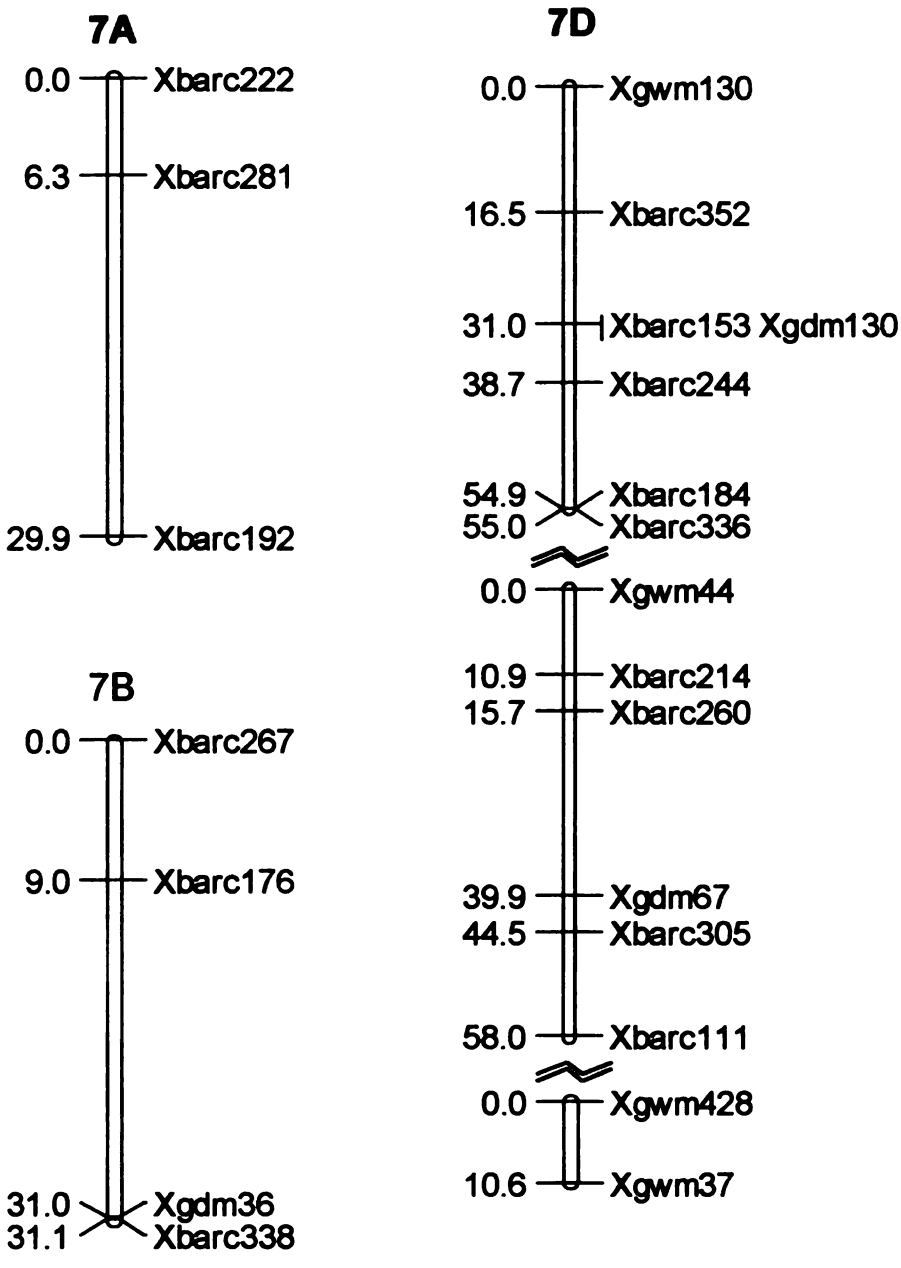
7A
0.0 Xbarc222
6.3 Xbarc281
29.9 Xbarc192
7B
0.0 Xbarc267
9.0 Xbarc176
31.0 Xgdm36
31.1 Xbarc338
7D
0.0 Xgwm130
16.5 Xbarc352
31.0 Xbarc153 Xgdm130
38.7 Xbarc244
54.9 Xbarc184
55.0 Xbarc336
0.0 Xgwm44
10.9 Xbarc214
15.7 Xbarc260
39.9 Xgdm67
44.5 Xbarc305
58.0 Xbarc111
0.0 Xgwm428
10.6 Xgwm37

Fig 3-1 (cont'd)

Fig 3-2. Frequency distributions of 108 dihaploid lines evaluated with several disease scoring methods. A) number of scabby spikelets at 7, 10, 14, 17 and 21dpi. B) percent of scabby spikelets at 7, 10, 14, 17 and 21dpi. C) AUDPC of the number of scabby spikelets at 10, 14, 17 and 21dpi. D) AUDPC of the percent of scabby spikelets at 10, 14, 17 and 21dpi. The means of CASS94 and Flycatcher for each rating system and each dpi are indicated with a star (CASS94) or circle (Flycatcher). The Least Significant Differences (LSD) values in the legends are based on $\alpha = 0.05$. The X-values of a given bin reflect the upper limit of that bin.

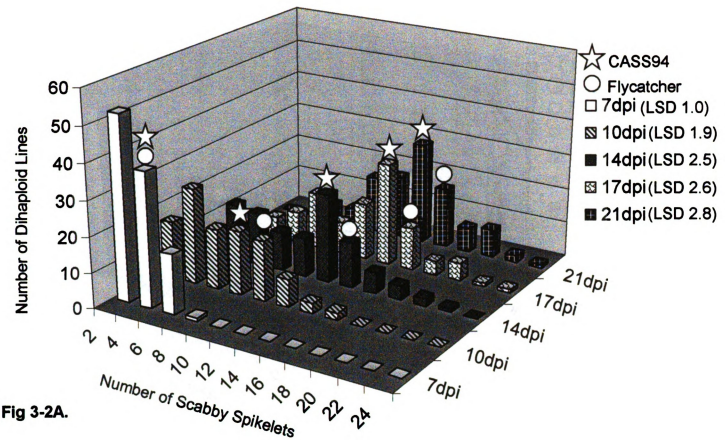
Number of Dihaploid Lines
60
50
40
30
20
10
0
2 4 6 8 10 12 14 16 18 20 22 24
Number of Scabby Spikelets
7dpi
10dpi
14dpi
17dpi
21dpi
CASS94
Flycatcher
7dpi (LSD 1.0)
10dpi (LSD 1.9)
14dpi (LSD 2.5)
17dpi (LSD 2.6)
21dpi (LSD 2.8)

Fig 3-2A.

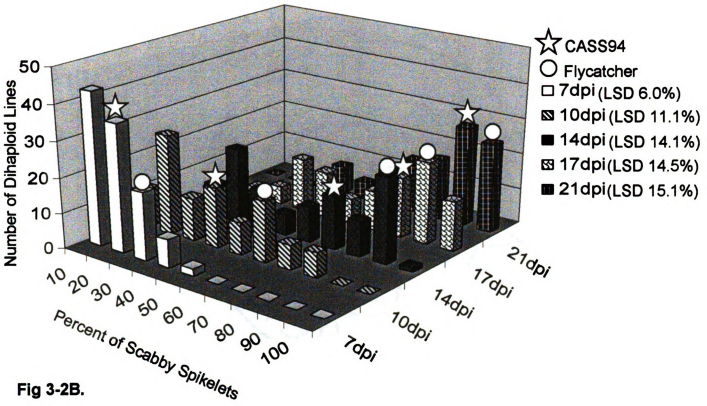
Number of Dihaploid Lines
50
40
30
20
10
0
10 20 30 40 50 60 70 80 90 100
Percent of Scabby Spikelets
7dpi
10dpi
14dpi
17dpi
21dpi
CASS94
Flycatcher
7dpi (LSD 6.0%)
10dpi (LSD 11.1%)
14dpi (LSD 14.1%)
17dpi (LSD 14.5%)
21dpi (LSD 15.1%)

Fig 3-2B.

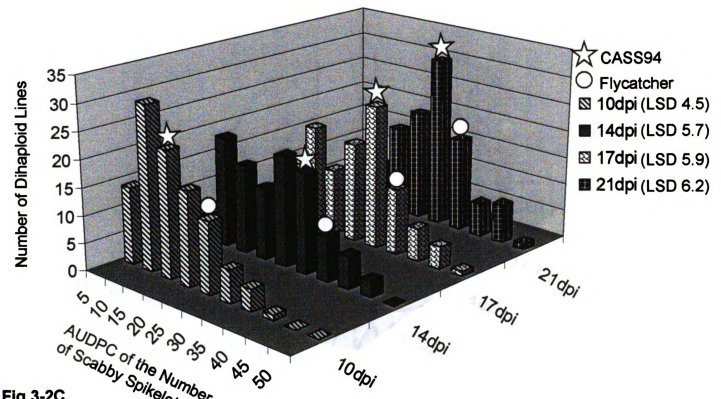
Number of Dihaploid Lines
35
30
25
20
15
10
5
0
5
10
15
20
25
30
35
40
45
50
AUDPC of the Number of Scabby Spikelets
10dpi
14dpi
17dpi
21dpi
CASS94
Flycatcher
10dpi (LSD 4.5)
14dpi (LSD 5.7)
17dpi (LSD 5.9)
21dpi (LSD 6.2)

Fig 3-2C.

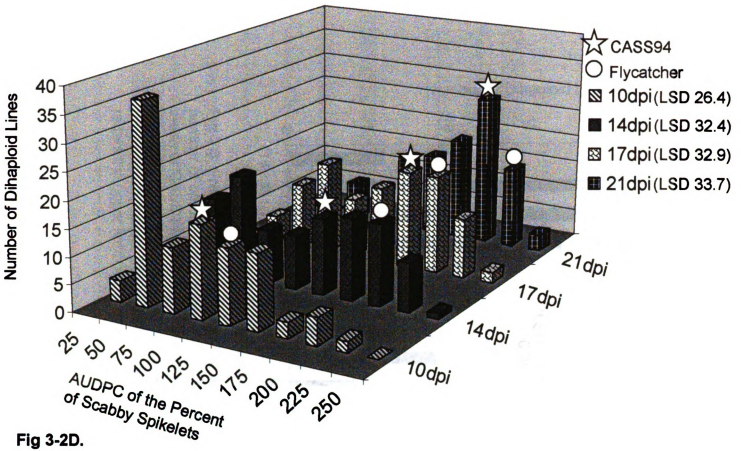
CASS94
Flycatcher
10dpi (LSD 26.4)
14dpi (LSD 32.4)
17dpi (LSD 32.9)
21dpi (LSD 33.7)
Number of Dihaploid Lines
0
5
10
15
20
25
30
35
40
25
50
75
100
125
150
175
200
225
250
AUDPC of the Percent of Scabby Spikelets
10dpi
14dpi
17dpi
21dpi

Fig 3-2D.

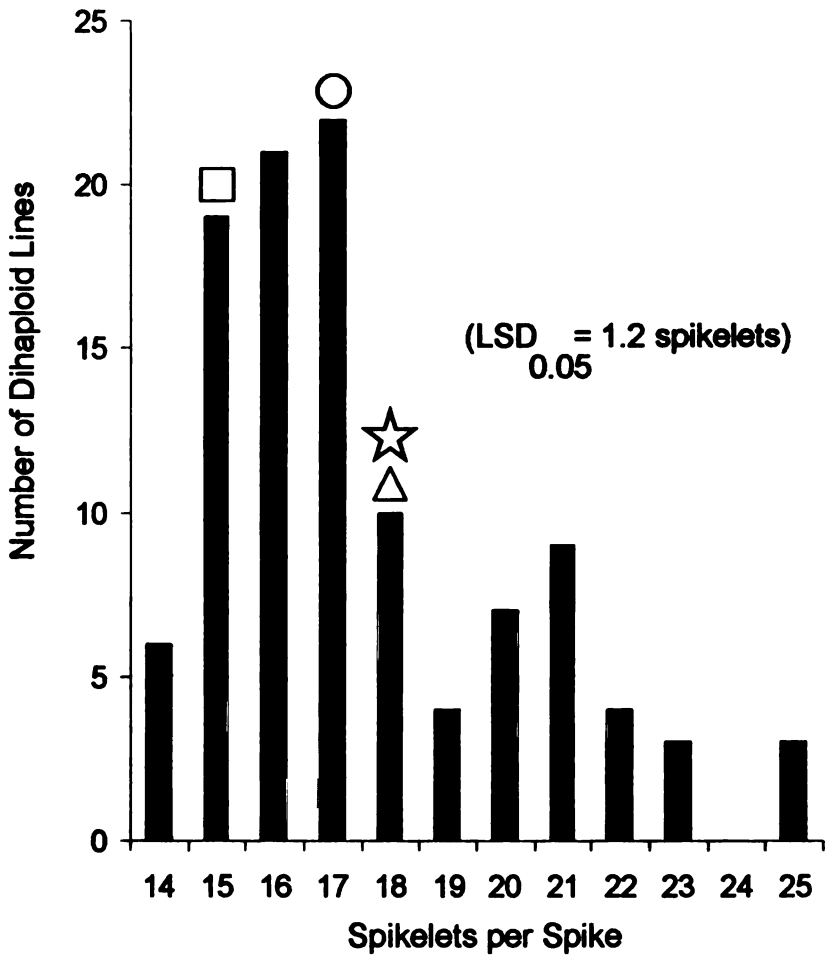

Fig 3-3. Frequency distribution of the average total number of spikelets per spike for 108 DH lines. The mean of CASS94 (star), Flycatcher (circle) W14 (triangle), and Norm (square) are shown. The LSD for $p < 0.05$ is indicated. The X-axes values for a given bin reflect the upper limit of that bin.

Figure 3-4. Combined linkage map and Composite Interval Mapping LOD graphs for a region of chromosome arm 2DL. LOD graphs for A) number of scabby spikelets at 7, 10, 14, 17, 21dpi. B) percent of scabby spikelets at 7, 10, 14, 17, 21dpi. C) AUDPC of the number of scabby spikelets at 10, 14, 17, 21dpi and D) AUDPC of the percent of scabby spikelets at 10, 14, 17, 21dpi. The maximum R^2 value for the QTL region from Composite Interval Mapping analysis is indicated next to the LOD graph key for each corresponding dpi. Distances on the linkage map are indicated in cM. The support interval of the QTL at each dpi are shown by black bar/line drawings placed between the linkage map and the LOD graphs. The inner 1-LOD support interval is indicated by a black bar, and the inner 2-LOD support interval is indicated by lines extending on either side of the 1-LOD support interval. For each LOD graph, a beaded horizontal line at LOD 3.0 is drawn as a reference. Dashed arrowed lines are drawn to show the corresponding position of the linkage map and the QTL graph.

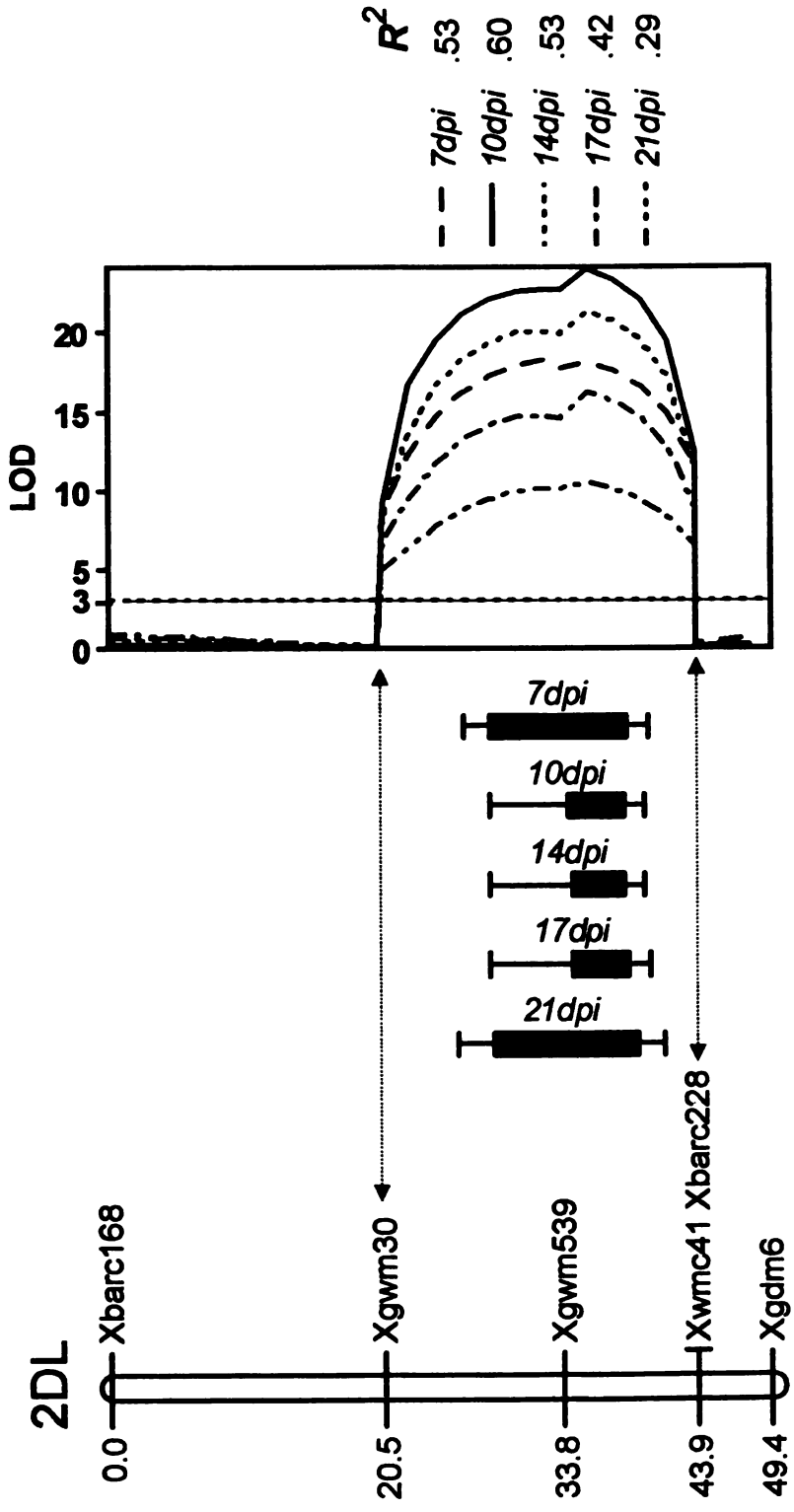

Fig 3-4A. 2DL QTL for the number of scabby spikelets.

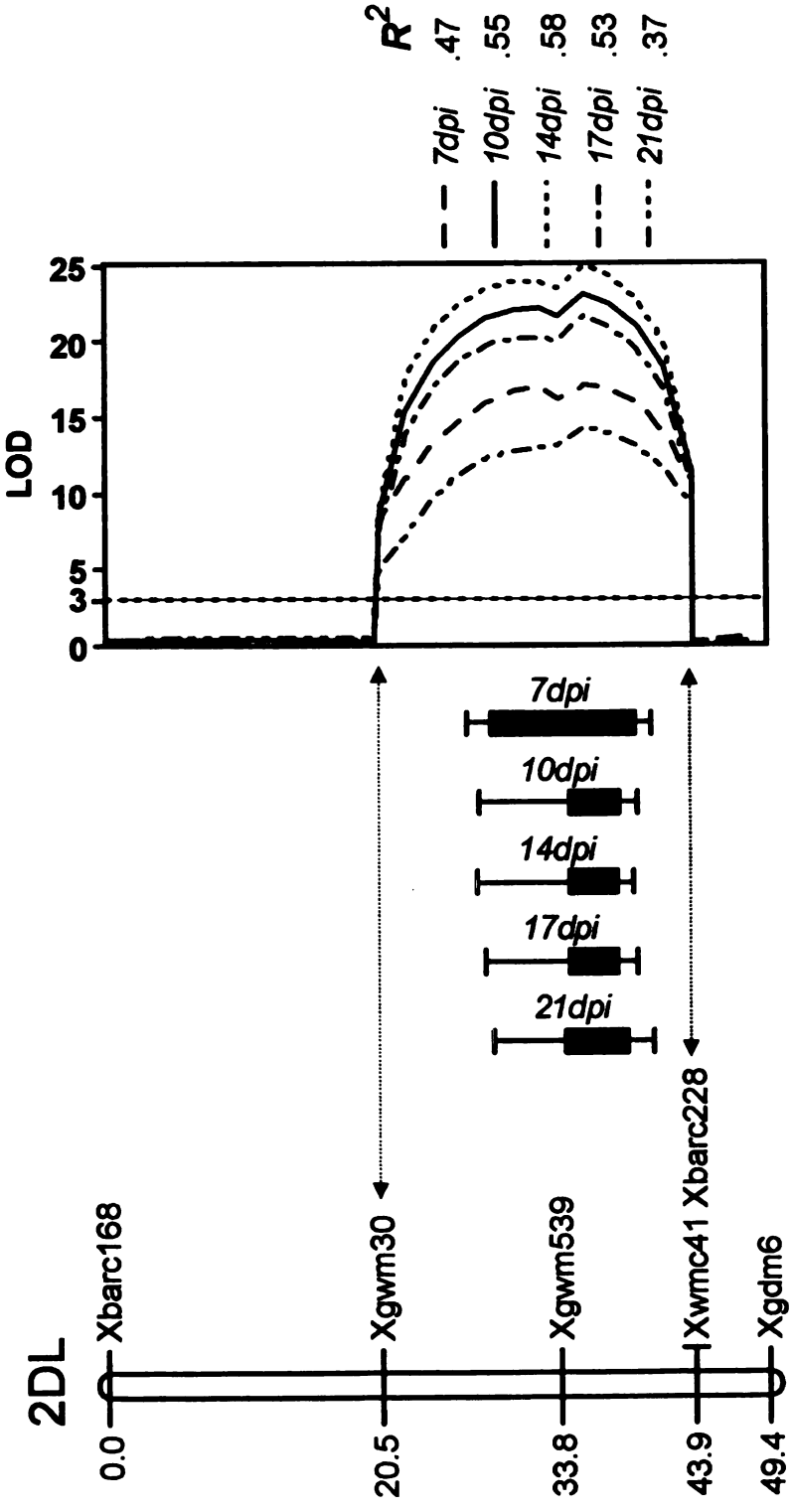

Fig 3-4B. 2DL QTL for the percent of scabby spikelets.

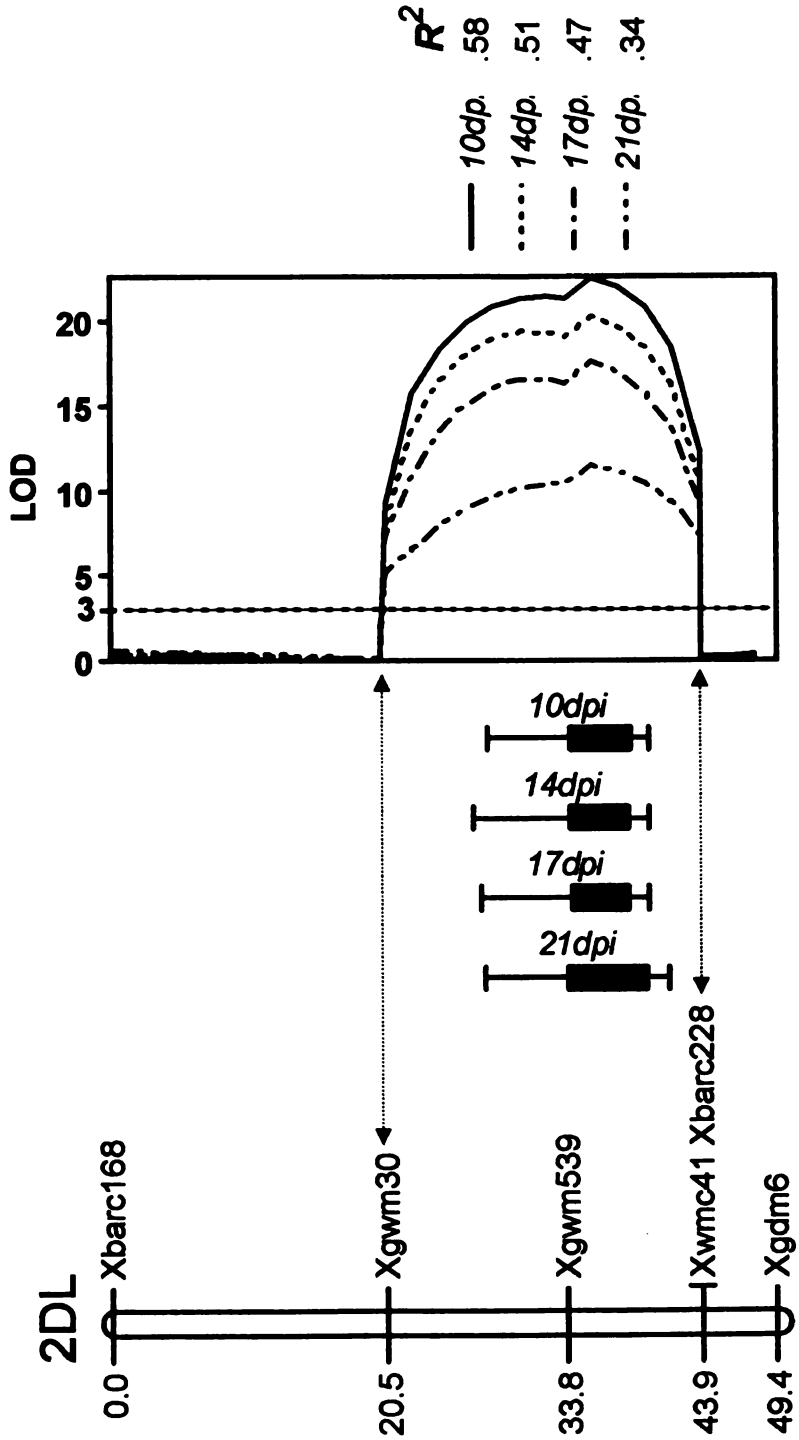

Fig 3-4C. 2DL QTL for the AUDPC of the number of scabby spikelets.

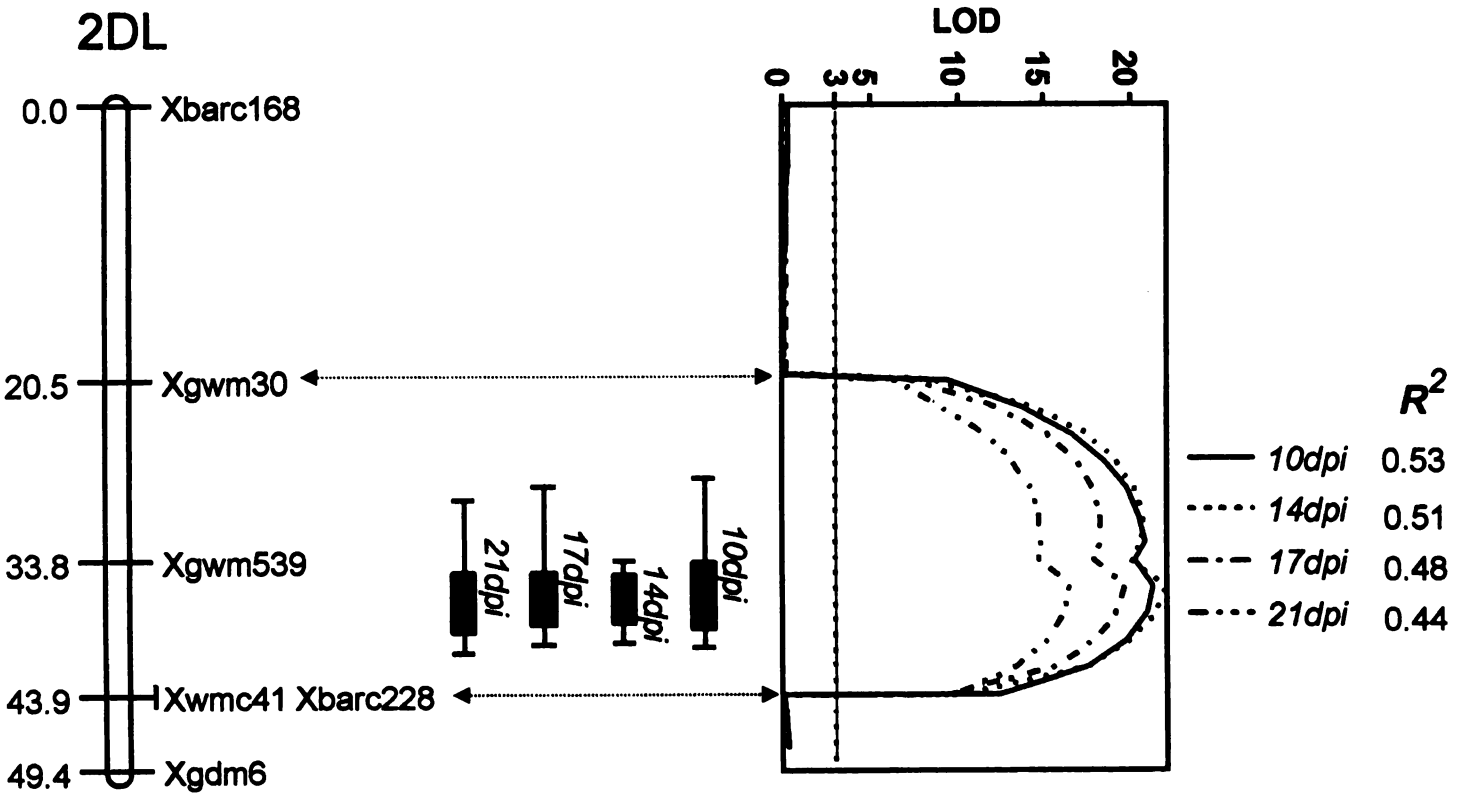

Fig 3-4D. 2DL QTL for the AUDPC of the percent of scabby spikelets.

Figure 3-5. Combined linkage maps and LOD graphs for CIM derived QTL on linkage groups of chromosome 4A, and chromosome arms 5AL and 5DL. LOD graphs are placed to the right of the corresponding linkage map. For each LOD graph, a key is shown for the dpi/measurement combinations. 10dpi-P is the percent of scabby spikelets at 10dpi. 14dpi-AP is the AUDPC of the percent of scabby spikelets at 14dpi. 21dpi-SS is the number of scabby spikelets at 21dpi. The maximum R^2 value for the QTL region according to Composite Interval Mapping results is indicated next to the LOD graph key for each corresponding dpi/measurement combination. Distances on the linkage map are indicated in cM. The support interval of the QTL at each dpi are shown by black bar/line drawings placed between the linkage map and the LOD graphs. The inner 1-LOD support interval is indicated by a black bar, and the inner 2-LOD support interval is indicated by lines extending on either side of the 1-LOD support interval. For each LOD graph, a beaded horizontal line at LOD 3.0 is drawn as a reference. Dashed arrowed lines are drawn to show the corresponding position of the linkage map and the QTL graph.

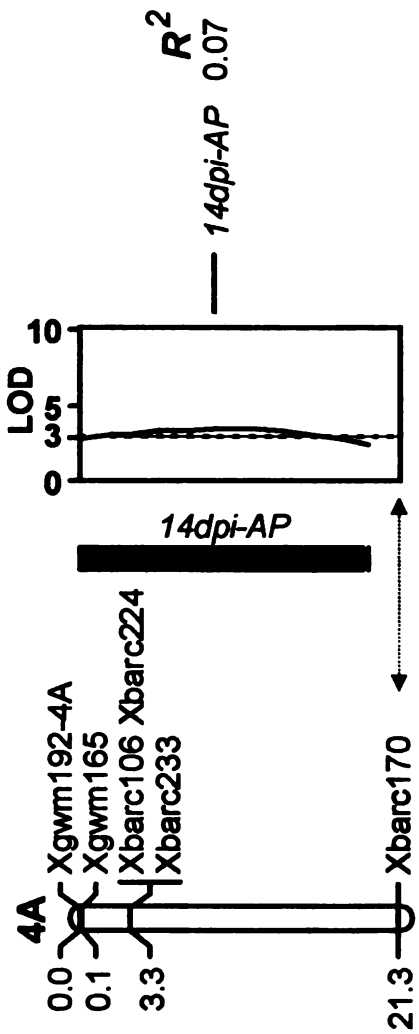

Fig 3-5: Graph of QTL on 4A, 5AL and 5DL.

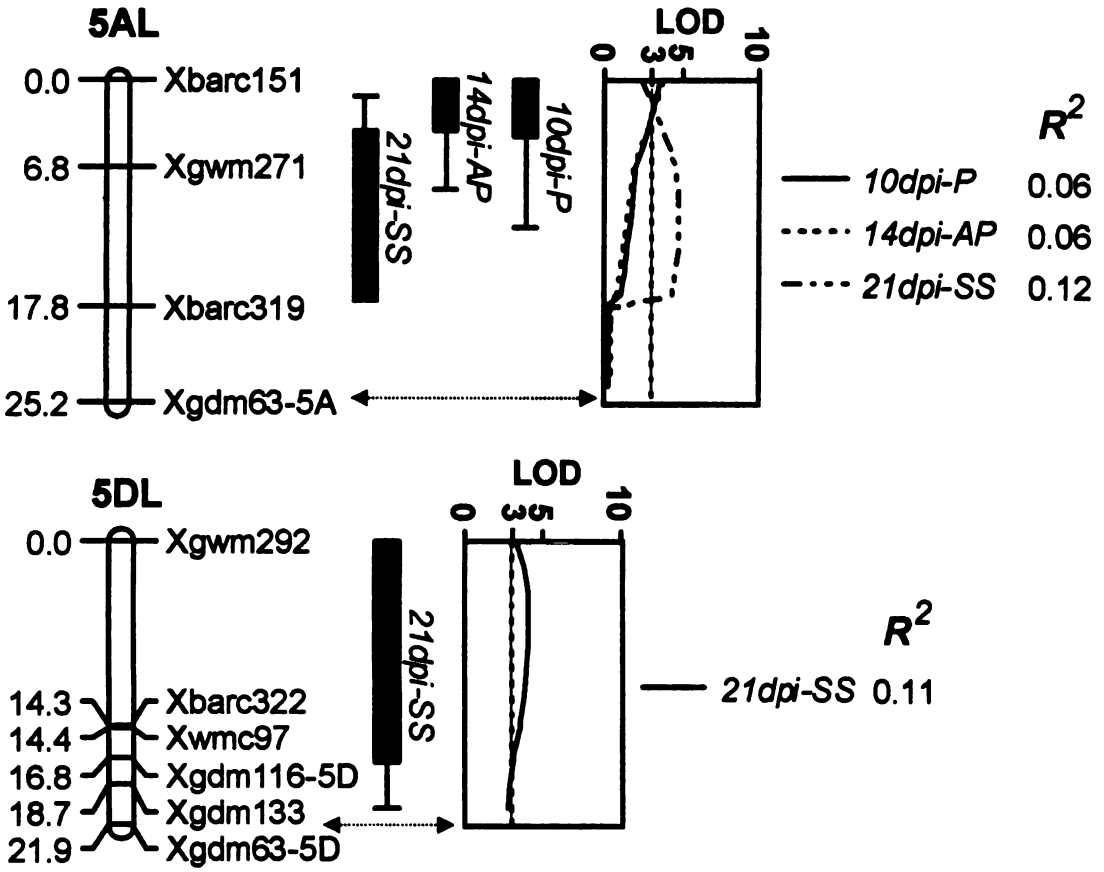
5AL
0.0
Xbarc151
6.8
Xgwm271
17.8
Xbarc319
25.2
Xgdm63-5A
21dpi-SS
14dpi-AP
10dpi-P
LOD
0
3
5
10
R^2
10dpi-P 0.06
14dpi-AP 0.06
21dpi-SS 0.12
5DL
0.0
Xgwm292
14.3
Xbarc322
14.4
Xwmc97
16.8
Xgdm116-5D
18.7
Xgdm133
21.9
Xgdm63-5D
21dpi-SS
LOD
0
3
5
10
R^2
21dpi-SS 0.11

Fig 3-5 (Cont'd) .

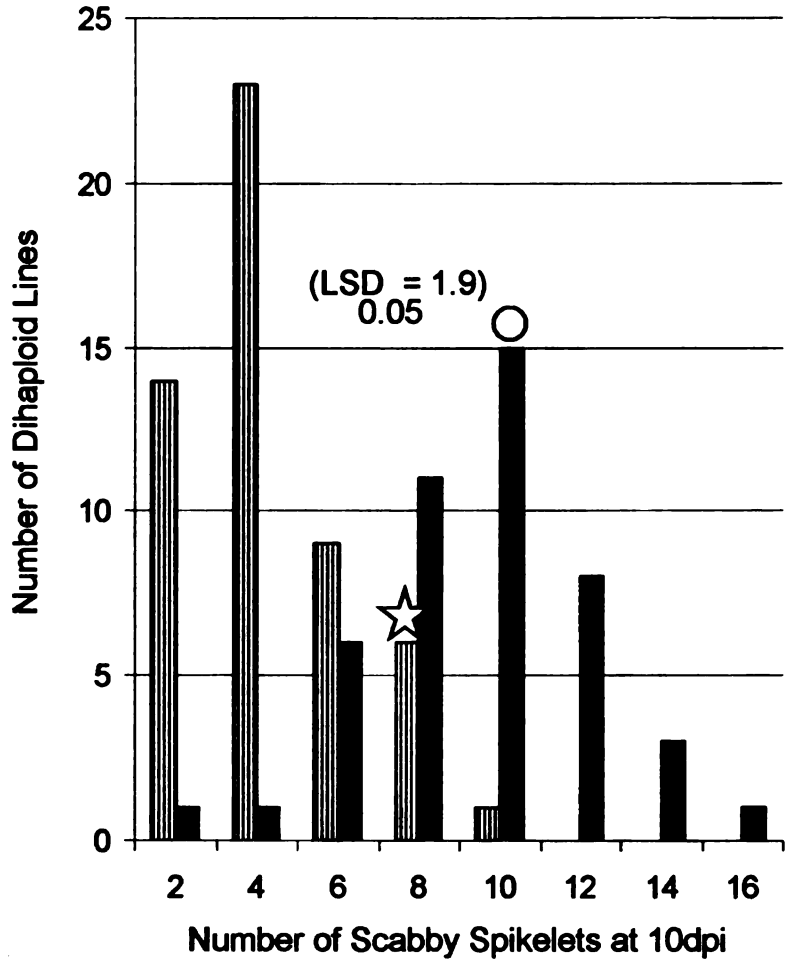

Fig 3-6. Frequency distribution of the mean number of scabby spikelets at 10dpi for 99 DH lines with either the CASS94 allele (striped bars) or the Flycatcher allele (black bars) for the SSR locus *Xgwm539*. The mean of CASS94 (star) and Flycatcher (circle) at 10dpi for the percentage of scabby spikelets are also indicated. The X-value for a given bin reflects the upper limit of that bin.

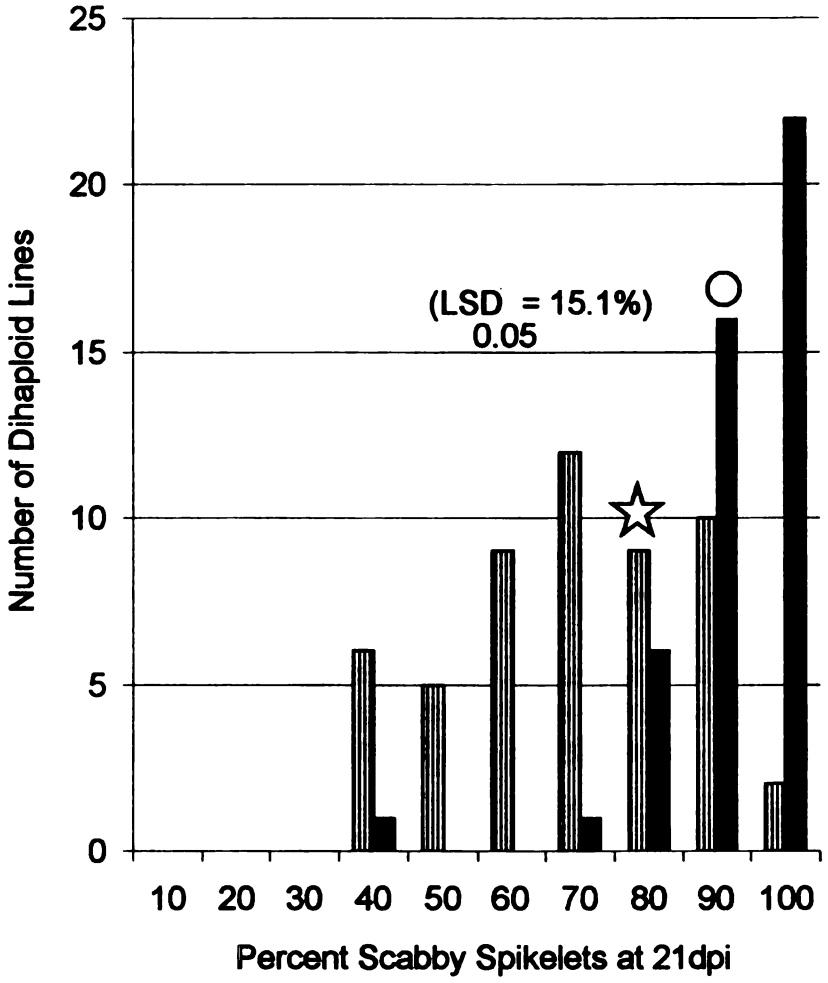

Fig 3-7. Frequency distribution of the mean percentage of scabby spikelets at 21dpi for 99 DH lines with either the CASS94 (striped bars) or the Flycatcher allele (black bars) for SSR locus *Xgwm539*. The mean of CASS94 (star) and Flycatcher (circle) at 21dpi for the percentage of scabby spikelets are also indicated. The X value for a given bin reflects the upper limit of that bin.

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