Genetic analysis of resistance to Fusarium head blight in wheat (*Triticum* spp.) using phenotypic characters and molecular markers

By

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ABSTRACT

Fusarium head blight (FHB), caused mainly by *Fusarium graminearum* (teleomorph: *Gibberella zeae*), is one of the most damaging diseases of wheat.

A 'Brio'/'TC 67' spring wheat population was used to map quantitative trait loci (QTLs) for resistance to FHB, and to study the association of morphological and developmental characteristics with FHB resistance. Interval mapping (IM) detected a major QTL on chromosome 5AL for resistance to disease severity (type II resistance) and Fusarium-damaged kernels (FDK) under greenhouse and field conditions, respectively. Inconsistent QTL(s) was also detected on chromosome 5BS for disease severity and index using field data. The associations of plant height and number of days to anthesis were negative with disease incidence, severity, index, and deoxynivalenol (DON) accumulation data under field conditions. However, number of days to anthesis was positively correlated with disease severity (greenhouse) and FDK (field). Awnedness had a negative effect on FHB, namely the presence of awns resulted in less disease in the population. Spike threshability also affected FHB so that the hard threshable genotypes represented lower disease.

Phylogenetic relationships of putative *F. graminearum* isolates from different sources were characterized using *Tri101* gene sequencing data. Canadian and Iranian isolates clustered in *F. graminearum* lineage 7 (=*F. graminearum sensu stricto*) within the *F. graminearum* clade while the isolates received from CIMMYT, Mexico were placed in *F. graminearum* lineage 3 (=*Fusarium boothii*) within the *Fg* clade or *Fusarium cerealis*. The PCR assay based on the *Tri12* gene revealed the presence of the NIV, 3-ADON, and 15-ADON chemotypes with 15-ADON being the predominant chemotype.

While we did not find the NIV chemotype among the Canadian isolates, it was the predominant chemotype among the Iranian isolates. High variation in aggressiveness was observed among and within *Fusarium* species tested, with the isolates of *F. graminearum sensu stricto* being the most aggressive and the NIV chemotype being the least aggressive.

The interactions between *Fusarium* isolates and wheat genotypes from different sources were investigated by inoculating isolates of *F. graminearum sensu stricto* and *F. boothii* on wheat genotypes. Significant differences were observed among the genotypes inoculated by single isolates. Results also showed significant interactions between *Fusarium* isolates and wheat genotypes. The *F. boothii* isolates from CIMMYT produced low disease symptom and infection on wheat genotypes regardless of the origin of the genotypes while *F. graminearum sensu stricto* isolates from Canada and Iran resulted in higher FHB scores.

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To my wife Mina

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INTRODUCTION

Introduction

Wheat is the most important cereal crop; it is widely grown in different parts of the world and and is increasing in production. Wheat, along with maize and rice, feeds much of the world, providing 44% of total edible dry matter and 40% of food crop energy consumed in developing countries {Dixon, 2005 #707}. Bread wheat, which accounts for 90% of total wheat production, is grown on a substantial scale in more than 70 countries {Lantica, 2005 #706}. Given the steady increases in wheat productivity during the past 40 years, it has continued to play a major role in global food security. However, global food security remains quite fragile because of challenges such as susceptibility to diseases and pests.

Wheat is susceptible to many diseases, the more destructive including rusts, bunts, powdery mildew, and fusarium head blight (FHB). Fusarium head blight is one of the most devastating diseases of wheat and other small grain crops in humid and semi-humid areas worldwide. Methods of control of FHB include agronomic practices, chemical control, biological control, and the use of resistant cultivars. Development of resistant cultivars is the most practical and economic approach for environmentally safe and sustainable control of the disease {Yang, 2005 #352}. The long-term effectiveness of resistant cultivars depends on the type of genetic resistance present in wheat genotypes, the nature of the pathogen, and the host-pathogen interactions.

Even though no complete resistance or immunity to FHB has been observed, genotypic variation is large and well-documented in wheat and its relatives. Although QTLs/genes from different sources have been mapped and in some cases successfully used in wheat breeding programs, finding new sources of resistance is needed to avoid

complete dependence on limited sources. *Triticum timopheevii* (Zhuk.) Zhuk. is a source of FHB resistance which has been used to introgress resistance into wheat {Fedak, 2004 #105}. Mapping and tagging the FHB resistance available in a wheat cultivar with an alien background such as *T. timopheevii* may be of great interest for use in wheat breeding programs.

Fusarium graminearum Schwabe (teleomorph: *Gibberella zeae* (Schwein.) Petch.) is the most dominant and widespread pathogen causing FHB on wheat and other small grain cereals worldwide. *Fusarium graminearum* was thought to be a single panmictic species spanning six continents until recently. Using phylogenetic analysis of DNA sequences from isolates of *F. graminearum* collected from around the world, 13 phylogenetically distinct and biogeographically structured lineages (=species) were discovered within the *F. graminearum* complex {O'Donnell, 2000 #247;O'Donnell, 2008 #713;O'Donnell, 2004 #248;Starkey, 2007 #306;Ward, 2002 #334;Yli-Mattila, 2009 #709}. These species which have been formally named, have different geographic distributions, differ in production of trichothecenes, and may differ in their ability to cause disease on particular crops. Genetic diversity studies of *F. graminearum* showed high genetic variation within individual field populations, populations sampled across a large-scale geographical zone, or within collections of isolates. In addition, *Fusarium* species produce trichothecenes which are divided into different categories. The aggressiveness of *F. graminearum* isolates depends on their capacity to produce trichothecenes {Mesterházy, 2002 #219;Miedaner, 2000 #224}. High variation in aggressiveness and/or pathogenicity has been observed among *F. graminearum* isolates from different geographical regions {Akinsanmi, 2004 #3;Bai, 1996 #24;Cullen, 1982 #88;Cumagun, 2004 #89;Mesterházy, 1984 #214;Miedaner, 1996 #223;Miedaner, 2000

#224;Miedaner, 1996 #225;Muthomi, 2000 #241;Walker, 2001 #323;Wu, 2005 #347}. Understanding the genetic profile and diversity of the pathogen may provide insights into the epidemiological and destructive potential of the pathogen, and may lead to an improvement in our strategies for control of the pathogen and management of the disease(s) caused by it.

Although different isolates of *Fusarium* may show variation in aggressiveness and there may be significant interactions between wheat cultivars and pathogen isolates, there is no evidence for stable pathogen races. Resistance to FHB in wheat is usually stable and resistant cultivars show consistent resistance to almost all isolates of *F. graminearum* worldwide. Based on reaction of wheat cultivars to different species of *Fusarium*, it has been concluded that resistance to certain isolates of *F. graminearum* as well as to other species of *Fusarium* was not strain-specific or species-specific in wheat cultivars {Mesterházy, 1981 #215}.

In this study, genetic analysis of resistance to fusarium head blight in wheat (*Triticum* spp.) using phenotypic characters and molecular markers was investigated. The present thesis consists of five chapters: chapter 1 provides a general literature review for FHB and all of the following chapters of the thesis, chapter 2 presents an overview to molecular mapping of quantitative trait loci for fusarium head blight resistance in a population of wheat with a *T. timopheevii* background, chapter 3 examines the molecular genetic diversity and variation for aggressiveness among *Fusarium graminearum* isolates from different sources, chapter 4 presents the results of host-pathogen interactions between selected wheat genotypes and *Fusarium* isolates from different sources, and chapter 5 provides the general discussion and conclusions.

CHAPTER 1

GENERAL LITERATURE REVIEW

Fusarium head blight

Introduction

Fusarium head blight (FHB), also called scab, is a devastating disease of wheat and other small grains in humid and semi-humid areas worldwide. This fungal disease can completely destroy a potentially high-yielding crop within a few weeks of harvest (McMullen et al. 1997).

FHB was first described just over a century ago and was considered a major threat to wheat and barley during the early years of the twentieth century (Dickson and Mains 1929). During recent decades there have been outbreaks of FHB in the United States and Canada (McMullen et al. 1997). The most extended episodes of epidemics have occurred in winter wheat and spring wheat growing areas of midwestern and eastern states of the United States as well as in Manitoba and Ontario in Canada (Kephart 1991; McMullen et al. 1997; Tuite et al. 1990; Wong et al. 1992). FHB has remained the most serious fungal disease of wheat in eastern Canada, Manitoba, and eastern Saskatchewan since 1993, resulting in millions of dollars of losses annually; its incidence has steadily spread to western parts of Canada (Gilbert and Tekauz 2000; Tekauz et al. 2000). In China, FHB can be found in two-thirds of the provinces, where it affects more than seven million hectares of wheat (Wang et al. 1982). Disease epidemics generally occur in the lower and middle reaches of the Yangtze Valley, coastal areas of southern China, and eastern parts of Heliongjiang province (Zhuang and Li 1993). In Iran, FHB is one of the most important diseases of wheat in the coastal northern and north-western wheat growing areas and sometimes in other parts of the country when rainfall is unusually high. Wheat FHB has also become a threat to wheat production in many other countries (Bai and

Shaner 1994; Ban 2001; Gilchrist et al. 1997; Mesterházy 2003; Reis 1990; Snijders 1990b; Snijders 1990d; Sutton 1982).

Fusarium head blight can significantly reduce grain yield and quality. Yield reduction results from shrivelled grains which may be eliminated from the combine because of their light weight. Diseased kernels which are not eliminated from the combine reduce grain weight. FHB causes indirect losses by reducing seed germination and causing seedling blight and poor stands (Chongo et al. 2001; Gilbert and Tekauz 1995; Sutton 1982; Tuite et al. 1990). In addition, FHB-infected grains may contain significant levels of mycotoxins such as deoxynivalenol (DON) and zearalenone which pose a serious threat to animal and human health and food safety (Bai and Shaner 1994; Desjardins et al. 1996; Marasas et al. 1984; McMullen et al. 1997; Miller et al. 1991; Parry et al. 1995; Snijders 1990b; Sutton 1982; Tuite et al. 1990). These mycotoxins have been associated with livestock toxicoses, feed refusal, diarrhoea, emesis, alimentary haemorrhaging, and contact dermatitis. Effects of the mycotoxins in human include toxicosis, nausea, vomiting, anorexia, and convulsions (Bennett and Klich 2003). Grains may be downgraded or rejected in commerce because of the presence of *Fusarium*-damaged kernels (FDK) in crop and/or contamination with one or more mycotoxins (McMullen et al. 1997; Tuite et al. 1990). Milling, baking, and pasta-making properties of wheat also are affected (Dexter et al. 1996; Dexter et al. 1997) as the pathogen destroys starch granules, cell walls, and endosperm proteins (Bechtel et al. 1985; Nightingale et al. 1999).

Symptoms

Initial infections appear as small, water-soaked, brownish spots at the base or middle of the glume, or on the rachis (Mathre 1982). Water soaking and discoloration may then spread in all directions from the point of infection (Figure 1.1). Other symptoms include tan to brown discoloration ('blight') of the rachis especially at the base of the spike, a pink or orange coloured mold along the edge of the glumes or at the base of the spikelets under moist conditions, and kernels that are shrivelled, white, and chalky ('tombstone') in appearance. Premature death or bleaching of the spikelets is also a common symptom, and is particularly clear on immature spikes where one or more spikelets or the entire spike is affected (Wiese 1987). Awns often become deformed, twisted and curved downward. During prolonged warm and moist weather conditions, spikelets on early-infected spikes appeared speckled as a result of the development of blue/black perithecia, giving the 'scabbed' appearance (Mathre 1982). Such perithecia are commonly associated with spikes infected with *Gibberella zeae* (Schwein.) Petch., the sexual stage of *Fusarium graminearum* Schwabe. When wheat spikes are severely infected by FHB, the spike may turn dark brown (Parry et al. 1995).

Figure 1.1. Symptoms of fusarium head blight on wheat head.

Photograph courtesy of Jacolyn Morrison, USDA, ARS, Cereal Disease Laboratory, St. Paul, MN.

The pathogens and geographical distribution

Smith (1884) in England made the first record of FHB and attributed the disease to the fungus *Fusisporium culmorum* W. G. Smith. In the United States, Chester (1890) and Arthur (1891) independently reported the disease and stated that 'scab' was becoming important in wheat. In Ohio, USA, Detmers (1892) also recorded the disease and attributed it to *Fusisporium culmorum.* In the 1920s, serious epidemics of the disease

caused predominantly by *F. graminearum* were recorded in wheat throughout the USA (Johnson et al. 1920; Koehler et al. 1924; Maclnnes and Fogelman 1923).

Since the first records, FHB has been reported in most wheat-growing areas of the world, and at least 17 different *Fusarium* species have been associated with the disease (Parry et al. 1995). In spite of the number of *Fusarium* species involved, three species are predominant in different parts of the world: *F. graminearum* (teleomorph: *G. zeae*), *Fusarium culmorum* (W. G. Smith) Sacc. initially named as *Fusisporium culmorum* with no known teleomorphic state, and *Fusarium avenaceum* (Corda ex Fries) Sacc. (teleomorph: *Gibberella avenacea* R. J. Cook). Their geographical distribution is related to their temperature requirements. In warmer regions of the world, including parts of the USA, Canada, Australia, and Central Europe, *F. graminearum* is the most important species causing FHB. In cooler regions of Northwest Europe, *F. culmorum* is the predominant species, and *Fusarium poae* (Peck) Wollenw. and *Microdochium nivale* (Fr.) Samuel *et* Hallett have a great importance. *F. avenaceum* has been isolated from diseased samples over a range of climates, but usually represents only a small proportion of *Fusarium* isolates (Parry et al. 1995).

Fusarium graminearum is the predominant species causing fusarium head blight in many countries (Clear and Abramson 1986; Schroeder and Christensen 1963; Sutton 1982; Wang et al. 1982; Wiese 1987). The pathogen also is associated with stalk and ear rot of corn and may cause a root rot of cereals (McMullen et al. 1997).

Epidemiology

Disease cycle

It is clearly understood from the disease cycle on small grain cereals how fusarium head blight relates to seed infection, seedling blight, and foot rot (Figure. 1.2). In the centre of the cycle is the initial source of *Fusarium* inoculum from the soil or cereal stubble and residue which survives as saprophytic mycelium, chlamydospores, or perithecia. Sowing cereal seed into *Fusarium*-infested soil may result in the infection of plants and the development of both seedling blight and foot rot. Later in the growing season, air-borne inoculum, usually in the form of conidia or ascospores, may infect the spikes of plants, resulting in FHB. Under conditions of high relative humidity (RH) or rain, infected spikes may produce pinkish mycelia and sporodochia, resulting in production of macroconidia. Later in the season, macroconidia may infect secondary tillers. *Fusarium*-infected grain obtained from the diseased spikes, if used as seed, may provide a source of inoculum for the development of seedling blight which completes the disease cycle (Parry et al. 1994).

When temperature and moisture are favourable, FHB infection occurs during anthesis, which is the growth stage most susceptible to infection (Andersen 1948; Arthur 1891; Atanasoff 1920; Caron 1993; Dickson et al. 1921; Lacey et al. 1999; Pugh et al. 1933; Rapilly et al. 1973; Strange and Smith 1971). Because of this short period of vulnerability, the fungus is generally limited to one infection cycle per season (Bai and Shaner 1994).

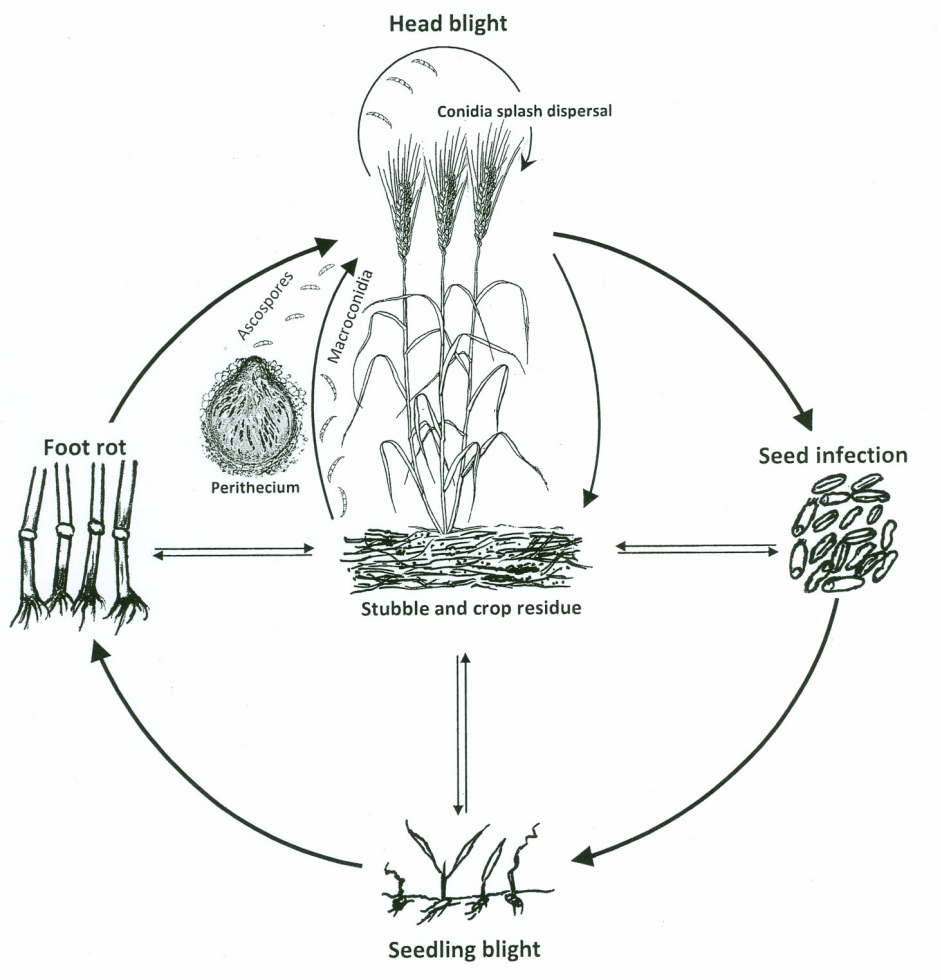

Figure 1.2. Fusarium head blight disease cycle on small grain cereals.

Sources of inoculum

Fusarium head blight pathogens survive on stubble and debris of wheat and other small grain cereals as well as in old maize stalks and ear pieces (Burgess and Griffin 1968; Gordon 1952; Gordon 1959; Hoffer et al. 1918; Shurtleff 1980; Warren and Kommedahl 1973). The previous crop and amount of crop residue on the soil surface are major factors related to local inoculum levels (Dill-Macky and Jones 2000; Teich and Hamilton 1985). In an investigation on the survival of *G. zeae* in infected wheat kernels, Inch and Gilbert (2003b) observed the survival of *G. zeae* and development of perithecia

on wheat kernels two years after being left on the soil surface or buried at 5 and 10 cm, but ascospores developed only in perithecia on the kernels left at the soil surface. Similar results were reported for survival and sporulation of *G. zeae* on wheat and maize tissues (Khonga and Sutton 1988). The rate of decomposition of residues is more rapid within the soil than above or on the soil surface (Dill-Macky 1999; Khonga and Sutton 1988; Todd et al. 2001). In conjunction with the lack of spore production within the soil, it can be concluded that buried residues contribute little to inoculum production (Gilbert and Fernando 2004). The fungi are present and survive in colonised crop residues, and may develop saprophytically on residues during the fall, winter, and spring (Sutton 1982). When maize and wheat are grown in rotation they provide an abundance of debris on which a primary source of *Fusarium* inoculum can develop (Sutton 1982). The fungi also survive saprophytically and parasitically on wheat leaves throughout the growing season (Ali and Francl 2001; Osborne et al. 2002). Other sources of inoculum include numerous plant hosts such as soybean (Martinelli et al. 2001), grasses and broadleaved weeds (Inch and Gilbert 2003a; Parry et al. 1995), and noncereal residues such as canola and field peas (Gilbert et al. 2003). However, the importance of weeds as a support for survival of *Fusarium* inoculum has not yet been determined (Jenkinson and Parry 1994a). Grains contaminated with the pathogens are another major source of inoculum, which may cause disease early in the growing season (Caron 1993; Cassini 1970). The soil may also be contaminated by FHB pathogens (Atanasoff 1920; Sutton 1982) but wet soil conditions do not favour fungal survival (Dickson 1923). Soil-borne infections take part less rapidly than seed-borne infections, so their attacks affect the collar and the upper parts of the roots (Cassini 1970). Probably the most obvious source of inoculum for the development of fusarium head blight epidemics originates from fusarium foot rot in a growing cereal

crop, but the relationship between fusarium foot rot and FHB is not very clear and needs further investigations (Parry et al. 1995).

Inoculum production

Conidia, chlamydospores, or hyphal fragments can serve as inoculum, but in the case of *G. zeae (F. graminearum)*, ascospores are also an important form of primary inoculum (Bai and Shaner 1994; Parry et al. 1995; Sutton 1982).

Mycelial growth and germination of macroconidia in *F. graminearum* occur in temperature ranges of 4-32 C with the optimum of 28 C and 28-32 C for mycelial growth and conidial germination, respectively (Andersen 1948). Perithecial production of *G. zeae* occurs in temperatures of 15-32 C with an optimum of 29 C (Caron 1993; Tschanz et al. 1976). Dufault et al. (2002a; 2002b) reported that an extended period of maize stalk wetness at temperatures between 15 and 25 C favoured perithecial development under both field and controlled conditions. The optimum temperature range of 28-32 C for production of macroconidia (Andersen 1948) is higher than that for the production of ascospores which is 25-28 C (Caron 1993; Ye 1980). Light is required for the production of perithecia in *G. zeae* (Tschanz et al. 1976). The recent shift to conservation tillage practices has resulted in increased amounts of crop residue on soil surface, which may increase the amount of inoculum and infection of wheat and other small grains (Bai and Shaner 1994; Dill-Macky and Jones 2000; Krupinsky et al. 2002). But where a large, regional source of atmospheric inoculum exists, tillage practices may not significantly affect FHB in individual fields (Schmale III et al. 2006). However, the effect of tillage management on FHB has not been demonstrated conclusively (Miller et al. 1998a; Sturz and Johnston 1985).

Inoculum release and dispersal

Relative humidity (RH) and rainfall are among the factors that favour the formation of perithecia (Caron 2000). Ascospore discharge is strongly associated with an increase in RH following the decrease in temperature that occurs at the end of the afternoon, and spores are released at night with peak numbers usually trapped between 16:00 and midnight (Paulitz 1996; Paulitz and Seaman 1994). In spite of this, ascospore release is inhibited by >5 mm rain, intermittent rain, or days with continuous RH>80% (Gilbert and Tekauz 2000). Paulitz (1996) reported that hourly spore counts ranged between 600 and 9000 ascospores/m^3 and that release occurred over a range of temperatures (11-30 C) and RH (60-95%). Mode of dispersal of *Fusarium* inoculum to spikes of cereals has not been demonstrated conclusively, but several alternatives have been proposed (Parry et al. 1995). Wind has long been considered the principal vector for spore dispersal, and observations indicate that it can play an important role in dispersal of *Fusarium* inoculum (Atanasoff 1920; Martin 1988; Parry et al. 1995). Wind is important in the transport of ascospores (Caron 1993; Gilbert and Tekauz 2000; Parry et al. 1995). A decline in seed infection within 5-22 m of the inoculum source in artificially inoculated field plots (Fernando et al. 1997) or in ascospore concentration within 60 m of a naturally overwintered source of inoculum (de Luna et al. 2002) showed wind-driven gradients over short distances. The idea that ascospores might be taken into the planetary boundary layer was proposed by Del Ponte et al. (2002). They recorded ascospore occurrence at altitudes of more than 180 m above ground, over lakes and regions far from farm fields, using remote-controlled model aircrafts fitted with spore traps. Long-distance dispersal of inoculum occurs when ascospores are transported by air streams in the atmosphere at high altitudes (Fernando et al. 2000; Maldonado-Ramirez et al. 2005). Rain is another factor

that plays an important role in the dispersal of *Fusarium* inoculum (Fernando et al. 2000; Hörberg 2002; Jenkinson and Parry 1994b; Millar and Colhoun 1969; Parry et al. 1995). Splashing transports spores especially macroconidia (Gilbert and Tekauz 2000). Champeil et al. (2004) concluded that splashing alone is sufficient to transfer a conidium from crop residues or stem base to the spike, assuming there is no obstacle. Another important environmental factor which is worthy of note for ascospore release is light. It appears that the process of ascospore release does not directly require light, as most ascospores are trapped during the night (Inch et al. 2000; Paulitz 1996; Schmale III et al. 2002). However, Trail et al. (2002) reported that under lab conditions, ascospore release was 8-30% greater in light than in complete darkness.

The possibility of systemic infection of spikes through foot and/or stem has long been the subject of debate (Champeil et al. 2004; Parry et al. 1995). Systemic infection of wheat spikes was disregarded earlier by Atanasoff (1920), who isolated *F. graminearum* from the peduncle segments taken from near the spikes, but not from those segments taken from near the flag leaf. Further evidence against the systemic infection of wheat spikes was provided by Bennett (1933), who failed to isolate either *F. avenaceum* or *F. culmorum* from segments above the second internode. In another study, the tops of plants produced from seeds inoculated with *M. nivale* and those grown from healthy seeds had similar numbers of perithecia, even though the plants grown from inoculated seeds had more perithecia at the base of the stem (Millar and Colhoun 1969). In addition, following inoculation of the base of the wheat stem, only 3% of plants displayed colonisation beyond the second node and no fungus were detected beyond the fifth node (Clement and Parry 1998). However, there are other findings that confirm the relationship between foot rot and head blight due to *Fusarium*. After inoculating seedlings of winter wheat below

soil level with *F. culmorum,* Jordan and Fielding (1988) re-isolated the pathogen from all intenodes and some spikes of plants. During similar studies with *F. avenaceum, F. culmorum, F. graminearum* and *M. nivale,* Hutcheon and Jordan (1992) later reported the colonisation of spikes of winter wheat. The systemic growth of *F. culmorum* in the stems of winter wheat has also been demonstrated by Snijders (1990e), who after inoculating the plants at soil level, re-isolated the pathogen from stem segments up to 70 cm above ground level.

Arthropod vectors such as insects and mites may be involved in *Fusarium* inoculum dispersal. During a survey of *Fusarium* species over Canada, Gordon (1959) isolated *F. avenaceum, F. culmorum* and *F. poae* from several insects including the common housefly (*Musca domestica* L.), clover leaf weevil [*Hypera punctata* (Fabricius)], and grasshoppers [*Melanoplus bivittatus* (Say)]. Windels et al. (1976) isolated seven *Fusarium* species including *F. graminearum* and *F. avenaceum* from picnic beetles [*Glischrochilus quadrisignatus* (Say)]. Other insects such as the orange wheat blossom midge [*Sitodiplosis mosellana* (Géhin)] may transmit *F. graminearum* in nature (Mongrain et al. 2000). Some mites also have been shown to play a role in the dispersal of *Fusarium* inoculum. For example, the mite *Siteroptes graminum* (Reuter) has been demonstrated to carry spores of *F. poae* (Cherewick and Robinson 1958; Cooper 1940). These observations show that insects and/or mites may play a role in dispersal of *Fusarium* inoculum.

Infection and colonisation of the spikes

Once *Fusarium* inoculum has been dispersed to the spike, several factors determine whether disease develops. Anthesis is the most susceptible growth stage of

cereals to *Fusarium* infection (Andersen 1948; Arthur 1891; Atanasoff 1920; Caron 1993; Dickson et al. 1921; Lacey et al. 1999; Pugh et al. 1933; Rapilly et al. 1973; Strange and Smith 1971) and susceptibility strongly decreases after the start of the dough stage (Caron 1993; Lacey et al. 1999; Pugh et al. 1933; Rapilly et al. 1973; Strange and Smith 1971). Findings show that the initial infection of spikes takes place via extruded anthers (Dickson et al. 1921; McKay and Loughnane 1945; Pugh et al. 1933; Strange and Smith 1971) and elimination of the anthers from wheat decreases the frequency of infection by *F. graminearum* (Andersen 1948; Strange and Smith 1971). Similarly, sterile wheat lines are less susceptible to head blight than fertile lines (Matsui et al. 2002). These findings, along with extensive colonisation of wheat anthers by *F. graminearum* observed by Andersen (1948) and Strange and Smith (1971), indicated that fungal growth is stimulated in these structures. Strange and Smith (1978) found that two substances-choline chloride and betaine hydrochloride-are much more concentrated in the anthers compared to other organs. They showed that these substances favour the development of hypha, but not the germination of spores in *F. avenaceum*, *F. culmorum*, and *F. graminearum* (Strange and Smith 1978). In a more recent study, Engle et al. (2004) found that hyphal growth and spore germination of *F. graminearum* were not significantly affected by choline, betaine, or a combination of both. Using a strain of *F. graminearum* inoculated on resistant and susceptible wheat cultivars, Miller et al. (2004) observed hyphae of the pathogen inside the floret at the point of inoculation with a particular affinity for the pollen and anthers of both cultivars.

The infection process in susceptible and resistant varieties is very similar (Kang and Buchenauer 2000). The pathogen first penetrates host tissues 36–48 h after inoculation (Kang and Buchenauer 2000). The first organs affected are the anthers (Pugh

et al. 1933), the lemma and the tip of the ovaries (Kang and Buchenauer 2000; Wanjiru et al. 2002), and glumes and the rachides (Schroeder and Christensen 1963). The penetration of the fungus into the spike is favoured by relatively low temperatures and high humidity (Rapilly et al. 1973). The hypha of *F. graminearum* and/or *F. culmorum* invade the host tissues predominantly by direct penetration (Kang and Buchenauer 2000) as well as through the stomata (Kang and Buchenauer 2000; Schroeder and Christensen 1963). The pathogens then propagate into the spike passing through and around the cells in their path (Kang and Buchenauer 2000, 2002; Pugh et al. 1933) and degrade the cells that they infect (Kang and Buchenauer 2000, 2002; Schroeder and Christensen 1963). They move mainly toward the rachis (Kang and Buchenauer 2000; Wanjiru et al. 2002) or toward the young grains which they invade via the parenchyma of the pericarp (Schroeder and Christensen 1963). Shortly after flowering, the parenchyma of the infected pericarp begins to break down, the nuclei and cytoplasm of the cells disappear, and the cell walls break down (Pugh et al. 1933).

Incubation and sporulation

Soon after infection, dark-brown, water-soaked spots appear on the glumes of infected florets. Later, entire florets become blighted. The fungus infects other spikelets internally through vascular bundles of the rachilla and rachis (Bushnell et al. 2003). Blight becomes more severe as the fungus spreads within the spike. Eventually the entire spike becomes blighted. The dark brown blight symptoms usually extend into the rachis even down into the stem tissue as the fungus spreads within the spike. The clogging of vascular tissues in the rachis can cause the spike to ripen prematurely, so that even grains not directly infected will be shrivelled as a result of shortage of water and nutrients (Bai

1995; Schroeder and Christensen 1963). Perithecia and conidia develop on the surface of spikelets and rachis under humid climatic conditions (Sutton 1982). The duration of the incubation period decreases with increasing relative humidity (Caron 1993). In conditions of saturating humidity, the duration of incubation required for the appearance of macroconidia of *F. culmorum* and *F. graminearum* on the spike was 12 days at 14 C, less than 5 days at 20 C, and less than 3 days in temperatures between 25 and 30 C (Caron 1993; Sutton 1982). More spores are formed after a long period of high humidity. This may then result in the infection of later crops, such as maize (Champeil et al. 2004). The timing of rain appears to be critical for the development of a head blight epidemic. For example, Mains et al. (1929) found that prolonged wet weather conditions during May and June following anthesis resulted in an epidemic of wheat scab in Indiana, USA, in 1928. Nakagawa et al. (1966) showed that the incidence of FHB caused by *F. graminearum* was significantly associated with rainfall during May in Japan where wheat reaches anthesis between mid-April and mid-May. In a review of FHB epidemics on winter wheat in the Netherlands, Snijders (1990b) found a strong correlation between the incidence of infected spikelets and the total rainfall during the period of June 11 to July 11, when wheat was in anthesis. Because of the short period of vulnerability of the plants to the fungi (anthesis period), the disease is generally limited to one infection cycle per season (Bai and Shaner 1994).

Sources of resistance

Arthur (1891) was the first to note differences in resistance/susceptibility to FHB among wheat cultivars. Considerable efforts since then have been made to find sources of resistance to use in breeding programs (Bai et al. 1989b; Hanson et al. 1950; Liu et al.

1989; Liu and Wang 1990; Wang et al. 1989). Most authors conclude that no wheat cultivar is immune, a few are moderately resistant, but most are susceptible.

Only a handful of resistance sources to FHB have been identified in common wheat (*Triticum aestivum* L., $2n = 6x = 42$, AABBDD). Reported sources of FHB resistance in spring wheat include 'Sumai 3' and its derivatives from China; 'Nobeokabouzu-komugi', 'Shinchunaga', 'Nyu Bai', and their relatives from Japan; and 'Frontana' and 'Encruzilhada' from Brazil (Bai et al. 1989b; Ban 2000; Ban and Suenaga 2000; Liu and Wang 1990; Mesterházy 1987; Schroeder and Christensen 1963; Wang et al. 1989; Yu et al. 2006).

'Sumai 3' which is derived from 'Funo' and 'Taiwanxiaomai', was reported to have high general combining ability for both FHB resistance and yield traits, and has been successfully used as a resistant parent in wheat breeding programs worldwide (Bai et al. 1990; Liu et al. 1991; Wang et al. 1989; Zhuang and Li 1993). 'Ning 7840' and 'Ning 8026' derived from 'Sumai 3' are moderate yielding wheat cultivars with excellent resistance to FHB as well as some resistance to leaf rust, stem rust, and powdery mildew (Wang et al. 1982; Zhou 1985). 'Ning 8623', 'Ning 8633', 'Ning 8675', 'Ning 8641', and some other lines derived from 'Sumai 3' possess moderate resistance to FHB and have higher yield potential, shorter stature, higher test weight, and better processing quality than 'Sumai 3' (Bai et al. 1989b). Some other derivatives of the Italian cultivar 'Funo' such as 'Yangmai 3', 'Yangmai 4', and 'Yangmai 5' which are moderately susceptible to FHB, have high yield potential and have been widely adopted for commercial production (Bai and Shaner 1994). Among Japanese resistance sources, 'Shinchunaga' which is an old cultivar selected from a natural mutation of landrace 'Nakanaga', has been successfully used as a resistant parent in improving FHB resistance in wheat breeding

programs in Japan (Ban 2000). Similar to Chinese FHB resistant landraces, Japanese sources all are inferior to 'Sumai 3' for various agronomic traits (Ban 2001). Two Brazilian cultivars, 'Frontana' and 'Encruzilhada', have been used as parents in some breeding programs (Ban 2001; Gilbert et al. 1997; Mesterházy 1997a; Singh and van Ginkel 1997).

From winter wheat germplasm, the cultivars 'Arina', 'Renan', and 'Praag-8' from Europe were reported as FHB resistance sources (Gervais et al. 2003; Ruckenbauer et al. 2001; Snijders 1990c).

In the United States, winter wheat cultivars 'Ernie' and 'Freedom' have a low disease incidence and severity in the field and have been used as parents in some U.S. breeding programs (Rudd et al. 2001). Novel FHB resistance was also postulated to be present in several recently released cultivars, including in the winter wheat cultivar 'Truman' (McKendry et al. 2005), and in two spring wheat cultivars 'Steele-ND' (Mergoum et al. 2005), and 'Glenn' (Mergoum et al. 2006).

Diploid and tetraploid wheat species usually are highly susceptible to FHB (Wan et al. 1997b). For example, durum wheat [*Triticum turgidum* L. subsp. *durum* (Desf.) Husn., $2n = 4x = 28$, AABB] is consistently more susceptible to FHB caused by *F. graminearum* and *F. culmorum* than common wheat (Atanasoff 1924; Hanson et al. 1950) and sources of resistance are limited in durum wheat (Buerstmayr et al. 2003b; Stack 1988; Stack et al. 2002).

A number of wild relatives of wheat have been identified as sources of resistance to FHB (Ban 1997; Buerstmayr et al. 2003b; Chen et al. 2001; Liu et al. 2000; Shen et al. 2004; Wan et al. 1997a; Wan et al. 1997b) and alien chromatin carrying resistance genes to FHB has been transferred from wild relatives to cultivated wheat (Chen and Liu 2000;

Fedak et al. 2003; Han and Fedak 2003; Liu et al. 2000). Olivera et al. (2003) evaluated the reaction of 82 accessions of *Aegilops sharonensis* Eig ($2n = 2x = 14$, S^lS^l) originating from Israel to FHB and found that 11 of them exhibited high levels of resistance. *Elymus giganteus* Vahl [syn.: *Leymus racemosus* (Lam.) Tzvel. subsp. *racemosus*, $2n = 4x = 28$, JJNN], *Roegneria kamoji* (Ohwi) Ohwi ex Keng [syns.: *Elymus kamoji* (Ohwi) S. L. Chen and *Agropyron kamoji* Ohwi, $2n = 6x = 42$, $S^{ts}S^{ts}H^{ts}H^{ts}Y^{ts}Y^{ts}$], and *Roegneria ciliaris* (Trin.) Nevski [syn.: *Elymus ciliaris* (Trin.) Tzvel. subsp. *ciliaris*, $2n = 4x = 28$, $S^cS^cY^cY^c$] have been shown to have resistance to FHB (Liu et al. 1989; Mujeeb-Kazi et al. 1983; Wang et al. 2001; Wang et al. 1986; Wang et al. 1991; Weng and Liu 1989; Weng and Liu 1991). The FHB resistance in *E. giganteus* is associated with three chromosomes (Chen et al. 1997). Ban (1997) evaluated four indigenous Japanese species in the genus *Elymus* and found that *Elymus humidus* (Ohwi *et* Sakamoto) Osada ($2n = 6x = 42$, SSHHYY) and *Elymus racemifer* (Steud.) Tzvel. ($2n = 4x = 28$, SSYY) exhibited a high level of resistance to FHB. Fedak (2000) also reported that the native Japanese species *E. humidus* was immune to FHB. This species exhibited FHB resistance at a level higher than 'Sumai 3' (Ban 1997; Cai et al. 2005). *Thinopyrum elongatum* (Host) D. R. Dewey [syn.: *Lophopyrum elongatum* (Host) A. Löve, $2n = 2x = 14$, EE] is known as another source of FHB resistance (Jauhar and Peterson 1998). Furthermore, Jauhar and Peterson (2001) identified FHB resistance in an accession of *Thinopyrum junceiforme* (A. Löve *et* D. Löve) A. Löve ($2n = 4x = 28$, $J_1J_1J_2J_2$). Finally, accessions of *Thinopyrum intermedium* (Host) Barkworth *et* D. R. Dewey ($2n = 6x = 42$), *Thinopyrum ponticum* (Podp.) Barkworth *et* D. R. Dewey ($2n = 10x = 70$), and *Thinopyrum junceum* (L.) A. Löve ($2n = 6x = 42$) have been identified with FHB resistance equal to that of 'Sumai 3' (Cai et al. 2005).

Relatives of common and durum wheat under the genus *Triticum* are genetically more closely related to them than the species in other genera under *Triticeae.* Some of the species in *Triticum* share genomes with common and durum wheat and have high crossability with them. Resistance to FHB has been found in some of these relatives. *Triticum tauschii* (Coss.) Schmalh. [syn.: *Aegilops tauschii* (Coss.), $2n = 2x = 14$, DD] has been reported to be a source of resistance to FHB (Gagkaeva 2003; Gilchrist et al. 1997). Fedak et al. (2004) also found 7 *Triticum speltoides* (Tausch) Gren. ex K. Richt. (syn.: *Aegilops speltoides* Tausch var. *speltoides*, $2n = 2x = 14$, BB) accessions resistant to FHB. In another study, Gagkaeva (2003) identified resistance to FHB in 252 accessions in 26 species of *Triticum*, including *Triticum aethiopicum* Jakubz. ($2n = 4x = 28$, AABB), *Triticum turanicum* Jakubz. ($2n = 4x = 28$, AABB), *Triticum urartu* Thum. ex Gandil. ($2n = 2x = 14$, AA), *Triticum timopheevii* (Zhuk.) Zhuk. ($2n = 4x = 28$, AAGG), *Triticum persicum* (Boiss.) Aitch. *et* Hemsl. ($2n = 4x = 28$, AABB), *Triticum ispahanicum* Heslot ($2n = 4x = 28$, AABB), *Triticum karamyschevii* Nevski ($2n = 4x = 28$, AABB), *Triticum vavilovii* Jakubz. ($2n = 6x = 42$, AABBDD), *Triticum dicoccoides* (Körn ex Asch. *et* Graebn.) Schweinf. ($2n = 4x = 28$, AABB), *Triticum sphaerococcum* Perc. ($2n = 6x = 42$, AABBDD), *Triticum militinae* Zhuk. *et* Migush. ($2n = 4x = 28$, AAGG), *Triticum dicoccum* Schrank ($2n = 4x = 28$, AABB), and *Triticum spelta* L. ($2n = 6x = 42$, AABBDD). The most resistant accessions were from the species *T. timopheevii*, *T. karamyschevii*, *T. militinae*, *T. persicum, T. dicoccum*, and *T. spelta*. Fedak et al. (2004) also found FHB resistance in *T. timopheevii* and *Triticum monococcum* L. ($2n = 2x = 28$, AA). Recently, Fedak et al. (2009) reported the introgression of FHB resistance from *T. monococcum*, *T. speltoides*, and *Triticum cylindricum* (Host) Ces. ($2n = 4x = 28$, CCDD) into bread wheat.

Tetraploid wheat genotypes have been evaluated for their reaction to FHB. Miller et al. (1998b) evaluated 282 wild emmer wheat [*Triticum turgidum* L. subsp. *dicoccoides* (Körn ex Asch. *et* Graebn.) Thell., $2n = 4x = 28$, AABB] accessions for reaction to FHB and identified 10 accessions that were more resistant than the best available durum wheat. Buerstmayr et al. (2003b) screened 151 wild emmer accessions originating from different areas of Israel and Turkey and identified eight accessions resistant to FHB. Oliver et al. (2007) evaluated 416 accessions of wild emmer wheat for reaction to FHB and found that there was wide variation in response to FHB, ranging from highly susceptible to highly resistant. In another study, Oliver et al. (2008) evaluated 376 accessions of five cultivated subspecies of *T. turgidum*, including Persian wheat [*T. turgidum* L. subsp. *carthlicum* (Nevski) A. Löve *et* D. Löve, $2n = 4x = 28$, AABB], cultivated emmer wheat [*T. turgidum* L. subsp. *dicoccum* (Schrank ex Schübl.) Thell., $2n = 4x = 28$, AABB], Polish wheat [*T. turgidum* L. subsp. *polonicum* (L.) Thell., $2n = 4x = 28$, AABB], Oriental wheat [*T. turgidum* L. subsp. *turanicum* (Jakubz.) A. Löve *et* D. Löve, $2n = 4x = 28$, AABB], and Poulard wheat (*T. turgidum* L. subsp. *turgidum*, $2n = 4x = 28$, AABB) in the greenhouse and field, and observed that 16 *T. turgidum* subsp. *carthlicum* and 4 *T. turgidum* subsp. *dicoccum* accessions were consistently resistant or moderately resistant to FHB. Furthermore, in the evaluation of 255 accessions of six tetraploid wheat species including Persian wheat, wild emmer wheat, cultivated emmer wheat, Polish wheat, oriental wheat, and poulard wheat, Cai et al. (2005) found one accession of Persian wheat and four accessions of cultivated emmer wheat with a high level of resistance to FHB. Resistance to FHB also has been occasionally identified among Persian wheat and cultivated emmer wheat by other workers (Clarke et al. 2004; Gagkaeva 2003; Gladysz et al. 2004; Somers et al. 2006).

Transfer of FHB resistance genes to wheat from alien genomes without homology to wheat genomes is more difficult compared to alien genomes that are homologous or closely related to the wheat genome (Cai et al. 2005). In addition, the resistance found in alien species is usually associated with undesirable traits which are difficult to remove from the progeny (Bai and Shaner 2004). Special chromosome manipulation is needed to introgress FHB resistance genes into wheat from distantly related alien species (Cai et al. 2005) and significant effort and time may be required for pre-breeding to remove these 'wild' characters (Bai and Shaner 2004).

Components of resistance

Schroeder and Christensen (1963) proposed two types of resistance in wheat: resistance to initial infection (now referred to as type I resistance) and resistance to spread of blight symptoms within a spike (now referred to as type II resistance). They found that the two types of resistance varied independently among cultivars. The first example of type II resistance was provided by Schroeder and Christensen (1963), who showed that hyphal growth could not be sustained in the resistant cultivar, 'Frontana'. Three other types of resistance to FHB have been proposed: decomposition or no accumulation of mycotoxins, resistance to kernel infection, and tolerance (Mesterházy 1995; Miller et al. 1985; Wang and Miller 1988).

Infected grain usually contains DON regardless of the degree of resistance of a cultivar to head blight. However, grain DON contents differ among cultivars (Bai et al. 2001b). Mesterházy (2002) reported toxin levels near zero in most resistant genotypes but very high toxin levels in susceptible cultivars, both caused by the same isolates and inoculum. Low DON accumulation in some wheat cultivars compared to other cultivars

grown in the same environment has been described as type III resistance (Miller and Arnison 1986; Miller et al. 1985). Low DON content in a kernel could result from three possible causes: (a) a low level of DON produced by the fungus, (b) a degradation of DON by plant enzymes during kernel development, or (c) a high level of DON in spike tissue other than kernels, but failure of DON to move into kernels during their development (Bai and Shaner 2004). Whether resistance to DON accumulation is independent of type I or type II is still unknown (Bai and Shaner 2004).

Resistance to kernel infection (type IV resistance) can be quantified by measuring the percentage of infected kernels. However, the degree of kernel infection may be reduced by the presence of type I or type II resistance in the plant, so this must be taken into account when attempting to measure resistance to kernel infection (Shaner 2002). Tolerance (type V resistance) can be measured by relative yield reduction when diseased and healthy plants of the same cultivar are compared in a similar experimental design (Bai and Shaner 2004).

Type I and type II resistance are commonly used but type III, type IV, and type V resistance have not been used consistently by researchers (Shaner 2002). Type II resistance has been extensively studied in wheat as it appears to be more stable and less affected by non genetic factors (Bai and Shaner 1994).

Molecular and biochemical mechanisms of resistance

Many attempts have been made to understand the mechanisms of resistance of wheat to FHB (Bai et al. 2001a; Chen et al. 1999; Desjardins et al. 1996; Mesterházy 1995; Miller et al. 1985; Pritsch et al. 2000; Pritsch et al. 2001), but the biochemical and molecular basis of resistance is mainly unknown (Bai and Shaner 2004). The expression

of pathogenesis-related proteins including PR-1, PR-2 (β-1,3-glucanases), PR-3 (chitinase), PR-4 (hevein-like protein), PR-5 (thaumatin-like proteins), and peroxidase was induced in both resistant and susceptible cultivars after point inoculation (Pritsch et al. 2001). These proteins were detected as early as 6-12 h after inoculation and reached the peak after 36-48 h (Pritsch et al. 2000). PR-4 and PR-5 transcripts expressed earlier and higher levels in 'Sumai 3' than in the susceptible cultivar 'Wheaton' (Pritsch et al. 2000). In another study, Li et al. (2001) found that β-1,3-glucanases and chitinases also accumulated faster in 'Sumai 3' than in its susceptible mutant. Expression of a rice thaumatin-like protein gene in wheat delayed FHB symptoms in wheat spikes inoculated with *Fusarium* (Chen et al. 1999). This phenomenon shows that pathogenesis-related genes in wheat are activated after fungal infection and they may play a role in general defence against *Fusarium* infection, even though they may not be the key factors responsible for resistance (Bai and Shaner 2004). Several other enzymes, such as superoxide dismutase, catalase, phenylalanine ammonia lyase, ascorbic acid peroxidase, and ascorbic acid oxidase have also been related to FHB resistance in wheat (Bai and Shaner 2004).

Preformed chemical compounds in FHB resistant and susceptible cultivars are different. Choline content in susceptible cultivar spikes was twice that of a resistant cultivar during anthesis (Li and Wu 1994). Content of chlorogenic acid (a phenolic compound) in the susceptible cultivar was also higher than that in the resistant cultivar (Ye et al. 1990).

DON produced by the fungus during fungal infection has been proposed as a virulence factor (Proctor et al. 1995). Aggressiveness of *F. graminearum* isolates also depends on their DON-producing capacity (Mesterházy 2002; Miedaner et al. 2000).

Disruption of the gene encoding trichodiene synthase (*Tri*5) in *F. graminearum*, an enzyme which catalyzes the first step in the DON biosynthetic pathway, reduced DON production and disease severity (Desjardins et al. 1996). Bai et al. (2001a) indicated that the DON-nonproducing isolates still could infect wheat spikes in both greenhouse and field conditions but could not spread beyond the initial infection, suggesting that DON is an aggressiveness factor, rather than a pathogenicity factor (Harris et al. 1999; Proctor et al. 1995). Bai and Shaner (2004) reached the conclusion that DON may not be essential for primary infection by the fungus, but may enhance symptom development and spread of the pathogen within a spike. If this is true, low DON content in an infected kernel or expression of a DON detoxificating gene from the fungus in wheat may improve wheat resistance (Bai and Shaner 2004). More recently, trichothecene 3-*O*-acetyltransferase (*Tri101*) gene has been successfully transferred into wheat (Okubara et al. 2002). *Tri101*, encoding an enzyme that catalyzes the conversion of toxic *Fusarium* trichothecenes including DON to less-toxic products, has been proposed as a metabolic self-protection mechanism in *F. graminearum* (Kimura et al. 1998). So, expression of *Tri101* may limit the accumulation of DON and enhance the level of resistance in wheat. After DON, Gpmk1, a mitogen-activated protein (MAP) kinase, is known as the second virulence factor in *F. graminearum* (Jenczmionka et al. 2003).

Resistance in wheat probably involves a complex network of signalling pathways (Bai and Shaner 2004). Application of large-scale gene analysis such as microarray analysis and global monitoring of pathogenesis-related genes may allow the identification of genome-wide gene expression, a better understanding of the molecular basis of wheat defence against infection by the pathogen, and facilitate discovery of critical pathways and key genes involved in these pathways (Bai and Shaner 2004).

Inheritance of resistance

Christensen et al. (1929) first showed that resistance to FHB was an inherited characteristic and observed transgressive resistance among progenies of 'Marquis' x 'Preston'. Hanson et al. (1950) crossed relatively resistant spring wheat cultivars with more susceptible cultivars and observed transgressive resistance among the progenies inoculated with a mixture of *Fusarium* species.

Inheritance of type II resistance in wheat has been extensively studied (Bai et al. 2000b; Bai et al. 1989a; Bai et al. 1990; Ban 2001; Buerstmayr et al. 1999; Liu et al. 1991; Nakagawa 1955). Many investigators consider FHB resistance to be quantitatively inherited and controlled by many minor genes (Chen 1983; Liao and Yu 1985; Snijders 1990d; Wu 1986; Yu 1990; Yu 1982), some researchers provide evidence of oligogenic control (Bai et al. 1989a; Bai et al. 1990; Li and Yu 1988; Nakagawa 1955), and others have shown that the resistance is controlled by a small number of major genes (Yang 1994). The number of major genes varies with varieties and they may have different effects (Yang 1994). It can be concluded that a few major genes accompanied by some minor genes control type II resistance (Bai and Shaner 1994; Bai et al. 1989a; Liao and Yu 1985; Nakagawa 1955; Van Ginkel et al. 1996).

Additive gene effects play a major role in the inheritance of type II resistance to FHB but non-additive gene effects might also be significant (Bai et al. 2000b; Bai et al. 1993; Bai et al. 1989a; Bai et al. 1989c; Chen 1983; Lin et al. 1992; Snijders 1990a, d; Wu et al. 1984; Zhang and Pan 1982). Dominance appears to be the most important component of the non-additive gene effect (Bai et al. 1990; Chen 1983; Snijders 1990d). Epistatic effects were considered significant in some studies (Bai et al. 2000b; Bai et al. 1993; Snijders 1990a) but not in another (Zhuang and Li 1993). Heritabilities are usually

high (Bai et al. 1989c; Chen 1983; Liao and Yu 1985), but there are exceptions (Zhang et al. 1990).

Using a set of diallel crosses among seven spring and winter genotypes with different levels of resistance (including 'Sumai 3', 'Xinzhongchang', and 'Wangshuibai'), Lin et al. (1992) indicated that inheritance of resistance to a strain of *F. graminearum* is governed by the additive-dominance model with additive gene action being the most important factor of resistance. The number of genes governing resistance in this population was estimated to vary from two to four. In an investigation, Singh et al. (1995) showed that the resistance of 'Frontana' is controlled by the additive interaction of a minimum of three minor genes. In this study transgressive segregants were identified, indicating that the susceptible (or moderately susceptible) parents also carry one (or two) minor genes. The combination of these genes with the genes in 'Frontana' produced the progenies with significantly better FHB resistance than that of 'Frontana' (Singh et al. 1995). Other classic genetics studies identified two resistance genes in 'Frontana', 'Ning 7840' (Van Ginkel et al. 1996), 'WZHHS', 'Sumai 3', and 'Ning 7840' (Bai et al. 1990), and three genes in 'WSB' and 'YGFZ' (Bai et al. 1990). There is evidence that different numbers of genes have been proposed in the same resistant cultivar in different studies (Lu et al. 2001). Kolb et al. (2001) mentioned several possible reasons for these inconsistent results including polygenic control of FHB resistance in wheat, effect of different genetic backgrounds, different types of resistance evaluated, genotype and environment interactions, heterogeneous sources of a resistant parent, or inoculation techniques used in different studies.

Nakagawa (1955) reported that three pairs of epistatic factors might control FHB resistance in some wheat cultivars. Major genes at different loci on a chromosome may

differ in their effects and may show complementation (Bai and Shaner 1994). Minor genes may function as modifiers of the major genes, as reported in resistance to stripe rust (Bai et al. 1989a; Lewellen et al. 1967).

Monosomic or chromosome substitution analysis indicate that resistance genes from different Chinese and Japanese wheat cultivars are distributed over the entire wheat genome except on chromosome 1A (Lu et al. 2001). 'Sumai 3' has FHB resistance genes on chromosomes 1B, 2A, 5A, 6D, and 7D (Yu 1982), 'Wangshuibai' on chromosomes 4A, 5A, 7A, 7B, and 4D (Liao and Yu 1985), and the cultivar 'PHJZM' on chromosomes 6D, 7A, 3B, 5B, and 6B (Yu 1990). The moderately susceptible cultivar 'HHDTB' has resistance genes on chromosomes 5D, 1B, 7B, and 4D (Bai and Shaner 1994) and the cultivar 'YGFZ' on chromosomes 3A and 4D (Yu 1990).

Li and Yu (1988) suggested that disease resistance could be measured in five ways: incubation period, time required for disease spread from the infection site to the rachis, daily rate of FHB progress before and after symptoms reach the rachis, and severity. They concluded that disease spread to the rachis was an important criterion in disease rating. Resistance at different stages of FHB development might be controlled by different genes in wheat. Li and Yu (1988) indicated that in cultivar 'WZHHS' resistance genes on chromosomes 1B, 2A, 3D, 4B, 6A, 6D, 6B, 7B, and 7D affected the incubation period; genes on 3D, 6A, and 7D controlled spread of the fungus from the inoculated spikelet to the rachis; and genes on 2A, 3D, 4D, 5B, 6B, and 7D were responsible for spread of the fungus to the entire spike. The accumulation of different resistance genes in plants that operate at different stages of disease development may enhance the overall resistance of a cultivar (Bai and Shaner 1994).

Resistance to FHB in wheat usually is stable and resistant cultivars show consistent resistance to almost all isolates of *F. graminearum* worldwide. Since its release 30 years ago, 'Sumai 3' and its derivatives are still the major sources of resistance to FHB in wheat breeding programs in China (Bai et al. 2003a; Lu et al. 2001) and International Maize and Wheat Improvement Centre (CIMMYT), Mexico (Bai and Shaner 2004). These resistance sources have also been extensively tested for FHB resistance in Japan, the United States, and many European countries with a worldwide collection of *F. graminearum* isolates (Bai 1995; Bai et al. 2003a; Ban 2001; Kolb et al. 2001; Mesterházy 2003). Failure of resistance to FHB in 'Sumai 3' source has not been reported; it is still the best source of type II resistance worldwide (Bai and Shaner 2004).

Although different isolates of *Fusarium* may differ widely in aggressiveness and there may be significant interactions between wheat cultivars and pathogen isolates, there is no evidence for stable pathogen races (Bai and Shaner 1996; Mesterházy 2003; Snijders and Van Eeuwijk 1991; Wang and Miller 1987), such as those found in cereal rust fungi, powdery mildew fungi, and some other specialized pathogens. Based on the test of reaction of wheat cultivars to different species of *Fusarium*, Mesterházy (1981) concluded that resistance to certain isolates of *F. graminearum* as well as to other species of *Fusarium* was not strain-specific or species-specific in wheat cultivars. The species of *Fusarium* that cause head blight in wheat can infect many other cereals and maize without showing specialization, and a host-specific, blight-causing *Fusarium* species has not been documented to date (Van Eeuwijk et al. 1995). It can be concluded that resistance to FHB is a horizontal or non-specific nature at least for the most prevalent species like *F. culmorum* and *F. graminearum* (Mesterházy et al. 1999; Snijders and Van Eeuwijk 1991; Van Eeuwijk et al. 1995). So the resistance genes in 'Sumai 3' and other sources of

resistance currently used in breeding programs are not expected to be overcome by new isolates of the pathogen in the near future. However, given the large genetic variability that exists in *Fusarium* spp. (Bowden and Leslie 1999), use of at least a few different resistance genes in a wheat breeding program would be a wise approach (Buerstmayr et al. 2009).

Pathogen profile (*Fusarium graminearum*)

The name *Fusarium graminearum* (teleomorph: *Gibberella zeae*) was used for a long time to describe a *Fusarium* species isolated from head blight affected wheat and barley (*Hordeum vulgare* L.), stalk rot affected maize (*Zea mays* L.), head scab affected pearl millet [*Pennisetum typhoides* (Burm f.) Stapf. and C. E. Hubbard.], and crown rot affected barley, oats (*Avena sativa* L.), and common wheat grass [*Agropyron scabrum* (R. Br.) P. Beauv.]. Later, two naturally occurring and morphologically distinct populations within *F. graminearum* were described by Purss (1969; 1971) and Francis and Burgess (1977). Two populations, originally designated as group 1 and group 2, were based on the inability or ability of cultures to form perithecia, respectively (Francis and Burgess 1977). Group 1 heterothallic fungi are normally associated with diseases of the crown while group 2 homothallic isolates are associated with diseases of aerial parts of plants (Burgess et al. 1975). Subsequent analysis based on both morphological features and DNA sequence data has led to renaming of group 1 *F. graminearum* as *Fusarium pseudograminearum* Aoki and O'Donnell (teleomorph: *Gibberella coronicola* Aoki and O'Donnell) (Aoki and O'Donnell 1999a, b).

Although the former group 2 population, *F. graminearum* (*G. zeae*), has the ability to reproduce both sexually and asexually, and both macroconidia and ascospores can infect cereal heads (Sutton 1982), the relative proportion of each reproduction system is not very clear. Since *G. zeae* isolates are haploid and homothallic, sexual reproduction can occur either by cross-or self-fertilization, but the relative frequency of outcrossing and selfing in nature is not well-known. Perithecia are readily produced in culture and on plant materials in the field as evidenced by the massive amounts of ascospores (Schmale III et al. 2006; Schmale III et al. 2005). Extensive sexual recombination should increase the level of variation in the *F. graminearum* (*G. zeae*) population (Burdon 1993).

Fusarium graminearum isolates demonstrate high variation in different features such as genotypic characteristics and phylogenetic profiles, genetic diversity, mycotoxin production and trichothecene chemotypes, pathogenicity/aggressiveness, vegetative compatibility groups (VCGs), and phenotypic characteristics. Better understanding of the pathogen profile is a key approach to deal with FHB and to employ appropriate strategies for disease control.

Molecular phylogenetics and the *Fusarium graminearum* complex

The FHB primary pathogen, *F. graminearum* (*G. zeae*), was thought to be a single species spanning six continents until the genealogical concordance phylogenetic species recognition (GCPSR) approach (Taylor et al. 2000) was used to investigate species limits using a global collection of FHB causing fungal isolates (O'Donnell et al. 2000; Ward et al. 2002). Results of the phylogenetic analysis using DNA sequences of six nuclear genes (7.1 kb) from 99 isolates of the *F. graminearum*, collected from a variety of substrates from around the world, revealed seven biogeographically structured lineages within *F.*

graminearum clade (referred to as the *Fg* clade) (O'Donnell et al. 2000). This suggests that the lineages within the *Fg* clade represent phylogenetically distinct species among which gene flow has been limited during their evolutionary history (O'Donnell et al. 2000). Using a 19-kb region of the trichothecene gene cluster from 39 isolates of *F. graminearum* representing the global genetic diversity of species in the *Fg* clade, Ward et al. (2002) identified all seven aforementioned lineages plus a new one named lineage 8 within the *Fg* clade.

O'Donnell et al. (2004) investigated species limits within the *Fg* clade through phylogenetic analyses of DNA sequences from portions of 11 nuclear genes (13.6 kb) and identified the eight previously known and a new phylogenetically distinct lineages (species) within the *Fg* clade. The 1–9 lineage designations used formerly have been abandoned as they were assigned new species names as follows: [1] *Fusarium austroamericanum,* [2] *Fusarium meridionale*, [3] *Fusarium boothii*, [4] *Fusarium mesoamericanum*, [5] *Fusarium acaciae-mearnsii*, [6] *Fusarium asiaticum*, [7] *Fusarium graminearum*, [8] *Fusarium cortaderiae*, and [9] *Fusarium brasilicum* (O'Donnell et al. 2004).

By employing more isolates of *Fg* clade and use of phylogenetic analysis of multilocus DNA sequence data from 13 genes (16.3 kb) together with analyses of their morphology, pathogenicity to wheat, and trichothecene toxin potential, Starkey et al. (2007) introduced two novel species within *F. graminearum* species complex: *Fusarium vorosii* and *Fusarium gerlachii*. Later two new species including *Fusarium aethiopicum* from Ethiopia (O'Donnell et al. 2008) and *Fusarium ussurianum* from the Russian Far East (Yli-Mattila et al. 2009) were reported.

So, the previously known *F. graminearum* 'group 2' is now known to be a monophyletic species complex consisting of at least 13 separate phylogenetic species. These new species have different geographic distributions, differ in production of trichothecene mycotoxins, and may differ in their ability to cause disease on particular crops (Cumagun et al. 2004; O'Donnell et al. 2000; O'Donnell et al. 2004).

The name *F. graminearum* (former lineage 7 in the *Fg* clade) which corresponds to the teleomorph *G. zeae*, was assigned to the major causal agent of FHB in wheat and barley, and appears to have a cosmopolitan distribution (O'Donnell et al. 2004). It looks to be the predominant species in the *Fg* clade found in Canada (K. O'Donnell, Pers. Comm.), USA (Burlakoti et al. 2008; Zeller et al. 2003, 2004), Argentina (Ramirez et al. 2007), and central Europe (Tóth et al. 2005). *Fusarium graminearum sensu stricto* isolates have also been detected from New Zealand (Monds et al. 2005) and several Asian countries, including China (Gale et al. 2002), Japan (Karugia et al. 2009; Suga et al. 2008), and Korea (Lee et al. 2009). *Fusarium asiaticum* is predominantly found in Asia (Gale et al. 2005; Gale et al. 2002; Karugia et al. 2009; Lee et al. 2009; O'Donnell et al. 2004; Suga et al. 2008) but has also been identified in very low numbers from samples originating from Brazil and the United States (Gale et al. 2005). *Fusarium mesoamericanum* is endemic to Central America, while *F. acaciae-mearnsii* appears to be endemic to Australia or less likely Africa (O'Donnell et al. 2004). *Fusarium meridionale, F. brasilicum, F. austroamericanum,* and *F. cortaderiae* are endemic to South America, but the endemic area of *F. boothii* is problematic given its distribution in Africa, Mexico, and Mesoamerica (O'Donnell et al. 2004).

Although the description of these species and the nomenclature system is yet to receive widespread acceptance (Miedaner et al. 2008), demonstration of fertile crosses

between lineage 7 and all other lineages and also between some others (Bowden et al. 2006) questions the validity of species designation for the interfertile lineages. However, inter-lineage hybridization must have been a rare event; otherwise the lineages could not have been established (Miedaner et al. 2008).

Genetic diversity of *Fusarium graminearum* populations

A population is defined as a group of individuals originating from a limited geographical area which are sharing a common gene pool (McDonald and McDermott 1993). Genetic diversity of a population is the result of all evolutionary processes that have influenced a population (McDonald and Linde 2002). Recombination, gene flow, and mutation increase genetic diversity, while selection and genetic drift decrease it. Understanding the nature of genetic diversity within populations, the level of population subdivision, and its association with phenotypic traits such as aggressiveness and mycotoxin production is essential to help in predicting the evolutionary potential of FHB pathogens with measures for disease control.

Recombination is the most obvious mechanism to shuffle and maintain high genetic diversity in populations (Miedaner et al. 2008). In *F. graminearum*, sexual recombination has been observed under laboratory conditions with a moderate level of outcrossing (Bowden and Leslie 1999), but under field conditions it is inferred only from high genotypic diversity which is detected using VCGs and molecular markers and by population estimates like linkage disequilibrium (Miedaner et al. 2008). Questions regarding sexual recombination can only be addressed if outcrossing is observed in the population (Gale et al. 2002). Even rare outcrossing events may contribute significantly to genetic diversity (Leslie and Klein 1996).

Gene flow breaks down boundaries that could isolate populations and introduces new genetic diversity into agricultural fields (McDonald and Linde 2002). The exchange of both genes and genotypes can contribute to gene flow between populations. Dispersal of sexual and asexual propagules plays an important role in gene flow to keep the genetic diversity in *F. graminearum* high (Miedaner et al. 2008).

Most studies have revealed a high level of genetic diversity in *F. graminearum* within individual field populations or populations sampled across a large-scale geographical zone. Using random amplified polymorphic DNA (RAPD) primers applied to 72 isolates of *F. graminearum* collected from three provinces of Canada (Quebec, Ontario, and Prince Edward Island), Dusabenyagasani et al. (1999) showed that all isolates were genetically distinct and most of the genetic variability among the isolates was explained by within-region variation. Carter et al. (2000) analyzed a collection of 62 *F. graminearum* isolates from maize, wheat, and rice from different locations in Nepal using molecular markers, and detected variation within the collection. Miedaner et al. (2001) detected high genetic variation within four field populations of *F. graminearum* from Germany, Hungary, and Canada using polymerase chain reaction (PCR)-based fingerprinting. In another study, Miedaner et al. (2001) found 84% of the molecular variance within a sampling area of approximately 1 m^2. All 225 isolates of the *Fg* clade collected from four wheat fields in Zhejiang, China belonged to *F. asiaticum* but there was high genotypic variation among the isolates (Gale et al. 2002). In Canadian *F. graminearum* populations, 92–97% (Mishra et al. 2004) and 75% (Fernando et al. 2006) of the molecular variation was associated with differences among isolates within populations. On the other hand, Ouellet and Seifert (1993) characterized *F. graminearum* isolates from Canada using RAPD and PCR, and demonstrated a relatively low amount of

genetic diversity among the isolates tested which could not be grouped according to host or geographic origin.

Analysis of biodiversity and phylogeny of *F. graminearum* isolates originating from Russia, China, Germany, and Finland using isozyme variation, β-tubulin and intergenic spacer (IGS) sequences demonstrated a high level of genetic diversity among the isolates (Gagkaeva and Yli-Mattila 2004). High genotypic variation has also been found among the isolates of *F. graminearum* from USA (Walker et al. 2001), Australia (Akinsanmi et al. 2006), and Europe (Waalwijk et al. 2003).

Amplified fragment length polymorphism (AFLP) analysis of large numbers of *G. zeae* isolates from different populations collected across USA indicated that all populations of the pathogen belonged to *F. graminearum sensu stricto*, and that the genetic identity among the populations and the estimated effective migration rate were high (Zeller et al. 2003, 2004). It is concluded that a large, homogeneous, interbreeding population of the pathogen is present over USA; genetic diversity results from a continuous recombination among inocula in the atmosphere which are most likely from multiple origins over large geographical distances (Zeller et al. 2003, 2004). Although the New York atmospheric populations of *G. zeae* were genotypically diverse, they were genetically similar and potentially part of a large, interbreeding population of the pathogen in North America (Schmale III et al. 2006). When New York populations were compared with those collected across the United States, the observed genetic identities among the populations was high. However, there was a significant negative correlation between genetic identity and geographic distance, suggesting that some genetic isolation may occur on a continental scale (Schmale III et al. 2006). Variable number tandem repeat (VNTR) markers showed that all populations sampled from barley, wheat, potato,

and sugar beet in the upper Midwest of the United States were assigned to *F. graminearum sensu stricto*, but gene and genotype diversity were high in all populations (Burlakoti et al. 2008).

Furthermore, little or no population subdivision has been observed among the isolates of *F. graminearum* sampled from fields separated by hundreds of kilometres in Europe (Naef and Defago 2006), China (Gale et al. 2002), and Canada (Fernando et al. 2006).

Based on AFLP analysis of 113 isolates of the *Fg* clade collected from Argentina, all isolates were assigned to *F. graminearum sensu stricto*, but a high genotypic variation was detected among the isolates (Ramirez et al. 2007). Using sequence characterized amplified regions (SCARs) and AFLP analyses of 437 *Fg* complex isolates from wheat spikes in China, two species of *Fusarium* were recovered: *F. graminearum sensu stricto* mainly from wheat growing in the cooler regions and *F. asiaticum* from warmer regions (Qu et al. 2008). However, more diversity was detected by AFLP, revealing several subgroups within each species.

AFLP and PCR analysis of 356 isolates of *Fg* complex from rice in Korea showed that 333 isolates belonged to *F. asiaticum* and 23 isolates to *F. graminearum sensu stricto* (Lee et al. 2009). Most isolates of the *Fg* complex sampled from a 500-m^2 experimental wheat field in Kumamoto Prefecture, Japan were classified as *F. asiaticum* with high gene diversity; only four isolates were classified as *F. graminearum sensu stricto* (Karugia et al. 2009).

Populations of *F. graminearum* are highly flexible in adapting to their environments. Impressive changes from *F. culmorum* to *F. graminearum* have been reported in the last decade in the Netherlands (Waalwijk et al. 2003), southern (Obst et al.

1997) and northern Germany (Miedaner et al. 2008), and south-west of England and south Wales (Jennings et al. 2004). The specific causes for these changes are unclear, however, the rapid evolutionary changes on large geographical scales demonstrate the high genetic flexibility of these fungal populations (Miedaner et al. 2008). However, the shift from *F. graminearum* to *F. culmorum* may have significant consequences for cereal production as *F. graminearum* is generally regarded to be more damaging pathogen than *F. culmorum* in terms of both yield loss and mycotoxin production (Jennings et al. 2004).

Mycotoxin production and trichothecene chemotypes

Fusarium head blight of cereals may result in contamination of cereal grains with mycotoxins such as trichothecenes and estrogenic toxins (Bai and Shaner 1994; Desjardins et al. 1996; Marasas et al. 1984; McMullen et al. 1997; Miller et al. 1991; Parry et al. 1995; Snijders 1990b; Sutton 1982; Tuite et al. 1990). The trichothecenes produced by *Fusarium* are divided into two broad categories based on the presence (B-trichothecenes) or absence (A-trichothecenes) of a keto group at the C-8 position of the trichothecene ring (Ueno et al. 1973). All *Fg* clade species are B-trichothecene producers (Ward et al. 2002). Trichothecenes are synthesized by a complex biosynthetic pathway that requires the coordinated expression of more than 14 trichothecene (*Tri*) genes (Peplow et al. 2003). Except the 3-*O*-acetyltransferase (*Tri101*) gene (Kimura et al. 1998), all other trichothecene genes are localized within a gene cluster (Brown et al. 2001). In *F. graminearum*, the ultimate product of the pathway is nivalenol (NIV); 4-deoxynivalenol (DON) is a pathway intermediate product (Lee et al. 2002).

Large variation for type and amount of mycotoxin production has been found in collections of *F. graminearum* isolates from different regions (Gang et al. 1998; Miedaner

et al. 2000). Under normal cultural conditions, a high variation in zearalenone production has been reported among the isolates of *G. zeae* (Caldwell 1968; Cullen et al. 1982; Eugenio 1968). Fifteen Canadian isolates of *F. graminearum* varied for ergostrol and mycotoxin production (Gilbert et al. 2001). Significant differences were found in *in vitro* production of DON and zearalenone among 66 isolates of *F. graminearum* collected from North Carolina (Walker et al. 2001). There are other reports describing variation in mycotoxin production among the isolates (Atanassov et al. 1994; Goswami and Kistler 2005; Walker et al. 2001).

Based on the type of trichothecenes produced, Ichinoe et al. (1983) reported two chemotaxonomic groups of *G. zeae* isolated from wheat and barley in Japan: (*i*) nivalenol and fusarenon-X producers and (*ii*) deoxynivalenol and 3-acetyldeoxynivalenol producers. Both groups were also identified among the isolates from wheat, barley, and cockspur in Italy (Logrieco et al. 1988) and wheat and maize in Australia (Blaney and Dodman 1988). Further differentiation was detected within *F. graminearum* with the identification of 15-acetyldeoxynivalenol, a new derivative of deoxynivalenol (Miller et al. 1983).

Miller et al. (1991) identified three strain-specific profiles of trichothecene chemotypes within *F. graminearum*: chemotype I (DON chemotype) produced DON and/or its acetylated derivatives, while chemotype II (NIV chemotype) produced nivalenol and/or its diacetylated derivatives. Furthermore, isolates of chemotype I were subclassified into two types: chemotype IA (3-ADON chemotype) which produced DON and 3-ADON metabolites, and chemotype IB (15-ADON chemotype) which produced DON and 15-ADON metabolites (Miller et al. 1991).

DON-producing isolates of *F. graminearum* appear to occur more frequently than NIV-producing isolates in many parts of the world: isolates of the pathogen collected from soil or cereals in the United States were classified mainly as 15-ADON producers (Abbas et al. 1986; Abramson et al. 1993; Gale et al. 2007; Mirocha et al. 1989), Argentinean isolates of the pathogen collected from wheat as DON, 15-ADON, and 3-ADON producers (Faifer et al. 1990), Uruguayan isolates from barley as chemotype IB (DON/15-ADON) (Pineiro et al. 1996), European isolates from wheat spikes mostly as DON producers (Waalwijk et al. 2003), Korean isolates from corn and barley as 15-ADON and NIV chemotypes (Moon et al. 1999; Seo et al. 1996), and the isolates collected from soil or cereals in China, Australia, New Zealand, Norway, and Poland mainly as 3-ADON producers (Mirocha et al. 1989). In other studies, the majority of isolates of *F. graminearum* collected from England and Wales (Jennings et al. 2004), central Europe (Tóth et al. 2005), and China (Ji et al. 2007) were recognized as 15-ADON chemotype. Ramirez et al. (2006) recognized all isolates of the pathogen gathered from wheat as DON producers (Ramirez et al. 2006). In an investigation conducted by Guo et al. (2008) on two wheat cultivars in 15 locations in Manitoba, Canada, from 2004 to 2005, the percentages of 3-ADON and 15-ADON chemotypes ranged from 0 to 95.7 and 4.3 to 100%, respectively. However, in Japan (Ichinoe et al. 1983; Suga et al. 2008), Korea (Kim et al. 1993), and Iran (Haratian et al. 2008) NIV-producing isolates appeared to be predominant.

There have also been published the results of investigations conducted exclusively on trichothecene chemotyping of *Fg* clade and *F. graminearum sensu stricto* isolates. Most of 712 *F. graminearum sensu stricto* isolates gathered from nine states of the United States belonged to 15-ADON chemotype, but genetically divergent groups of isolates

mainly as 3-ADON chemotype were also identified in some locations of Minnesota and North Dakota (Gale et al. 2007). They cited it as a reason to reject the hypothesis that *F. graminearum sensu stricto* in the United States consists of a single population. Phylogenetic analyses and trichothecene chemotyping of 298 isolates of *Fg* clade collected from wheat and barley in Japan revealed the presence and differential distribution of *F. graminearum sensu stricto* and *F. asiaticum* in Japan, and different chemotype compositions among the isolates: all isolates of *F. graminearum sensu stricto* were of a 15- or 3-ADON chemotype, while most isolates of *F. asiaticum* were of NIV chemotype (Suga et al. 2008). Chemical analyses of trichothecenes in 356 isolates of the *Fg* complex from rice in Korea showed that 325 and 31 isolates had nivalenol and deoxynivalenol, respectively (Lee et al. 2009). PCR assays of 82 isolates of the *Fg* clade obtained from wheat kernels in Brazil to characterize the trichothecenes present showed that 76 isolates were of the 15-ADON chemotype, 6 isolates of the NIV chemotype, and none of the isolates were of the 3-ADON chemotype. DNA sequence analysis suggested that the 15-ADON and NIV chemotype isolates were *F. graminearum sensu stricto* and *F. meridionale*, respectively (Scoz et al. 2009). Out of a total of 183 *Fg* complex isolates from Japan, 80 isolates were of the NIV type, while 103 isolates, including all four *F. graminearum sensu stricto* isolates, were of the 3-ADON type, and no 15-ADON type isolate was detected (Karugia et al. 2009). Analysis of the trichothecene chemotype distribution among the isolates of *F. graminearum sensu stricto* from wheat in Argentina revealed that 15-acetyldeoxynivalenol was the most common chemotype (Alvarez et al. 2009).

Recently a significant shift from DON- to NIV-producing *F. graminearum* in northwestern Europe (Waalwijk et al. 2003) and from the original 15-ADON to 3-ADON

chemotype in North America (Ward et al. 2008) has been demonstrated. Analysis of FHB pathogen diversity in North America in 2008 revealed that there was a significant population structure associated with trichothecene chemotypes and that 3-ADON producing *F. graminearum* isolates are prevalent (Ward et al. 2008). In western Canada for example, the 3-ADON chemotype frequency increased more than 14-fold between 1998 and 2004 (Ward et al. 2008).

By analysis of a large field population of *F. graminearum* (>500 isolates) from Nepal using SCARs, Desjardins et al. (2004) identified three groups that were genetically distinct and polymorphic for trichothecene production: DON producers, NIV producers, and DON and NIV producers. They reported that the ability to cause FHB differed between SCAR groups and trichothecene chemotypes: DON producers were more virulent than NIV producers. There are also several reports supporting that DON-producing isolates are more aggressive toward plants than NIV-producing isolates (Cumagun et al. 2004; Desjardins et al. 2004; Goswami and Kistler 2005; Logrieco et al. 1990; Miedaner et al. 2000; Muthomi et al. 2000). The relationship between chemotype and pathogenicity has not been established (Logrieco et al. 1990; Perkowski et al. 1997) but Carter et al. (2002) reported the influence of mycotoxin chemotype in determining pathogenicity of isolates at the seedling stage on a particular host. In a test of 31 isolates belonging to eight species of the *Fg* clade, pathogenicity was not influenced by the type of mycotoxin produced, but a significant correlation was observed between the amount of the dominant trichothecene (DON and its acetylated forms or NIV) produced and the level of aggressiveness on wheat (Goswami and Kistler 2005).

The chemotype differences may have important fitness consequences for the fungi (Alexander et al. 1998; Kimura et al. 1998). Although DNA sequence analysis indicates

that NIV production is an ancestral trait, the worldwide distribution of DON and of DON-producing isolates of *F. graminearum* today suggests that DON production may have some selective advantage for this pathogen (Desjardins et al. 2004). This may also be true for the ability of 3-ADON and 15-ADON chemotypes to dominate ecological zones. The isolates from 3-ADON populations produced more trichothecene and had higher reproductivity and growth rates compared to the isolates from the 15-ADON populations (Ward et al. 2008).

Trichothecene chemotypes do not correlate highly with the *Fg* clade phylogeny (O'Donnell et al. 2000; Ward et al. 2002), indicating that each of these chemotypes has multiple independent evolutionary origins or that their evolutionary history is different from what is predicted by the *Fg* clade phylogeny (Ward et al. 2002). Mycotoxin analysis of New Zealand *Fg* clade isolates showed that *F. graminearum sensu stricto* isolates produced either NIV or DON, but *F. cortaderiae* isolates produced only NIV (Monds et al. 2005). Analysis of 299 isolates of the *Fg* clade representing all regions in China showed that 231 isolates were from *F. asiaticum* with 3-ADON, 15-ADON, and NIV chemotypes and 3-ADON being the predominant chemotype. However, 68 isolates assigned to *F. graminearum sensu stricto* consisted only of the 15-ADON chemotype (Zhang et al. 2007).

Variation in pathogenicity/aggressiveness

A large variation in pathogenicity of *G. zeae* isolates, from non-pathogenic to consistently pathogenic, has been reported in field trials (Cullen et al. 1982). Walker et al. (2001) observed significant differences in pathogenicity among *F. graminearum* isolates collected from North Carolina. Using coleoptile and floret inoculations for pathogenicity

assays, Wu et al. (2005) observed significant differences in pathogenicity among the 58 isolates of *F. graminearum* from China and detected a high positive correlation between coleoptile and floret inoculations.

High variation in aggressiveness has also been found among *F. graminearum* isolates from different geographical regions (Akinsanmi et al. 2004; Bai and Shaner 1996; Mesterházy 1984; Miedaner et al. 1996, 2000 #224; Muthomi et al. 2000). Miedaner and Schilling (1996) reported significant variation for aggressiveness among the isolates of *F. graminearum* from a single field. A significant quantitative variation for aggressiveness was observed within the individual field populations of *F. graminearum* from Germany and among the isolates from a world collection tested on young winter rye in the greenhouse (Miedaner et al. 2001). Gilbert et al. (2001) observed high variation in aggressiveness among Canadian isolates of *F. graminearum*, with disease severity ranging from 17.2 to 39.1 for single-floret injection and 39.1 to 69.0 for spray inoculation. All *F. graminearum* isolates from central Europe were found to be highly pathogenic in *in vitro* aggressiveness tests (Tóth et al. 2005). There are more reports describing variation in aggressiveness among the isolates of *F. graminearum* (Cumagun et al. 2004; Goswami and Kistler 2005; Xue et al. 2004).

Vegetative compatibility groups (VCGs) and phenotypic variation

Vegetative compatibility groups (VCGs) have been used in fungal pathogens to assess the level of pathogen variability and obtain additional insights into their population structure (Leslie 1993). VCG variation is very high within *F. graminearum* even at the local level.

Bowden and Leslie (1992) found 24 different VCGs among 24 isolates of *F. graminearum* collected from 23 wheat fields in Kansas, USA. In another investigation, 19 VCGs were detected among 26 isolates sampled from wheat spikes in a 0.25 m^2 section of a single wheat field (Bowden and Leslie 1994), indicating that *F. graminearum* infecting wheat is genetically highly variable even within a very small area. Similarly, McCallum et al. (2001) identified 34 VCGs among 43 isolates of *F. graminearum* collected from barley spikes throughout Manitoba.

Diversity in VCGs have been detected among the isolates of *F. graminearum* from Canada (Fernando et al. 2006; Gilbert et al. 2001; McCallum et al. 2001), USA (Bowden and Leslie 1994; Zeller et al. 2003), Argentina (Ramirez et al. 2006), China (Chen et al. 2007b), Korea (Moon et al. 1999), and Iran (Naseri et al. 2000).

Mapping of QTLs for fusarium head blight resistance

Plant material

In quantitative trait loci (QTL) mapping, segregating populations derived from a cross of contrasting parents are used. Frequently used populations are recombinant inbred lines (RIL), doubled haploid (DH) lines, or populations derived from backcrosses. Use of introgression lines or intervarietal substitution lines developed by a backcrossing method and other sets of genotypes such as cultivars, breeding lines, or introduced germplasm is another option (Buerstmayr et al. 2009).

In QTL mapping the basic principle is to detect correlations between genotypes and phenotypes in a population or sample of individuals on the basis of linkage disequilibrium (Breseghello and Sorrells 2006; Gupta et al. 2005; Rostoks et al. 2006).

Phenotyping

In this procedure the goal is to determine the level of genetic resistance of every line in the mapping population as precisely as possible. The level of FHB in wheat genotypes is determined by the host resistance factors, the pathogen aggressiveness, and the environment. The influence of environment on disease establishment and development can lead to significant genotype-by-environment (GxE) interactions (Campbell and Lipps 1998; Fuentes et al. 2005), which may significantly bias QTL estimates (Ma et al. 2006a). Field and/or greenhouse evaluations are conducted under optimum environmental conditions for disease development to detect the real reaction of genotypes in experiments. A uniform inoculation method, inoculum pressure, experimental condition during disease development, and scoring method are applied to all genotypes of the mapping population during QTL studies.

Type I resistance is more difficult to evaluate and therefore fewer reports have been published on the QTLs controlling type I resistance (Buerstmayr et al. 2009). As an indicator of type I resistance, disease incidence (percentage of spikes with disease symptoms) is measured in spray or naturally inoculated plots or pots. As a scale for type II resistance, disease severity (percentage of diseased spikelets per unit area) is typically measured following single-floret inoculation, conidial spray or grain-spawn inoculation. Other disease-related traits including level of mycotoxins (mostly DON), percentage of FDK in harvested samples, and amount of yield or yield components relative to non-inoculated controls are usually measured using relevant scoring methods.

Morphological and developmental characteristics such as plant height (Draeger et al. 2007; Klahr et al. 2007; Mesterházy 1995; Paillard et al. 2004; Schmale III et al. 2005), head compactness (Schmale III et al. 2005), flower opening (Gilsinger et al. 2005),

or heading date (Klahr et al. 2007; Miedaner et al. 2006; Wilde et al. 2007) may affect the response of genotypes to the pathogen. Separating pleiotropic effects of genes involved in morphological or developmental traits on FHB reaction from the effects of true resistance genes which may be linked to such morphological or developmental genes is not always easy and sometimes causes difficulty in QTL mapping (Buerstmayr et al. 2009). The choice of the pathogen species or isolates for inoculation has also been discussed.

The number of lines in the mapping population is very important. It has been shown that using more lines is always better than using fewer lines (Beavis 1998) and a limited population size may lead to underestimation of QTL number, overestimation of QTL effects, and failure to quantify QTL interactions (Vales et al. 2005a). If QTL of moderate to small individual effects contribute to trait expression, a large number of lines are needed for precise QTL estimation (Vales et al. 2005b). Although more than 300 lines would be desirable to map quantitative traits controlled by multiple loci, because of practical limitations, more than 300 lines are rarely used in QTL mapping in plants (Melchinger et al. 2004; Schön et al. 2004). Most studies to date have used 100–200 lines. Populations of less than 100 lines are considered too low to detect anything except large effect QTLs for FHB resistance (Buerstmayr et al. 2009).

The number and design of the phenotyping experiments is very important in successful QTL mapping. At least two independent experiments (locations or years) are necessary to estimate the repeatability of the resistance evaluation and determine the stability of QTL estimates across environments (Buerstmayr et al. 2009).

Genotyping

Genotypic information of each line in the mapping population is obtained using different molecular markers. The type and number of markers applied depends on the equipment and resources available.

The first DNA marker generation exploited is called restriction fragment length polymorphisms (RFLPs). The main advantages of RFLP markers are their codominance and high reproducibility (Weising et al. 2005). During the 1990s, RFLPs were very popular, but PCR-based markers have become dominant in recent years. RAPD, DNA amplification fingerprinting (DAF), and arbitrary primed PCR (AP-PCR) all use primers of arbitrary nucleotide sequence to amplify anonymous PCR fragments from genomic template DNA (Weising et al. 2005). The RAPD procedure introduced by Williams et al. (1990), is technically the simplest version and is independent of any prior DNA sequence information. Despite a number of drawbacks, RAPDs are still widely used. Microsatellites, also known as simple sequence repeats (SSRs), consist of tandemly repeated short DNA sequence motifs. They frequently are size-polymorphic in a population, due to a variable number of tandem repeats (Weising et al. 2005). The popularity of nuclear microsatellites originates from several important advantages including their codominant inheritance, high abundance, enormous extent of allelic diversity, and the ease of assessing size variation by PCR with pairs of flanking primers (Weising et al. 2005). AFLP technology represents a combination of RFLP analysis and PCR. AFLP can be applied to all organisms without previous sequence information and generally results in highly informative fingerprints (Weising et al. 2005). It is one of the most popular and powerful technologies to detect DNA polymorphism. Other techniques such as cleaved amplified polymorphic sequences (CAPS), SCARs, microsatellite-primed

PCR (MP-PCR), target region amplification polymorphism (TRAP), randomly amplified microsatellites (RAMS), secondary digest AFLP (SDAFLP), and single-strand conformation polymorphism (SSCP) may be used to detect DNA variation. Markers based on single nucleotide polymorphisms (SNPs) may become more popular in the future (Buerstmayr et al. 2009).

Adequate number and appropriate choice of markers should be considered in QTL mapping to achieve full coverage of the genome (*e.g.* no gaps >20 cM) especially in the suspected QTL regions. Although any part of the wheat genome can be mapped using a thousand SSR markers which are now available in the public domain, the development of a dense map in hexaploid wheat is still demanding (Buerstmayr et al. 2009).

Molecular markers tightly linked to resistance genes provide a powerful alternative tool for tracing resistance genes (Bai et al. 2003b). Exploitation of molecular markers associated with FHB resistance genes has mainly focused on type II FHB resistance (Anderson et al. 2001; Bai et al. 1999; Buerstmayr et al. 2002; Waldron et al. 1999; Yang et al. 2003; Zhou et al. 2002). Development of DNA marker-assisted screening for the presence of resistance genes may make selection for resistance more efficient in breeding programs (Bai et al. 1999; Kolb et al. 2001).

QTLs for FHB resistance

A broad spectrum of FHB sources of resistance from spring wheat, winter wheat, tetraploid wheat, and wild relatives of wheat have been used for QTL mapping to find and use QTLs for resistance to FHB in wheat breeding programs.

QTLs from Sumai 3 and its derivatives

The first two QTL mapping studies which published by Waldron et al. (1999) and Bai et al. (1999) were both based on populations derived from Chinese cultivars with high type II resistance to FHB. Waldron et al. (1999) found five QTLs associated with type II resistance in a RIL mapping population derived from a cross between 'Sumai 3' (resistant) and 'Stoa' (moderately susceptible) in single-floret-inoculated greenhouse tests. The QTL with the largest effect, originated from 'Sumai 3' and mapped to chromosome 3BS, was designated as *Qfhs.ndsu-3BS*. Two other major effect QTLs, derived from 'Stoa' and mapped to chromosomes 2AL and 4BL, and two minor effect QTLs derived from 'Sumai 3' and mapped to separate regions on chromosome 6BS were detected. Bai et al. (1999) identified 11 AFLP markers tightly linked to a major QTL for type II resistance on chromosome 3BS in a RIL population derived from 'Ning 7840'/'Clark' which was evaluated using single-floret inoculation in the greenhouse. 'Ning 7840' is a 'Sumai 3'-derived resistant parent with the pedigree 'Aurora'/'Anhui11'//'Sumai 3' and 'Clark' is extremely susceptible to disease spread in the spike. The aforesaid QTL was also associated with low DON accumulation in infected kernels (Bai et al. 2000a).

In two RIL populations of wheat including 'Sumai 3' x 'Stoa' and 'ND2603' ('Sumai 3' x 'Wheaton') x 'Butte 86' evaluated in single-floret-inoculated greenhouse tests, Anderson et al. (2001) detected two 'Sumai 3'-derived QTLs for type II resistance consist of the *Qfhs.ndsu-3BS* major QTL and a QTL on chromosome 6BS in both populations, of which *Qfhs.ndsu-3BS* QTL explained 41.6% and 24.8% of phenotypic variation in two populations, respectively. The authors also detected two new QTLs on chromosomes 3AL and 6AS in 'ND2603'/'Butte 86' population and two other QTLs on

chromosomes 2AL and 4BS originating from 'Stoa' in 'Sumai 3' x 'Stoa' population, all for type II resistance. In another RIL population of wheat from the cross 'Sumai 3' x 'Stoa' evaluated for kernel shattering (KS) and FHB in field trials, Zhang and Mergoum (2007) revealed four QTLs for FHB infection on chromosomes 2B, 3B, and 7A, three of them (on 2B and 7A) coincided with and/or linked to the KS QTLs with opposite allele effects in the corresponding genomic regions, which may explain the negative correlation (r = -0.29 and $P < 0.01$) between the KS and FHB infection.

Buerstmayr et al. (2002; 2003a) used RFLP, AFLP, and SSR markers to map QTLs for type I and type II FHB resistance in the field in a DH population derived from 'CM-82036' x 'Remus', in which 'CM-82036' was a selection from the cross of 'Sumai 3'x'Thornbird' from the CIMMYT wheat program. They detected two QTLs for resistance to visual disease severity on chromosomes 3B (*Qfhs.ndsu-3BS*) and 5A (*Qfhs.ifa-5A*) which explained 29 and 20% of the phenotypic variation in the population, respectively. These QTLs plus an additional QTL detected on 1B all originated from 'CM-82036'. Using spray inoculations, the effects of *Qfhs.ndsu-3BS* and *Qfhs.ifa-5A* were in a comparable range, but by use of single-floret inoculation, *Qfhs.ndsu-3BS* showed a much larger effect than *Qfhs.ifa-5A* (Buerstmayr et al. 2002; Buerstmayr et al. 2003a). Based on their results from experiments using different inoculation methods, they concluded that *Qfhs.ifa-5A* may contribute mainly to type I resistance and to a lesser extent to type II resistance, whereas *Qfhs.ndsu-3BS* appears to play a role primarily in type II resistance (Buerstmayr et al. 2003a). Similar conclusions were drawn by Chen et al. (2006) who evaluated a 'W14' x 'Pioneer Brand 2684' DH population and found that the 3BS QTL had a larger effect on resistance than the 5AS QTL in the single-floret-inoculated greenhouse test, whereas, the 5AS QTL had a larger effect in the spray-

inoculated field experiment. The QTLs on 3B and 5A were also detected in five different breeding populations with 'CM-82036' as a resistant parent (Angerer et al. 2003). Using SSR and AFLP markers in a 'Ning 7840'/'Clark' RIL population evaluated in single-floret-inoculated greenhouse experiments, Zhou et al. (2002) detected one major QTL on 3BS and two QTLs with minor effects on 2BL and 2AS, all derived from 'Ning 7840' and all for type II resistance.

Using polymorphic SSR primers, in a DH population derived from 'Wuhan-1'/'Maringa' which later was corrected to'Wuhan-1'/'Nyu Bai' (McCartney et al. 2007), Somers et al. (2003) detected three QTLs on chromosomes 2DL, 3BS, and 4B for type II resistance in the single-floret-inoculated test in the greenhouse and two QTLs on chromosomes 2DS and 5AS for low DON content in the field. QTLs on 2DL and 3BS reduced disease severity by 32% in the greenhouse, QTLs on 3BS and 4B showed a 27% decrease in FHB in the field, and QTLs on 3BS and 5AS significantly reduced DON accumulation in harvested grains from field.

Yang et al. (2005b) evaluated a DH population from the cross of 'DH181' (a resistant line selected from the cross of 'Sumai 3' x 'HY368') and 'AC Foremost' (susceptible cultivar) in the field (spray inoculation) and greenhouse (single-floret inoculation), and reported seven QTLs for type I resistance, four QTLs for type II resistance, and six QTLs for resistance to kernel infection. QTLs on 2DS, 3BS, and 6BS were associated with all three traits.

Recently, Ma et al. (2006b) found a major QTL on 3BS and smaller effect QTLs on 2D, 4D, and 6A for resistance to disease severity in a RIL population from the cross of 'CS-SM3-7ADS' (a 'Chinese Spring'-'Sumai 3' chromosome 7A substitution line which is highly resistant to FHB) and 'Annong 8455' (a FHB susceptible cultivar) evaluated in

the field and greenhouse using point inoculation. All QTLs were derived from 'CS-SM3-7ADS'.

Because of its high breeding potential, the chromosomal segment covering *Qfhs.ndsu-3BS* was further fine mapped with AFLP, sequence tagged sites (STS), and SSR markers for marker-assisted selection (Cuthbert et al. 2006; Guo et al. 2003; Liu and Anderson 2003a; Liu and Anderson 2003b; Liu et al. 2006). Lemmens et al. (2005) found that wheat lines carrying *Qfhs.ndsu-3BS* were able to convert DON into the less phytotoxic DON-3-*O*-glycoside and hypothesized that *Qfhs.ndsu-3BS* either encodes a DON-glucosyltransferase or regulates the expression or activity of such an enzyme.

The *Qfhs.ndsu-3BS* QTL was recently re-named *Fhb1* (Liu et al. 2006). In high resolution mapping populations segregating for *Fhb1*, this locus was mapped as a single Mendelian gene with high precision (Cuthbert et al. 2006). Flanking STS markers covering *Fhb1* within a 1.2-cM interval are now available (Cuthbert et al. 2006; Lin et al. 2006). The QTL on 6BS, a significant type II resistance QTL originated from 'Sumai 3' or related lines (Anderson et al. 2001; Lin et al. 2004; Shen et al. 2003b; Waldron et al. 1999; Yang et al. 2005b), was named *Fhb2* and mapped as a single Mendelian factor with high precision in a fine mapping population (Cuthbert et al. 2007).

There are other Asian FHB resistance sources which their type II resistance is largely assigned to *Fhb1*: 'Huapei 57-2' (Bourdoncle and Ohm 2003) which has no pedigree reported for it, 'Ning 894037' which is a somaclonal variant from the FHB susceptible cultivar 'Yangmai 3' (Shen et al. 2003b) but has the same marker haplotype as 'Sumai 3' at five SSR markers around *Fhb1* (Liu and Anderson 2003a), 'W14' (Chen et al. 2006), and 'CJ 9306' (Jiang et al. 2007a; Jiang et al. 2007b) which both are highly FHB resistant lines derived from a cross involving 'Sumai 3' and another resistant line

(Chen et al. 2006; Jiang et al. 2007a; Jiang et al. 2007b). It is possible that these sources of resistance possess the same resistance allele as 'Sumai 3' at *Fhb1* (Buerstmayr et al. 2009).

Although 'Sumai 3' has been shown to have the alleles to enhance FHB resistance at several QTLs, it also has negative alleles at some loci, *i.e.* alleles that reduce the level of resistance to FHB in plants and make them more susceptible. A study of the 'Sumai 3' x 'Stoa' population showed that 'Sumai 3' contributed susceptible alleles for the QTLs on chromosomes 2AL and 4B (Anderson et al. 2001; Waldron et al. 1999). In two populations of 'Sumai-3' x 'Nobeokabozu-komugi' and 'Sumai 3' x 'Gamenya', Handa et al. (2008) identified and mapped a multidrug resistance-associated protein (MRP) gene on chromosome 2DS. The initial expression level of the MRP homologue was higher in the susceptible parent 'Gamenya' than in 'Sumai 3', and even after induction by FHB inoculation the expression level of the 'Sumai 3' MRP was still the same as that of the 'Gamenya' MRP before induction. Their study indicated that the MRP allele associated with the QTLs for both type II resistance and low-level DON content and additional effect to *Fhb1* of 'Sumai 3'. Therefore, the possible susceptible 'Sumai 3' allele for MRP should be excluded in order to obtain a higher level of FHB resistance in 'Sumai 3' in breeding programs (Handa et al. 2008). The FHB resistance QTL region of chromosome 2DS is also flanking the reduced height gene *rht8*/*Rht8* locus and the 'Sumai 3' allele at this region decreases plant height by about 10 cm, indicating that 'Sumai 3' possesses a semi-dwarf allele at this locus (Handa et al. 2008). In conclusion, Handa et al. (2008) hypothesized that the FHB resistance QTL on chromosome 2DS is a resistance gene complex consisting of specific gene(s) like MRP to control type II resistance by detoxification of DON and *rht8/Rht8* to control morphological traits and affecting type I

resistance. In a similar chromosomal region on 2DS, resistance QTL for type II resistance were detected from the susceptible cultivar 'Alondra' in a RIL population of 'Ning 894037' x 'Alondra' in both field and greenhouse experiments (Shen et al. 2003b).

'Sumai 3' and its derivatives are the best-known sources of resistance to FHB and they have been used widely in wheat breeding around the world. Mapping QTLs for FHB resistance in 'Sumai 3' derived populations identified several major and minor effect QTLs on different chromosomes for type I and type II resistance, low DON accumulation, and kernel infection. Major effect QTLs on chromosomes 3BS and 6BS for type II resistance and on chromosome 5A for type I resistance are potential factors of resistance which can be used individually or along with other major or minor QTLs to improve wheat resistance to FHB.

QTLs from Wangshuibai and its derivatives

The Chinese landrace 'Wangshuibai', which possesses high FHB resistance, has received considerable attention as an alternative source of resistance for wheat breeding. As 'Wangshuibai' had no evident association with 'Sumai 3' in its pedigree, the expectation was to find novel QTLs in 'Wangshuibai' (Buerstmayr et al. 2009). This was supported by the finding that several SSR and AFLP markers linked to the 3BS QTL on 'Wangshuibai' showed the same allele sizes as 'Nyu Bai' (McCartney et al. 2004) but slightly different allele sizes than 'Sumai 3' (Bai et al. 2003b; Liu and Anderson 2003a; McCartney et al. 2004).

In different mapping studies for type II resistance in 'Wangshuibai', the largest effect was found on 3BS which explained 6–37.3% of phenotypic variation (Lin et al. 2004; Ma et al. 2006b; Yu et al. 2008; Zhang et al. 2004; Zhou et al. 2004). Similarly,

Mardi et al. (2005) found a significant QTL on 3BS and a QTL on 2DL for FHB severity in a 'Wangshuibai' x 'Seri 82' RIL population evaluated in spray-inoculated field tests. Jia et al. (2005) reported six QTLs for disease severity on chromosomes 2D, 3BS, 4B, 5B, and 7A including the 3BS QTL in naturally infected trials in 'Wangshuibai' x 'Alondra'"s" DH population. In a RIL population of the cross of 'Wangshuibai' x 'Nanda 2419', three major effect QTLs for type II resistance on chromosomes 2B, 3B, and 6B were detected in single-floret-inoculated field trials (Lin et al. 2004) and three significant QTLs for type I resistance on chromosomes 4B, 5A, and 5B in spray-inoculated field experiments (Lin et al. 2006). They concluded that 'Wangshuibai' is a useful source for both type I and type II resistance. In a population of 'Wangshuibai' x 'Falat' evaluated for type II resistance in single-floret-inoculated greenhouse tests, Najaphy et al. (2006) identified a QTL region on chromosome 3B and another QTL on chromosome 2A accounting for 16% and 9.1% of phenotypic variation, respectively. Finally, Li et al. (2008) identified five QTLs associated with FDK in spray-inoculated field trials in a RIL population developed from the cross 'Nanda 2419' x 'Wangshuibai'.

Although 'Wangshuibai' and some other Asian FHB resistance sources seem to be genetically unrelated to 'Sumai 3', they possess QTLs with the same sequence of *Fhb1* as 'Sumai 3' (Buerstmayr et al. 2009). In spite of this, they can be used as an alternative or complementary source of resistance QTLs in wheat breeding programs.

QTLs from other spring wheat sources

In a study conducted on the 'Chokwang'/'Clark' RIL mapping population which was evaluated using single-floret inoculation in the greenhouse, the Korean cultivar 'Chokwang' was found to carry significant type II FHB resistance QTLs on chromosomes

4BL and 5DL, plus a QTL with marginal effect on 3BS (Yang et al. 2005a). This cultivar seems to carry QTLs different from those in 'Sumai 3' and its relatives and therefore has high potential in wheat breeding programs as a source of resistance genes (Buerstmayr et al. 2009).

The Brazilian cultivar 'Frontana' was identified as a source of resistance to FHB by Schroeder and Christensen (1963). An extensive mapping study using a DH population derived from a 'Frontana' x 'Remus' cross using single-floret and spray inoculations in the field detected two major effect QTLs on chromosomes 3A and 5A for resistance to disease severity, and less stable QTLs on 1B, 2A, 2B, 4B, 5A, and 6B (Steiner et al. 2004). In this study, the contribution of QTLs towards resistance to fungal penetration (disease severity and incidence) and fungal spread was 25% and ≤10%, respectively, indicating that FHB resistance in 'Frontana' primarily inhibits fungal penetration (Steiner et al. 2004). In a RIL population of 'Frontana' x 'Falat', Mardi et al. (2006) confirmed the 3AL QTL of 'Frontana' and detected three additional QTLs associated with FHB resistance on chromosomes 1BL, 3AL, and 7AS. In summary, 'Frontana' seems to be a source of moderate type I resistance which is possibly partly based on morphological or developmental traits, such as hard glumes and narrow flower opening (Buerstmayr et al. 2009).

Given that spring wheat resistance sources such as 'Chokwang' and 'Frontana' carry FHB resistance QTLs which are different from those found in 'Sumai 3' and other Asian sources, introgression of resistance QTLs from them along with QTLs from 'Sumai 3' and the related sources may lead to pyramiding resistance QTLs and the development of wheat lines with an enhanced level and stability of resistance.

QTLs from winter wheat

Less emphasis has been placed on molecular genetic analysis of winter wheat varieties for FHB resistance compared to the large investments that went into mapping of spring wheat resistance sources (Buerstmayr et al. 2009), a reflection of the importance of spring wheat in the world and/or outbreaks of severe FHB epidemics on spring wheat. As a result, the most FHB resistant lines are found in spring wheat.

A RIL winter wheat population derived from the cross of 'Sincron' (susceptible) and 'F1054W' (moderately resistant) was evaluated in a single-floret-inoculated field experiment in Romania for FHB resistance and was analyzed with several storage protein markers (Ittu et al. 2000). Two storage protein markers (*GliR1* on T1BL.1RS translocation chromosome and *GliD1b* on chromosome 1D) were associated with type II FHB resistance derived from 'Sincron', suggesting the location of two FHB QTLs on these chromosomes. Gervais et al. (2003) analyzed 'Renan' x 'Recital' winter wheat RIL mapping population evaluated under spray-inoculated field conditions and detected three QTLs with larger effects (one QLT on 2B and two QTLs on 5A) and a few QTLs with smaller effects on 2A, 3A, 3B, 5A, 5D, and 6D, all for resistance to disease severity. Association was observed between one of the FHB resistance QTLs on 5A and the B1 gene controlling the presence of awns, and there was overlap of some FHB QTLs with plant height QTLs (2BS, 5A) and/or flowering date QTLs (2BS). Shen et al. (2003a) analyzed type II resistance in RILs of a cross between 'F201R' (resistant) and 'Patterson' (susceptible) in a single-floret-inoculated greenhouse experiment. They found three QTLs derived from 'F201R' on chromosomes 1B, 3A, and 5A, and one QTL derived from 'Patterson' on chromosome 3D. Gilsinger et al. (2005) evaluated 100 RILs from the cross 'Patterson' x 'Goldfield' for FHB incidence, flower opening width, and flower opening

duration in field. They found four markers which had significant association with QTLs on chromosomes 2B and 7B controlling low FHB incidence, and that the QTL with major effect for low FHB incidence was detected in the region of markers *Xbarc200–Xgwm210* on chromosome 2BS. There was a significant association between low FHB incidence QTL on 2B and narrow flower opening in the population (Gilsinger et al. 2005).

The Swiss cultivar 'Arina' has long been known for its moderate FHB resistance (Buerstmayr et al. 1996; Snijders 1990c) and has been used in three independent QTL mapping studies to date: 240 RILs from the cross 'Arina' x 'Forno' (Paillard et al. 2004), 93 DHs from the cross 'Arina' x 'NK93604' (Semagn et al. 2007), and 116 DHs from the cross 'Arina' x 'Riband' (Draeger et al. 2007). In the 'Arina' x 'Forno' cross, assessed in spray-inoculated field experiments, three main effect QTLs for resistance to disease severity were detected on the long arms of chromosomes 6DL, 5BL, and 4AL, of which 5BL QTL originated from the susceptible parent 'Forno'. Five smaller effect QTLs for FHB resistance were also detected on chromosomes 2AL, 3AL, 3BL, 3DS, and 5AL. The QTLs on 2AL, 5AL, 5BL, and 6DL overlapped with plant height and/or heading time, indicating either linkage or pleiotropy between disease severity and morphological/developmental traits (Paillard et al. 2004). In the 'Arina' x 'NK93604' population, evaluated under spray-inoculated field conditions, two QTLs on chromosomes 1BL and 6BS originated from 'Arina' and two QTLs on 1AL and 7AL from 'NK93604' were detected for resistance to disease severity. Two QTLs, both derived from'NK93604' on chromosomes 1AL and 2AS were identified for low DON content (Semagn et al. 2007). Finally, in the 'Arina' x 'Riband' population, evaluated in spray-inoculated field and polytunnel experiments, 10 QTLs were detected for different traits associated with resistance to FHB severity, but only the effect of the QTL on

chromosome 4DS, co-localised with the semi-dwarfing locus *Rht-D1*, was significant and stable (Draeger et al. 2007). The semi-dwarf allele *Rht-D1b* inherited by 'Riband' contributed to significantly increased susceptibility not due to plant height per se, rather to either linkage of FHB susceptibility genes in some intervals and/or a pleiotropic physiological effect of the dwarfing allele at *Rht-D1b* (Draeger et al. 2007). The association of *Rht-D1b* allele with increased susceptibility to FHB was verified in an independent mapping study based on the population derived from 'Rialto' x 'Spark' which was evaluated under spray-inoculated field conditions (Srinivasachary et al. 2008). There is additional evidence showing that presence of *Rht-D1b* significantly impairs FHB resistance (Buerstmayr et al. 2008; Gosman et al. 2007). Further research is needed to clarify whether the association of *Rht- D1b* with susceptibility to FHB is due to linkage or pleiotropy and to determine the relationship of other widely used dwarfing genes like *Rht-B1b* and *Rht8* with FHB resistance (Buerstmayr et al. 2009). Surprisingly, almost no QTLs from the results of the three independent studies using 'Arina' were coincident. A large number of QTLs in the 'Arina' mapping populations were derived from the susceptible parents, indicating that 'Arina'"s" resistance may not be detected in marker assisted selection (MAS) (Buerstmayr et al. 2009).

In a winter wheat RIL mapping population developed from the cross 'Dream' x 'Lynx' and evaluated in a spray-inoculated field experiment, Schmolke et al. (2005) detected four QTLs for resistance to disease severity: three were derived from FHB resistant 'Dream' (2BL, 6AL, 7BS) and the fourth QTL was associated with the T1BL.1RS translocation chromosome present in the susceptible parent 'Lynx'. The QTL on 6AL chromosome were associated to plant height and compactness and the QTL on 7BS with heading date (Schmolke et al. 2005). Häberle et al. (2007) verified the presence

of the two major QTLs mapped on chromosomes 6AL and 7BS in a 'Dream' x 'Lynx' population and their phenotypic effects on resistance to FHB. They found that both QTLs were directly associated with plant height and designated them as *Qfhs.lfl-6AL* and *Qfhs.lfl-7BS*, respectively (Häberle et al. 2007). Klahr et al. (2007) tested a winter wheat RIL population derived from the cross 'Ritmo' (susceptible) x 'Cansas' (moderately resistant) in four spray-inoculated field experiments and detected QTLs associated with FHB severity on seven chromosome segments (1BS, 1DS, 3B, 3DL, 5BL, 7BS, and 7AL), two of which strongly overlapped with plant height and/or heading date QTLs (5BL, 7AL) indicating disease escape effects rather than physiological resistance at these two QTLs. The 1DS QTL primarily appeared to be involved in resistance to fungal penetration, whereas the other QTLs mainly contributed to resistance to fungal spread. However, the QTL on 5BL (*Qfhs.whs-5B*) was later relocalised to chromosome 1BL and renamed as *Qfhs.lfl-1BL* (Häberle et al. 2009). In lines derived from the cross 'Ritmo'/'Cansas' which were evaluated in four spray-inoculated experiments, *Qfhs.lfl-1BL* reduced FHB severity by 42% (Häberle et al. 2009). Liu et al. (2007) used RILs from the cross of the moderately resistant winter wheat 'Ernie' with the susceptible breeding line 'MO94-317' to map QTL for resistance to fungal spread and found stable QTLs on chromosomes 2B, 3B, 4BL, and 5A. None of these QTLs were associated with presence or absence of awns, earliness, or the number of spikelets per spike. Finally, Schmolke et al. (2008) reported two QTLs on chromosomes 1A (resistant allele from the susceptible parent 'Hussar') and 2BL (resistant allele from the resistant parent 'G16-92') for disease severity in the mapping population evaluated in spray-inoculated field tests. While the 1A QTL was associated with plant height, the 2BL QTL was inherited independently of morphological traits.

As mentioned above, several major and minor effect QTLs for resistance against disease incidence, disease severity, and DON accumulation on different chromosomes have been identified in winter wheat. In spite of the fact that fusarium head blight in winter wheat may not be as important as in spring wheat, mapping QTLs for FHB resistance and finding new sources of resistance among winter wheat genotypes may provide additional QTLs available to use both in winter wheat and spring wheat breeding programs wherever FHB is a problem.

QTLs in tetraploid wheat

The need for improving FHB resistance in tetraploid durum wheat is at least as urgent as for hexaploid wheat as durum wheat is almost exclusively used for human consumption and susceptibility to FHB can lead to a high risk to human health (Buerstmayr et al. 2009). Because of the limited variation for FHB resistance available in *T. turgidum* subsp. *durum*, its cultivated or wild relatives such as *T. turgidum* subsp. *dicoccum* and *T. turgidum* subsp. *dicoccoides* may provide alternative sources of resistance to FHB (Buerstmayr et al. 2003b; Oliver et al. 2007).

It has been shown that the 3A chromosome from the wild emmer (*T. turgidum* subsp. *dicoccoides*) accession 'FA-15-3' (syn.: 'Israel A') provides resistance to fusarium head blight (Ban and Watanabe 2001; Stack et al. 2002). Otto et al. (2002) developed a single chromosome RIL population for the 3A chromosome of 'FA-15-3' from the cross of 'Langdon' x 'Langdon' (*T. turgidum* subsp. *dicoccoides*-3A). A QTL for fungal spread, *Qfhs.ndsu-3AS*, was found near *Xgwm2* on 3AS in this population which explained 55% of the genetic variation for type II resistance. Recently, this QTL region was saturated with additional markers including *Xmwg14* and *Xbcd828*. The QTL region

of about 10 cM is flanked by two target region amplification polymorphism (TRAP) markers and peaks near two SSRs (*Xgwm2*, *Xbarc45*), a region not homoeologous to *Fhb1* (Chen et al. 2007a). Based on the fact that this QTL expressed in other genetic backgrounds but not in 'Israel A' or *T. turgidum* subsp. *dicoccoides* possessing both 2A and 3A chromosomes, a gene on chromosome 2A was proposed to suppress the FHB resistance of the 3A QTL (Garvin et al. 2003). To determine regions of chromosome 2A from 'Israel A' associated with the increased FHB susceptibility, Garvin et al. (2009) mapped a recombinant inbred chromosome line population of the cross 'Landon' x 'Langdon' (*T. turgidum* subsp. *dicoccoides*-2A) evaluated in single-floret-inoculated experiments in the greenhouse. QTL mapping identified a region on the long arm of chromosome 2A that was associated with FHB, and the best SSR marker in this region accounted for 21-26% of the variation for FHB resistance, with the 'Israel A' marker alleles associated with increased FHB susceptibility.

Screening of chromosome 7A substitution lines for reaction to FHB in the greenhouse showed that chromosome 7A possesses FHB resistance genes. In a RIL population derived from a cross of 'Langdon' x 'Langdon' (*T. turgidum* subsp. *dicoccoides*-7A), Kumar et al. (2007) mapped a significant QTL for fungal spread on chromosome 7AL,in a chromosomal region where several QTLs in hexaploid wheat also have been found.

In a DH mapping population derived from the cross of the *T. turgidum* subsp. *durum* cultivar 'Strongfield' (susceptible) with the *T. turgidum* subsp. *carthlicum* cultivar 'Blackbird' (resistant) which was evaluated for type II resistance in the greenhouse, Somers et al. (2006) found two significant QTLs on chromosomes 2BL and 6BS derived from 'Strongfield' and 'Blackbird', respectively. Their results showed that the 6BS QTL

in ‘Blackbird’ was coincident with the ‘Sumai 3’ derived gene, *Fhb2*. In another study on a DH population from the cross ‘Strongfield’ x ‘Blackbird’ evaluated under artificially-inoculated field conditions, Singh et al. (2008) detected a QTL on chromosome 1AS (Blackbird) explaining up to 24% of the phenotypic variation for FHB incidence, up to 15% for FHB severity, and up to 15% for the 1-9 disease rating scale.

Because of the importance of durum wheat in food industry and the relative susceptibility of durum genotypes to FHB, developing FHB-resistant durum wheat varieties is challenging and needed in FHB-prone parts of the world. Identification and introgression of resistance QTLs from durum and other tetraploid wheat genotypes to cultivated and commercial durum lines would be a wise approach as they are genetically close and may exhibit less linkage drag problems.

QTLs from wild relatives of wheat

In a single chromosome recombinant population for chromosome 4A developed from the cross ‘Hobbit-sib’ x ‘Hobbit-sib’ (*T. macha*-4A), Steed et al. (2005) detected a QTL for type I resistance, which was co-segregating with *Xgwm165* on the short arm of chromosome 4A derived from *T. macha.* Shen and Ohm (2007) also detected a QTL for type II resistance, located in the distal region of the long arm of 7el2, in a segregating mapping population derived from the cross of two chromosome substitution lines of different origins (7el1 and 7el2) both containing the introgressed *Th. ponticum* chromatin but with different reactions to *F. graminearum*.

Several further alien species such as *E. humidus*, *E. racemifer*, *R. kamoji*, and *L. racemosus* are potential donors of FHB resistance genes but as yet they have not been genetically mapped (Ban 1997; Chen et al. 2005; Oliver et al. 2005). Mapping and

tagging of FHB resistance present in alien species would be of great interest for use in wheat breeding programs.

CHAPTER 2

MOLECULAR MAPPING OF QUANTITATIVE TRAIT LOCI FOR FUSARIUM HEAD BLIGHT RESISTANCE IN A POPULATION OF WHEAT WITH *TRITICUM TIMOPHEEVII* BACKGROUND

Molecular mapping of quantitative trait loci for Fusarium head blight resistance in a population of wheat with *Triticum timopheevii* background

Summary

A population of recombinant inbred lines (RILs) derived from the cross of 'Brio' (a moderately susceptible bread wheat cultivar) and 'TC 67' (a *Triticum timopheevii* derived FHB-resistant line) was used to map quantitative trait loci (QTLs) for FHB resistance using microsatellite molecular markers, and to study the association between FHB resistance traits and some morphological/developmental characteristics under greenhouse and field conditions. Interval mapping (IM) detected a major QTL on chromosome 5AL that explained 14.4% of the phenotypic variation for disease severity (type II resistance) in the greenhouse and 19.2-23.0% for *Fusarium*-damaged kernels (FDK) under field conditions. Inconsistent QTL(s) on chromosome 5BS were also detected for disease severity and index (field) using single marker analysis (SMA). The association of plant height and number of days to anthesis with disease incidence, severity, index, and deoxynivalenol (DON) accumulation was negative and statistically significant, but values were low. However, number of days to anthesis was positively correlated with FDK (field) and disease severity (greenhouse). Awnedness had a negative effect on FHB, namely the presence of awns resulted in less disease in the population. Spike threshability also affected FHB so that the hard threshable genotypes represented lower disease. The 'Brio'/'TC 67' population, especially the lines carrying the major QTL detected in this study along with the linked SSR loci, provide an opportunity for breeding FHB-resistant wheat varieties.

Introduction

Fusarium head blight (FHB), caused mainly by *Fusarium graminearum* Schwabe [teleomorph: *Gibberella zeae* (Schwein.) Petch.], is one of the most important diseases of wheat, in areas where the weather is warm and humid after wheat has headed. It attacks during anthesis causing severe yield reduction and decreased grain quality (Bai and Shaner 1994). In addition, infected grain may contain mycotoxins such as deoxynivalenol (DON) and zearalenone (ZEA) which are harmful to animal and human health (Bai and Shaner 1994; Desjardins et al. 1996; Ehling et al. 1997; Marasas et al. 1984; McMullen et al. 1997; Miller et al. 1991; Parry et al. 1995; Snijders 1990b; Sutton 1982; Tanaka et al. 1988; Tuite et al. 1990; Yoshizawa and Jin 1995). Grain may be downgraded or rejected in commerce because of the presence of *Fusarium*-damaged kernels (FDK) and/or contamination with mycotoxins (McMullen et al. 1997; Tuite et al. 1990).

Chemical and agronomic measures for disease control are either not available or not feasible. Development of resistant cultivars is the most practical and economic approach for environmentally safe and sustainable long-term control (Yang et al. 2005b). However, breeding wheat for resistance to FHB is difficult because of the polygenic control of resistance, our limited knowledge of gene interactions, genotype x environment interactions, and the high cost of phenotyping (Bai and Shaner 1994; del Blanco et al. 2003; McMullen et al. 1997; Somers et al. 2003; Yang et al. 2005b; Yang 1994). No complete resistance or immunity to FHB has been reported, but genotypic variation is large and well-documented in wheat and its relatives. Among well-known sources of resistance to FHB are ‘Sumai 3’ and its derivatives from China, ‘Nobeokabouzu-komugi’, ‘Shinchunaga’, ‘Nyu Bai’ and their relatives from Japan, and ‘Frontana’ and

'Encruzilhada' from Brazil (Bai et al. 1989b; Ban 2000; Ban and Suenaga 2000; Liu and Wang 1990; Mesterházy 1987; Schroeder and Christensen 1963; Wang et al. 1989; Yu et al. 2006). FHB-resistant relatives of common wheat (*Triticum aestivum* L.) and durum wheat [*Triticum turgidum* L. subsp. *durum* (Desf.)] such as *Triticum timopheevii* (Zhuk.) Zhuk., *Triticum monococcum* L., *Triticum dicoccum* Schrank, and *Triticum dicoccoides* (Körn ex Asch. *et* Graebn.) Schweinf. are genetically more closely related to cultivated wheat sharing genomes and having high crossability. In some cases alien chromatin carrying FHB resistance genes has been transferred to cultivated wheat (Cao et al. 2009; Chen and Liu 2000; Fedak et al. 2003; Han and Fedak 2003; Liu et al. 2000). However, the resistance found in alien species is usually associated with undesirable characteristics which are difficult to remove from the genome (Bai and Shaner 2004).

Five types of resistance to FHB have been proposed: (I) resistance to initial infection, (II) resistance to spread of infection (Schroeder and Christensen 1963), (III) resistance to toxin accumulation, (IV) resistance to kernel infection, and (V) tolerance (Mesterházy 1995; Miller et al. 1985; Wang and Miller 1988). It has also been recognized that resistance to FHB in wheat involves active and passive mechanisms (Mesterházy 1995). Various morphological and agronomic traits such as heading date, plant height, head compactness, and flower opening have been shown to be associated with resistance to FHB in wheat. These traits which are passive resistance mechanisms (Mesterházy 1995), can result in apparent resistance by increasing the probability that the host escapes infection rather than by reducing disease as a result of host defence response (Kolb et al. 2001).

Type I resistance is more difficult to evaluate and therefore fewer reports have been published on genetic factors controlling type I resistance (Buerstmayr et al. 2009).

As an indicator of type I resistance, disease incidence (percentage of spikes with disease symptoms) in spray or naturally inoculated plots or pots is measured (Buerstmayr et al. 2009). Type II resistance which is most often evaluated by point inoculation under controlled conditions in the greenhouse, has been extensively studied in wheat as it appears to be more stable and less affected by non genetic factors (Bai and Shaner 1994). Injecting a conidial suspension of the pathogen into a floret of a flowering spike and measuring disease severity/spread (percentage of diseased spikelets per spike) is commonly used for evaluation of type II resistance (Bai et al. 1999; Waldron et al. 1999). Disease severity has also been used as a measure of total FHB resistance in spray-inoculated experiments (Buerstmayr et al. 2009).

Results of classical and cytogenetic studies show that resistance to FHB in wheat is quantitatively inherited and that the underlying quantitative trait loci (QTLs) are distributed over the entire genome. Molecular markers provide an approach to study quantitative traits such as FHB resistance in wheat and to trace genes that confer head blight resistance. Different molecular markers such as restriction fragment length polymorphisms (RFLPs), random amplified polymorphic DNAs (RAPDs), amplified fragment length polymorphisms (AFLPs), and microsatellites have been used to map FHB resistance QTLs. The basic principle in QTL mapping is to detect correlations between genotypes and phenotypes in a population on the basis of linkage disequilibrium (Breseghello and Sorrells 2006; Gupta et al. 2005; Rostoks et al. 2006). Once linkage is established between a marker and a QTL, this QTL can be introduced into germplasm using marker-assisted selection (del Blanco et al. 2003).

'Sumai 3' and its derivatives have been used widely for the development of mapping populations and QTL analysis studies. In addition to several minor QTLs on

chromosomes 1B, 2AS, 2B, 2DS, 3AL, 5A, 6AS, 7A, and 7BL, a major QTL for type II resistance was detected on chromosome 3BS (*Qfhs.ndsu-3BS*) which explained up to 60% of the phenotypic variation following single-floret inoculation (Anderson et al. 2001; Bai et al. 1999; Buerstmayr et al. 2002; Shen et al. 2003b; Waldron et al. 1999; Yang et al. 2005b; Zhang and Mergoum 2007; Zhou et al. 2002) and about 30% of the phenotypic variation after spray-inoculation in the field (Buerstmayr et al. 2003a, 2003 #55). Wheat lines carrying *Qfhs.ndsu-3BS* QTL have shown resistance against DON accumulation (Lemmens et al. 2005), disease incidence, or kernel infection (Yang et al. 2005b). Other QTLs for resistance to disease incidence or kernel infection originating from 'Sumai 3' and its derivatives have also been reported on chromosomes 1DL, 2DS, 3BC, 4DL, and 5AS (Yang et al. 2005b). Because of its high breeding potential, the chromosomal segment covering *Qfhs.ndsu-3BS* was further fine mapped using different markers (Cuthbert et al. 2006; Guo et al. 2003; Liu and Anderson 2003a; Liu and Anderson 2003b; Liu et al. 2006). This QTL was recently re-named *Fhb1* (Liu et al. 2006). Another major QTL from 'Sumai 3' or related lines was reported on 6BS for type II resistance (Anderson et al. 2001; Lin et al. 2004; Shen et al. 2003b; Waldron et al. 1999; Yang et al. 2005b) which later was characterized as *Fhb2*. (Cuthbert et al. 2007). This QTL also reduces disease incidence or FDK in wheat genotypes (Cuthbert et al. 2007; Yang et al. 2005b).

'Wangshuibai' which is another FHB-resistant Chinese wheat landrace with no evident association with 'Sumai 3' in its pedigree, has received considerable attention as an alternative source of resistance. In different mapping studies for type II resistance in 'Wangshuibai', the largest effect was found on 3BS with up to 37% phenotypic variation (Jia et al. 2005; Lin et al. 2004; Lin et al. 2006; Ma et al. 2006b; Mardi et al. 2005; Yu et

al. 2008; Zhang et al. 2004; Zhou et al. 2004). Wheat lines carrying this QTL have shown correlations with reduced DON accumulation or disease incidence (Ma et al. 2006b; Yu et al. 2008). Several other QTLs in 'Wangshiubai' or its derivatives have also been detected on chromosomes 1B, 2A, 2D, 3B, 3DL, 4B, 5B, 6B, and 7A for type II resistance (Jia et al. 2005; Lin et al. 2004; Lin et al. 2006; Ma et al. 2006b; Mardi et al. 2005; Najaphy et al. 2006; Yu et al. 2008; Zhou et al. 2004) or on 2A, 2D, 3AS, 4B, 5A, 5B, and 5DL for resistance to disease incidence or DON content (Lin et al. 2006; Ma et al. 2006b; Yu et al. 2008). Li et al. (2008) identified five QTLs associated with FDK in a population of 'Nanda 2419' x 'Wangshuibai', four of which originated from 'Wangshuibai'. Although 'Wangshuibai' seems to be genetically unrelated to 'Sumai 3', it possesses QTLs with the same sequence of *Fhb1* as 'Sumai 3' (Buerstmayr et al. 2009).

The Brazilian cultivar 'Frontana' carries two QTLs with major effects on chromosomes 3A and 5A for disease resistance and less stable QTLs on 2B, 4B, 5A, and 6B (Steiner et al. 2004). In another study, Mardi et al. (2006) confirmed the 3AL QTL of 'Frontana' and detected two additional QTLs associated with FHB resistance on chromosomes 3AL and 7AS. 'Frontana' seems to be a source of moderate type I resistance which is possibly partly based on morphological or developmental traits, such as hard glumes and narrow flower opening (Buerstmayr et al. 2009).

Although QTLs from different sources of resistance such as 'Sumai 3', 'Wangshuibai', 'Frontana', winter wheat, durum wheat, and wild relatives of wheat have been mapped and in some cases successfully used in wheat breeding programs, finding new sources of resistance is needed to avoid complete dependence on limited sources. Introgression of additional resistance genes and pyramiding FHB resistance QTLs in wheat lines may lead to development of wheat lines/cultivars with an enhanced level and

stability of resistance to prevent economic damage under high disease pressure. *Triticum timopheevii* is a source of FHB resistance which has been used to introgress resistance into wheat (Fedak et al. 2004). Mapping and tagging of FHB resistance available in wheat cultivars with an alien background such as *T. timopheevii* may be of great interest for use in wheat breeding programs.

Association of morphological and developmental traits with FHB resistance also is of great importance in breeding wheat for disease resistance and applying strategies for disease control. In general, short-statured, awned genotypes with a short peduncle and a compact spike are more susceptible to FHB than tall lines that are awnless and have a long peduncle and a lax head (Hilton et al. 1999; Mesterházy 1995; Parry et al. 1995; Rudd et al. 2001), even though there are exceptions to these general rules.

The reports from at least one decade ago show that there is a relationship between plant height and resistance to FHB in wheat (Hilton et al. 1999; Mesterházy 1995). Buerstmayr et al. (2000) found negative correlations between plant height and FHB symptoms in two different populations of wheat. In a DH wheat population derived from 'Wuhan-1'/'Maringa' which later was corrected to'Wuhan-1'/'Nyu Bai' (McCartney et al. 2007), Somers et al. (2003) showed that taller and later plants had less FHB infection under field conditions. They detected a QTL on chromosome 2DS for low DON accumulation which coincided with a major gene for plant height.

The negative correlation between FHB resistance and plant height or flowering date and the co-localization of FHB resistance QTLs and the QTLs for plant height and/or flowering date have been reported in populations from the crosses of 'Renan'/'Recital' (Gervais et al. 2003) and 'Arina'/'Forno' (Paillard et al. 2004). Steiner et al. (2004) found significant negative correlations between plant height and either disease incidence or

disease severity but the correlation between date of anthesis and resistance traits was positive. The 4DS QTL from 'Arina' co-localised with the semi-dwarfing locus *Rht-D1* (Draeger et al. 2007). The association of the *Rht-D1b* allele with increased susceptibility to FHB later was verified in a mapping population derived from 'Rialto' x 'Spark' (Srinivasachary et al. 2008). In a population of 'Dream'/'Lynx', two QTLs for plant height, four QTLs for heading date, and three QTLs for ear compactness were identified of which the 6AL QTL for height overlapped with QTLs for FHB resistance and ear compactness and the 7BS heading date QTL overlapped with an FHB resistance QTL (Schmolke et al. 2005). FHB resistance was significantly correlated with plant height and heading date in 'Cansas'/'Ritmo' population and overlapping QTLs for all three traits were observed (Klahr et al. 2007). Co-localizations have also been found between a QTL for disease severity resistance and a QTL for plant height in the resistant cultivar 'G16-92' (Schmolke et al. 2008) and between an FHB resistance QTL and a QTL for plant height and heading date in the mapping population derived from 'Pelikan'/'G93010' (Häberle et al. 2009).

The linkage between FHB resistance and awnedness was first reported by Snijders (1990a) in winter wheat infected with *Fusarium culmorum* (W. G. Smith) Sacc. Recently, Ban and Suenaga (2000) demonstrated that one of the resistance genes in the FHB resistant Chinese wheat cultivar 'Sumai 3' may be linked in repulsion to the dominant suppressor *B1* gene for awnedness. Gervais et al. (2003) also showed that the FHB resistance QTL located on the long arm of chromosome 5A was linked to the gene *B1* in a population of 'Renan'/'Recital'. Mesterházy (1995) stated that the presence of awns in wheat enhances the development of FHB.

Compactness of wheat spikes is another characteristic which is considered to have association with FHB. Steiner et al. (2004) observed a significant negative but low correlation between FHB and wheat spike compactness in a population derived from the cross 'Frontana'/'Remus'. They also found QTLs for spike compactness on chromosomes 1A and 7A and in a non-determined location. In contrast, Mesterházy et al. (1995) reported that plants with a dense head tend to be more susceptible to spike diseases because of micro-climatic conditions. In a population of 'G16-92'/'Hussar', a QTL for ear compactness was detected on chromosome 5A (Schmolke et al. 2008).

It also seems that wheat plants with a narrow flower opening and/or a short duration of flower opening will have a lower incidence of FHB by reducing the area and time in which *Fusarium* spores can enter the floret and initiate infection (Gilsinger et al. 2005). A major QTL associated with narrow flower opening and low FHB incidence was found on chromosome 2B in a population of 'Patterson'/'Goldfield' which explained 29% of the phenotypic variation for FHB incidence (Gilsinger et al. 2005).

Agronomic traits may play a role of markers in wheat breeding especially in preliminary screening and may be used as positive/negative markers to select FHB resistant genotypes in wheat breeding programs. Molecular markers associated with agronomic traits can also be identified and used for marker assisted selection (MAS) to break undesired associations between FHB resistance and other agronomic traits (Zhang et al. 2004).

The objective of the present study was to map FHB resistance QTLs in a population derived from the cross of 'Brio' (a moderately susceptible bread wheat cultivar) and 'TC 67' (a *T. timopheevii* derived FHB-resistant line) using microsatellite molecular markers, and to study the association between FHB resistance traits and some

morphological and developmental traits such as plant height, number of days to anthesis, and spike threshability.

Materials and methods

Plant materials

As shown in Figure 2.1, the origin of the mapping population goes back to a cross between the susceptible spring wheat cultivar 'Crocus' (*T. aestivum*, $2n = 6x = 42$, AABBDD) and a resistant accession of *T. timopheevii* ($2n = 4x = 28$, A^tA^tGG, PI 343447), and a backcross to 'Crocus' (Cao et al. 2009). 'Crocus' which is near-isogenic to the cultivar 'Columbus', has three crossability genes *Kr1*, *Kr2*, and *Kr3* derived from 'Chinese Spring' (Zale and Scoles 1999).

'Crocus' (PI 606243) was crossed to *T. timopheevii* (PI 343447) as the male parent in the greenhouse, and the F_1 plants were backcrossed with 'Crocus' (Figure 2.1). A population of 1500 F_2 plants was established and 535 BC_1 F_7 lines (*T. aestivum*, $2n = 6x = 42$, AABBDD) were developed in the greenhouse using single seed descent (SSD). One hundred lines were selected on the basis of plant fertility and agronomic traits and were evaluated for reaction to FHB in the greenhouse and field FHB nursery. The line 'TC 67' was selected from this population, on the basis of its superior FHB reaction and reasonable agronomic traits (Cao et al. 2009).

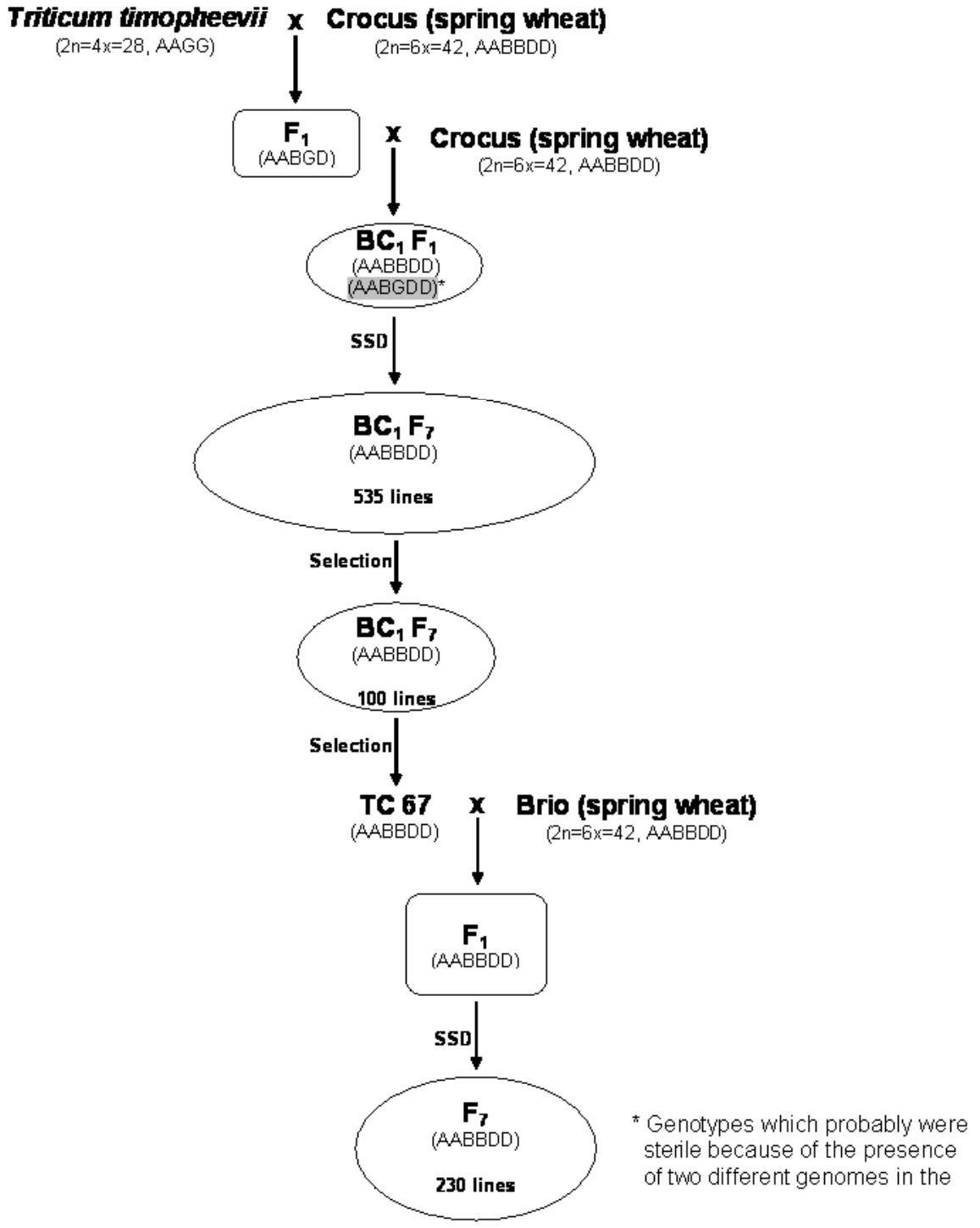

Figure 2.1. Development of the mapping population 'Brio'/'TC 67' using single seed descent used in the present study.

Later, the moderately susceptible wheat cultivar 'Brio' (*T. aestivum*, $2n = 6x = 42$, AABBDD) with the pedigree of Columbus/S68147//Laval19/Columbus was crossed to 'TC 67'. An F_7 mapping population consisting of 230 recombinant inbred lines (RILs) developed using SSD from the cross of 'Brio' and 'TC 67' was used in this study (Figure 2.1).

Greenhouse evaluation

The genotypes were evaluated for resistance to fungal spread within the spike (type II resistance) following single-floret inoculation in the greenhouse of the Cereal Research Centre, Winnipeg, Manitoba in 2007. The experimental layout was a randomized complete block design with three replicates and the 16 x 13 x 13 cm^3 pots were used as experimental plots. The greenhouse was maintained under conditions of 16 h light (25 C) and 8 h dark (20 C) supplemented with incandescent high pressure sodium lights (OSRAM SYLVANIA LTD, Mississauga, ON, Canada). Wheat plants were treated with a combination of propiconazole and spinosad one month after the seeding date to control powdery mildew and thrips.

A mixture of four highly aggressive isolates of *F. graminearum* (J. Gilbert, Pers. Comm.) including M6-04-4, M9-04-6, M1-04-1, and M8-04-3 stored at Cereal Research Centre (CRC), Winnipeg, Manitoba, was used for inoculum production and the greenhouse inoculations. The method used by Afshari-Azad (Afshari-Azad 1992) was modified as follows and used for inoculum production: 2.5 g of blended straw from wheat and barley was added to 125 ml tap water in a 250 ml flask, and autoclaved two times with a 24 h interval. A small plug of PDA containing the fungal isolate was added to the culture, and the culture was shaken for 96 h at 120 rpm at 25-30 C. The culture was

passed through a cheese cloth and the suspension diluted to 5 x 10^4 macroconidia/ml for inoculations. As spikes reached 50% anthesis, they were inoculated by injecting a 10-µl droplet of conidial suspension (5 x 10^4 macroconidia/ml) into the floret in a spikelet positioned 1/3 from the top of the spike using a micropipette (Figure 2.2). At least five spikes in each pot (replication) were inoculated and the spikes were covered with 20 x 5 cm^2 glassine bags (Seedburo Equipment Co., Chicago, IL, USA) for 48 h to maintain constant high humidity. Disease severity in the inoculated spikes was measured as the percentage of diseased spikelets per spike 21 days after inoculation.

Figure 2.2. Single-floret inoculation of wheat genotypes in the greenhouse.

Field evaluation

The mapping population and the parental lines were evaluated for resistance to initial infection (type I resistance), disease severity (a combination of type I and type II resistances), disease index (type I and II resistances), DON accumulation (type III resistance), and FDK (type IV resistance) in spray-inoculated field trials in two locations (Carman and Glenlea) in Manitoba, Canada in 2006 and 2007. The experimental design in both locations in 2006 was a randomized complete block design and in 2007 a 16 x 15 lattice design, each with three replicates. Plots consisted of 1 m (Carman) or 1.5 m (Glenlea) length rows with 30 cm row spacing. Sowing density was ≈ 5 g of seed per plot. Sowing date was May 29-30 and June 5 in Carman and Glenlea, respectively in 2006 and May 9 in both locations in 2007. Appropriate measures for fertilizing the nurseries and control of weed and insects were applied.

A mixture of the following isolates was used for inoculations in Carman in the first year: 40/04, 71/04, 98/04, 136/04, MSDS 3/03, and EMMB 19/03. The same isolates were used in Glenlea with the exception that instead of the last two isolates the isolates M1-04-5 and M3-04-3 (originally received from Canadian Grain Commission) were used. The isolates M1-04-1, M6-04-4, M8-04-3, and M9-04-6 were used in both locations in the second year. Actively growing cultures of *F. graminearum* on potato dextrose agar (Difco™, Sparks, Maryland, USA) were blended, added to liquid carboxymethyl cellulose (CMC) sodium culture media (Sigma®, St. Louis, MO, USA), and incubated under aeration for 5–7 days at room temperature. Concentrations of inoculum were determined using a haemocytometer and adjusted to 5 x 10^4 macroconidia/ml.

Plots were spray-inoculated individually when 50% of the plants had reached anthesis using a CO_2-powered backpack sprayer (Figure 2.3), and repeated 2 or 3 days

later. Nurseries were mist-irrigated (Carman) or sprinkler-irrigated (Glenlea) for 1 h after inoculation but in Carman the mist system operated for 12 more hours on the basis of 5 min per hour. Three weeks after inoculation, the genotypes were scored for disease incidence and severity. Disease incidence was determined as the percentage of diseased spikes in plots and disease severity according to a 0-100% scale for the visually infected spikelets on a whole-plot basis. The FHB index was calculated as the product of disease incidence x disease severity divided by 100. Rows were sickle harvested at maturity and were threshed using a Wintersteiger Nursery Master Elite combine (Wintersteiger AG, Ried, Austria). The threshing mechanism was set at a normal setting on the combine; however the wind speed was decreased and sieves were opened to ensure that FDK were retained in the harvested samples. A wheat head thresher (Precision Machine Co. Inc, Lincoln, NE, USA) later was used to thresh wheat genotypes which were not well threshed using Wintersteiger combine. *Fusarium*-damaged kernels were assessed by counting the visually damaged kernels in three random sub-samples of 100 grains from each plot. DON accumulation was measured using an ELISA method described by Sinha and Savard (1995).

Agronomic traits

Measurements were taken for plant height and presence/absence of awns for each line in the greenhouse, for spike threshability in the field, and for number of days to anthesis in both environments. Field data were collected from two locations (Carman and Glenlea) in 2006. Plant height was measured as the distance from the soil surface to the top of the head without awns. Number of days to anthesis was measured as the number of days from seeding date to 50% anthesis in the field and as the average number of days

from seeding to anthesis in the first five spikes reaching anthesis. Spike threshability was scored using a 1-3 scale (Wise et al. 2001): 1 = free threshing so that naked seeds dropped free of the glumes when spikes were crushed manually, 2 = not free threshing but glumes could be torn off with forceps to free a seed, and 3 = not free threshing and glumes could only be removed by scraping).

Figure 2.3. Spray inoculation of the *Fusarium* nurseries using backpack sprayer.

Statistical analysis of phenotypic data

All statistical analyses were performed using SAS® 9.2 (SAS Institute Inc., Raleigh, NC, USA). The Spearman correlation coefficients were calculated for every trait on the least squares means of the RILs using the PROC CORR. Before conducting the

analysis of variance (ANOVA), all greenhouse and field data were tested for normality using the PROC UNIVARIATE. As the residuals of the dependent variables did not follow a normal distribution, an arcsine transformation was applied to the data. The correlation between variances and means were plotted for transformed data using variance-by-mean plots to check the independence of means and variances. Analyses of variances were performed on transformed data of each trait using the PROC MIXED based on a randomized complete block design. Genotype effect was considered fixed in the statistical model while location, year, and block effects were considered random. Regression analysis between resistance traits and agronomic characteristics or between the markers and QTLs were estimated using the PROC REG procedure. Broad-sense heritabilities for RILs were estimated from ANOVA (Hallauer and Miranda 1981) using the formulae $h^2 = \sigma_G^2 \big/ [\sigma_G^2 + (\sigma_e^2 / r)]$ for single location-year or greenhouse data and $h^2 = \sigma_G^2 \big/ [\sigma_G^2 + (\sigma_{GL}^2 / l) + (\sigma_{GY}^2 / y) + (\sigma_{GYL}^2 / yl) + (\sigma_e^2 / ryl)]$ for combined data of two locations in two years, where σ_G^2 is the genotypic variance, σ_{GL}^2 is genotype x location variance, σ_{GY}^2 is genotype x year variance, σ_{GYL}^2 is genotype x location x year variance, σ_e^2 is residual variance, *r* is the number of replications (blocks), *l* is number of locations, and *y* is the number of years.

DNA preparation, PCR amplification, and genotypic data collection

The leaf tissue for DNA extraction was harvested two weeks after seeding the wheat genotypes in the growth cabinet and lyophilized for 48 h. DNA was extracted from 230 RILs, the parents of the population ('Brio' and 'TC 67'), and the parents of 'TC 67'

('Crocus' and the *T. timopheevii* line PI 343447) using the modified procedure developed by Warner et al. (2002) and quantified by fluorometry using Hoechst 33258 stain.

For PCR amplification, the forward primer had a 19-bp fluorescent labelled M13 primer (5´-CACGACGTTGTAAAACGAC) at the 5´ end. A universal fluorescent labelled M13 primer homologous to the same sequence added to each forward primer was also added to the PCR reaction (Schuelke 2000; Somers et al. 2004). The PCR reaction was performed in a 10 µl volume, containing 5 µl template DNA at 10 ng/µl, 1.5 mM $MgCl_2$, 0.8 mM of each dNTP (Invitrogen™, Carlsbad, CA, USA), 0.02 pmol/µl forward primer, 0.2 pmol/µl reverse primer (Invitrogen™), 1.8 pmol/µl M13 primer fluorescently labelled with FAM, HEX, or NED (Applied Biosystems, Foster City, CA, USA), 1x PCR buffer, and 1 unit/µl *Taq* DNA polymerase. The PCR products were amplified in a PTC-200 thermal cycler (MJ Research, Waltham, MA, USA) with the following cycling program: 1) 94 C for 2 min (initial denaturing step), 2) 31 cycles of 95 C for 1 min (for DNA denaturation), 0.5 C/s to 51/61 C, 51/61 C for 30 s (for primer annealing), 0.5 C/s to 73 C, and 73 C for 1 min (for primer extension), 3) 73 C for 5 min (for final extension), and 4) 4 C to hold the program.

SSR amplification products were multiplexed by combining 0.5 µl of FAM-, 0.6 µl of HEX-, and 0.5 µl of NED-labelled PCR products with 5.0 µl of a 4% mixture of GeneScan™ 500-ROX (Applied Biosystems) in Hi-Di formamide (Applied Biosystems). The multiplex was denatured for 10 min at 95 C and quickly chilled on ice for 5 min. The denatured sample was loaded on an ABI PRISM® 3130xl Genetic Analyzer (Applied Biosystems) and fragment analysis was performed with GeneScan® 3.7.1 (Applied Biosystems). Data collected by fluorescent capillary electrophoresis was converted to a gel-like image using Genographer 2.0 (http://hordeum.msu.montana.edu/genographer/).

The images were formatted using Canvas™ 11 and the final images were printed and scored manually.

SSR markers and bulked segregant analysis

A total of 851 SSR primer combinations stored at Cereal Research Centre, Winnipeg, MB, Canada, including *Xwmc*, *Xgwm*, *Xbarc*, *Xcfd*, *Xcfa*, and *Xgdm* covering all 21 wheat chromosomes were used (Table 2.1). All primers first were screened for polymorphism on the two parents and two bulk DNA samples consisting of either resistant or susceptible genotypes.

Based on the least squares means of the genotypes for disease severity under both greenhouse and field conditions two bulks of DNA samples were formed from either nine resistant or nine susceptible RILs by pooling equal amounts of diluted DNA for SSR analysis (10 ng/µl) from each of the selected lines. SSR markers which were polymorphic among the parental and bulk DNA samples were screened on the individual DNA samples of the bulks. The markers for which the fragments of the individual DNA samples were similar to the fragments of the bulks (similarity ≥ 90%), were used to genotype the entire mapping population.

Table 2.1. Type, number, and source of the primers used in the study.

Primer type	Number	Source
Xwmc	353	Wheat Microsatellite Consortium (Gupta et al. 2002)
Xgwm	230	IPK, Gatersleben, Germany (Pestsova et al. 2000; Röder et al. 1998)
Xbarc	123	USDA-ARS, Beltsville, MD, USA (Song et al. 2002; Song et al. 2005)
Xcfd	97	INRA, Paris, France (Guyomarc'h et al. 2002; Sourdille et al. 2003)
Xcfa	28	INRA, Paris, France (Guyomarc'h et al. 2002; Sourdille et al. 2003)
Xgdm	20	IPK, Gatersleben, Germany (Pestsova et al. 2000; Röder et al. 1998)

Construction of the linkage map and QTL mapping

Three polymorphic SSR markers from chromosome 5A were used to construct a genetic linkage map. MAPMAKER/EXP 3.0b (Lander et al. 1987) was used to estimate the distance between the markers. A Kosambi map function (Kosambi 1944) was applied to calculate the distance between the ordered markers. Linkage group(s) were established using a minimum logarithm of odds (LOD) threshold of 3.0.

Least squares means of arcsine transformed data of the traits were used for QTL analysis. Interval mapping (IM) was conducted with QTL Cartographer v. 1.17e (Basten et al. 1997) to detect the association of SSR markers and QTLs on the A genome. A QTL was declared significant if it achieved a LOD score > 3.0. To detect the association of the markers and QTLs on B genome, single marker analysis (SMA) option of QTL Cartographer was used to determine whether the markers were linked to a QTL and then a regression analysis was applied using PROC REG procedure of SAS to estimate the coefficients of determinations (R^2) for the linked markers and QTLs.

Results

FHB resistance

Thirty two data sets consisting of disease incidence, severity, index, FDK, and DON accumulation collected from the greenhouse or field were used for single-environment or combined data analysis. Analyses of variance of data showed significant differences ($P < 0.05$) among the RILs for almost all resistance traits. The exceptions were disease severity and index combined data of two locations in two years (results not shown). Analyses of variance for disease severity data (greenhouse), FDK simple data of

Glenlea-2006, Glenlea-2007, and Carman-2007, and FDK combined data of Carman+Glenlea-2006+2007 for which SSR markers linked to QTLs were detected (refer to QTL mapping section), are shown in Tables 2.2 and 2.3, respectively.

Significant differences were observed among the genotypes in the RIL population using analyses of variance of disease severity (greenhouse) and FDK single location-year data (Tables 2.2, 2.3a, b, and c). For the combined data of FDK from two locations in two years, the effects of genotype, genotype x location, genotype x location x year, and block were significant (Table 2.3d).

A high range of variation was observed in disease severity (greenhouse), incidence, severity, index, FDK, and DON accumulation (field) among the RILs and the frequency of distribution of all traits studied in the population was continuous, indicating polygenic and quantitative inheritance of resistance to FHB. Means, ranges, and heritabilities of disease severity data (greenhouse) and of FDK using single location-year and combined data over locations and years for the RIL population are presented in Table 2.4 and frequency distributions of these traits are shown in Figure 2.4.

Table 2.2. Analysis of variance of fusarium head blight disease severity data (type II resistance) collected on 230 recombinant inbred lines from the cross 'Brio'/'TC 67' under greenhouse conditions[a].

Sources of Variation	df	SS	MS	F Value	Pr > F
Genotype	229	186.4929	0.8144	7.60	< 0.0001
Block	2	0.7563	0.3781	1.98	0.1796
Spike (Block)	12	2.3052	0.1921	1.79	0.0437
Residual	3031	324.6026	0.1071	-	-

[a] Arcsine transformed data were used for data analysis.

Table 2.3. Analysis of variance of *Fusarium*-damaged kernels single location-year and combined data of two locations in two years collected on 230 recombinant inbred lines from the cross 'Brio'/'TC 67'[a].

a) Glenlea-2006

Sources of Variation	df	SS	MS	F Value	Pr > F
Genotype	201	3.5309	0.0176	4.09	< 0.0001
Block	2	0.2553	0.1276	29.72	< 0.0001
Residual	346	1.4862	0.0043	-	-

b) Carman-2007

Sources of Variation	df	SS	MS	F Value	Pr > F
Genotype	222	6.7015	0.0302	2.45	< 0.0001
Block	2	0.5692	0.2846	23.14	< 0.0001
Residual	428	5.2647	0.0123	-	-

c) Glenlea-2007

Sources of Variation	df	SS	MS	F Value	Pr > F
Genotype	208	2.6289	0.0126	3.12	< 0.0001
Block	2	0.1548	0.0774	19.11	< 0.0001
Residual	351	1.4220	0.0041	-	-

d) Carman+Glenlea-2006+2007

Sources of Variation	df	SS	MS	F Value	Pr > F
Genotype	225	9.3715	0.0417	2.34	< 0.0001
Location	1	8.6281	8.6281	11.49	0.1789
Year	1	1.7309	1.7309	2.37	0.3690
Location x Year	1	0.7320	0.7320	5.01	0.0523
Genotype x Location	214	4.9458	0.0231	1.54	0.0011
Genotype x Year	202	2.0198	0.0100	0.66	0.9978
Genotype x Location x Year	188	2.8397	0.0151	1.86	< 0.0001
Block (Location x Year)	8	1.2262	0.1533	18.90	< 0.0001
Residual	1471	11.9288	0.0081	-	-

[a] Arcsine transformed data were used for data analysis.

Means of disease severity in the greenhouse ranged from 4.51% to 98.70% with the mean of 35.08% for the population and values of 30.92% and 4.98% for 'Brio' and

'TC 67', respectively. Among the FDK field data set, Carman-2007 had the highest FDK with an overall mean of 14.19% for the population and the highest variation of FDK in the population with a range of 1.49-42-47%. Mean values of FDK for 'Brio' and 'TC 67' in Carman-2007 were 15.58% and 5.55%, respectively. Glenlea-2006 had the lowest population mean of 3.76% with a range of means of 0.30-25.36% among the genotypes and mean values of 5.81% for 'Brio' and 1.45% for 'TC 67'. FDK means for the genotypes in Glenlea-2007 ranged from 0.24 to 17.35% with the means of 4.37% for the population, 4.98% for 'Brio', and 0.56% for 'TC 67'. For the FDK combined data of two locations in two years, means of genotypes varied in a range of 1.65-22.33% with the population mean of 7.41% and mean values of 6.34% and 2.36% for 'Brio' and 'TC 67', respectively. The majority of the RILs exceeded the disease level of 'Brio' in which the disease value was close to the mean of the population (Figure 2.4). Transgressive segregants were found within the population for all traits in the experiments (Table 2.4 and Figure 2.4).

Heritability which measures the proportion of the phenotypic variance that is due to genetic effects, varied from 0.67 to 0.96 for the traits under greenhouse and field conditions (Table 2.4).

Table 2.4. Means and ranges of fusarium head blight disease severity data (type II resistance) under greenhouse conditions and *Fusarium*-damaged kernels using single location-year and combined data of two locations in two years among 230 recombinant inbred lines from the cross 'Brio'/'TC 67'[a].

Trait	Parents means[b]		Population mean[b]	Range of RILs means[b]	Heritability[c]
	Brio	TC 67			
Disease severity (greenhouse)	30.92	4.98	35.08	4.51-98.70	0.96
FDK (Glenlea-2006)	5.81	1.45	3.76	0.30-25.36	0.92
FDK (Carman-2007)	15.58	5.55	14.19	1.49-42.47	0.88
FDK (Glenlea-2007)	4.98	0.56	4.37	0.24-17.35	0.90
FDK (Carman+Glenlea-2006+2007)	6.34	2.36	7.41	1.65-22.33	0.67

[a] Disease severity and FDK are presented here using a 0-100% score.

[b] Arcsine back-transformed.

[c] Estimated using variances represented in Tables 2.2 and 2.3.

Correlations among FHB resistance traits

The correlations among FHB resistance traits using field (Carman and Glenlea in 2006) and greenhouse data are shown in Table 2.5. High positive correlations were observed among disease incidence, severity, and index under field conditions (0.67-0.91) while they had a range of correlations, none to intermediate, with FDK, DON accumulation (field), and disease severity (greenhouse). The correlations among FDK, DON accumulation (field) and disease severity (greenhouse) were also weak (0.26-0.37). Similar results were observed for the association among disease traits using single location-year data (data not shown).

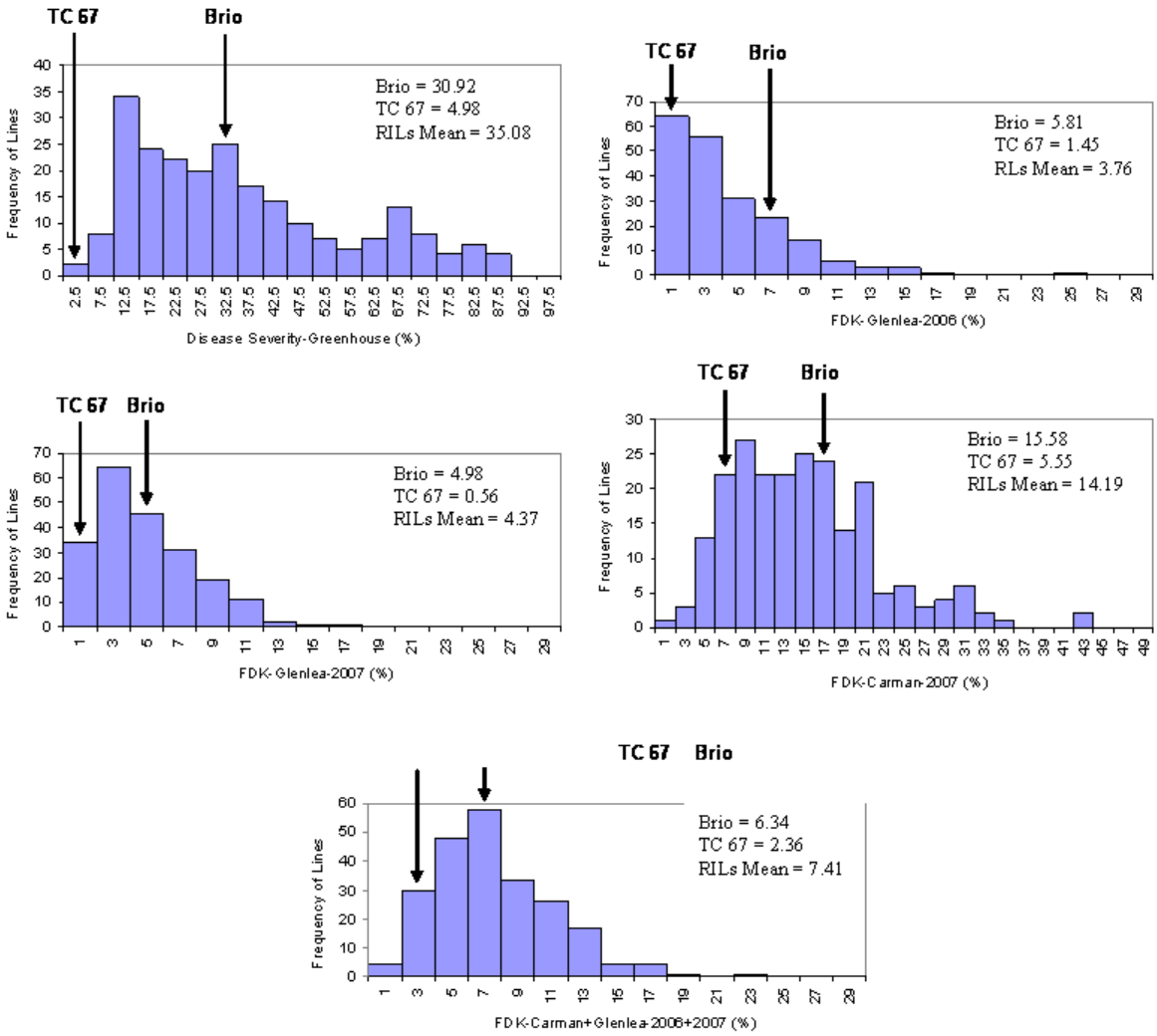

Figure 2.4. Frequency distribution of fusarium head blight disease severity (type II resistance) collected under greenhouse conditions and *Fusarium*-damaged kernels using single location-year and combined data of two locations in two years among 230 recombinant inbred lines from the cross 'Brio'/'TC 67'.

Means are back-transformed from least squares means of arcsine-transformed data. Values of the parental lines are indicated by arrows.

Table 2.5. Spearman correlation coefficients among fusarium head blight resistance traits using combined data of two locations in one year and greenhouse data among 230 recombinant inbred lines from the cross 'Brio'/'TC 67'[a].

Trait	Disease severity (F[b])	Disease index (F)	FDK (F)	DON (F)	Disease severity (G[c])
Disease incidence (F)	0.67**	0.91**	0.26**	0.50**	0.25**
Disease severity (F)	-	0.89**	0.12	0.42**	0.31**
Disease index (F)	-	-	0.20**	0.48**	0.30**
FDK (F)	-	-	-	0.26**	0.37**
DON (F)	-	-	-	-	0.26**

[a] Disease incidence, severity, index, FDK, and DON obtained from field experiments are based on least squares means (LS means) of arcsine transformed data at two locations, Carman and Glenlea, Manitoba, Canada in 2006 and disease severity from the greenhouse based on LS means of arcsine transformed data .

[b] Field (combined data of Carman and Glenlea in 2006)

[c] Greenhouse

** Significant at $P < 0.01$ probability level.

Agronomic traits

Analyses of variance for plant height from greenhouse-grown plants and number of days to anthesis from the greenhouse and field (Carman and Glenlea in 2006) experiments are shown in Table 2.6. Significant differences were observed among the genotypes for plant height and number of days to anthesis in the greenhouse (Tables 2.6a and b, respectively). In addition to the effect of genotype, the effects of location and genotype x location were significant for the combined data of number of days to anthesis over two locations in the field (Table 2.6c).

Table 2.6. Analysis of variance of agronomic traits using greenhouse and combined data of two locations in one year collected on 230 recombinant inbred lines from the cross 'Brio'/'TC 67'.

a) Plant height (greenhouse)

Sources of Variation	df	SS	MS	F Value	Pr > F
Genotype	230	83989	365.1674	4.13	< 0.0001
Block	2	3347.4892	1673.7446	18.93	< 0.0001
Residual	414	36600	88.4060	-	-

b) Number of days to anthesis (greenhouse)[a]

Sources of Variation	df	SS	MS	F Value	Pr > F
Genotype	229	33.0065	0.4141	51.24	< 0.0001
Block	2	6.4242	3.2121	7.71	0.0070
Spike (Block)	12	5.0880	0.4240	150.75	< 0.0001
Residual	3027	8.5137	0.0028	-	-

c) Number of days to anthesis (Carman+Glenlea-2006)[a]

Sources of Variation	df	SS	MS	F Value	Pr > F
Genotype	212	17.3910	0.0820	15.59	< 0.0001
Location	1	0.2377	0.2377	37.15	< 0.0001
Genotype x Location	204	1.0961	0.0054	2.15	< 0.0001
Block (Location)	4	0.0145	0.0036	1.45	0.2159
Residual	795	1.9867	0.0025	-	-

[a] Logarithmic transformed data were used for data analysis.

There was high variation in the heights and number of days to anthesis among the genotypes of the RIL population in both environments and the frequency of distribution of both traits was continuous indicating polygenic and quantitative inheritance of the traits (Figure 2.5). Means of plant heights in the greenhouse ranged from 72.33 to 130 cm with the mean of 101.83 cm for the population and the values of 73.83 and 130.00 cm for 'Brio' and 'TC 67', respectively. Means of number of days to anthesis for the genotypes in the greenhouse ranged from 60 to 95 days with an overall mean of 78 day for the population and values of 66 for 'Brio' and 84 for 'TC 67'. Finally, for the combined data

of days to anthesis over two locations in the field, means of the genotypes varied in a range of 37-80 days with the population mean of 55 days and the values of 40 and 71 for 'Brio' and 'TC 67', respectively. So, overall, under field conditions genotypes matured 23 days earlier than in the greenhouse. Transgressive segregants were found within the population for the number of days to anthesis in both environments (Figure 2.5).

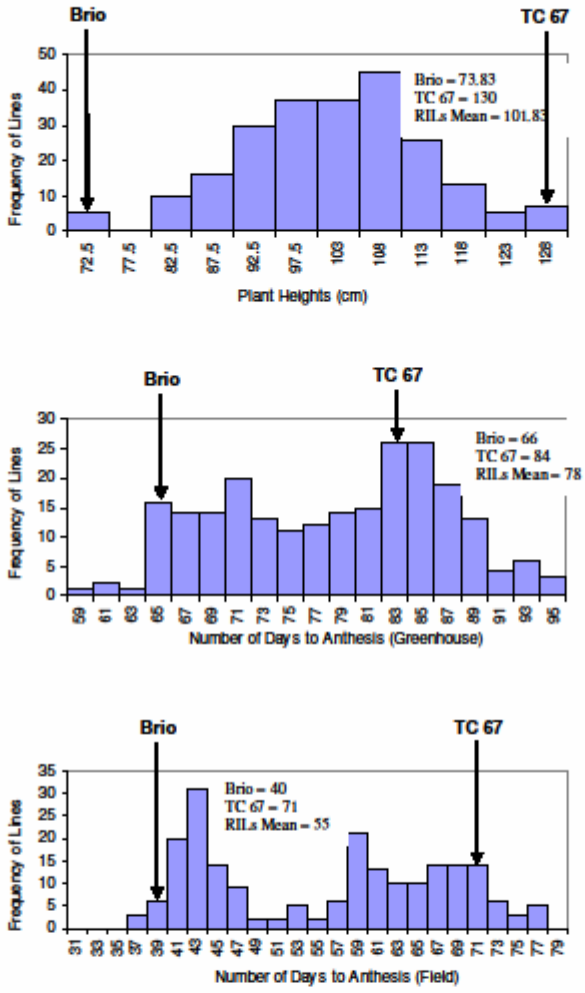

Figure 2.5. Frequency distribution of agronomic traits using greenhouse and combined data of two locations in one year among 230 recombinant inbred lines from the cross 'Brio'/'TC 67'. Means are back-transformed from least squares means of arcsine-transformed data. Values of the parental lines are indicated by arrows.

Association between agronomic traits and resistance to FHB

The association of plant height (greenhouse) and number of days to anthesis (greenhouse and field) with disease resistance traits measured in the greenhouse or field was determined and shown in Table 2.7. In general, these traits were not well correlated with disease. However, the associations of plant height and number of days to anthesis with disease incidence, severity, index, and DON accumulation (field) were negative. Furthermore, number of days to anthesis was positively correlated with FDK (field) and disease severity (greenhouse).

Table 2.7. Spearman correlation coefficients between agronomic traits and fusarium head blight among 230 recombinant inbred lines from the cross 'Brio'/'TC 67' using field and greenhouse data[a].

Trait	Disease incidence (F[b])	Disease severity (F)	Disease index (F)	FDK (F)	DON (F)	Disease severity (G[c])
Plant height-G	-0.21**	-0.26**	-0.27**	0.05	-0.31**	0.04
Days to anthesis-G	-0.19**	-0.33**	-0.29**	0.27**	-0.33**	0.18**
Days to anthesis-F	-0.22**	-0.40**	-0.34**	0.25**	-0.41**	0.15*

[a] Disease incidence, severity, index, FDK, and DON obtained from field experiments are based on least square means (LS means) of arcsine transformed data of two locations, Carman and Glenlea, Manitoba, Canada in 2006 and disease severity from the greenhouse based on LS means of arcsine transformed data.

[b] Field (combined data of Carman and Glenlea in 2006)

[c] Greenhouse

* Significant at $P < 0.05$ probability level.

** Significant at $P < 0.01$ probability level.

Regression analysis showed that in general there were significant associations between awnedness and all disease resistance traits using single location-year or

combined data set of field experiments (Table 2.8). The effect of awnedness on disease severity using greenhouse data was also significant. Awnedness consistently affected disease incidence and FDK in field conditions with higher coefficient of determination (R^2) values for FDK (Table 2.8). Awnedness explained 5-14% of the phenotypic variation observed for FDK in the population using different data sets. Results showed that awnedness had a negative effect on FHB, namely the presence of awns resulted in low disease in the population (data not shown).

Table 2.8. Coefficient of determination (R^2) values from regression analysis of awnedness and fusarium head blight resistance traits on 230 recombinant inbred lines from the cross 'Brio'/'TC 67' using field and greenhouse data sets.

Data set	Incidence (F)	Severity (F)	Index (F)	FDK (F)	DON (F)	Severity (G)
Carman-2006	0.03**	ns	ns	0.05**	0.03*	.
Glenlea-2006	0.08**	0.04**	0.06**	0.08**	0.07**	.
Carman-2007	0.04**	0.02*	0.04**	0.10**	.	.
Glenlea-2007	0.04**	ns	ns	0.08**	.	.
Carman+Glenlea-2006+2007	0.11**	0.05**	0.10**	0.14**	.	.
Greenhouse	.	.	.	.	.	0.02*

* Significant at $P < 0.05$ probability level.

** Significant at $P < 0.01$ probability level.

The effect of spike threshability on FHB was also investigated. Regression analysis showed that spike threshability was significantly associated with all FHB resistance traits using single location-year and combined data from field trials (Table 2.9). Spike threshability consistently affected FDK development under field conditions with

higher R^2 values. It explained 4-22% of the phenotypic variation for FDK in the population using different data sets. Similarly, spike threshability was associated with disease severity in the greenhouse by explaining 18% of the phenotypic variation observed in the population. Results showed that the hard threshable genotypes represented lower disease (data not shown).

No stable association between awnedness or spike threshability with number of days to anthesis or plant height was observed either in the field or under greenhouse conditions (Tables 2.8 and 2.9).

Table 2.9. Coefficient of determination (R^2) values from regression analysis of spike threshability and fusarium head blight resistance traits on 230 recombinant inbred lines from the cross 'Brio'/'TC 67' using field and greenhouse data sets.

Data set	Incidence (F)	Severity (F)	Index (F)	FDK (F)	DON (F)	Severity (G)
Carman-2006	0.03*	ns	ns	0.04**	ns	.
Glenlea-2006	0.03*	0.05**	0.04**	0.22**	0.11**	.
Carman-2007	0.05**	0.04**	0.06**	0.15**	.	.
Glenlea-2007	ns	ns	ns	0.21**	.	.
Carman+Glenlea-2006+2007	0.06**	0.05**	0.06**	0.22**	.	.
Greenhouse	.	.	.	.	.	0.18**

* Significant at $P < 0.05$ probability level.

** Significant at $P < 0.01$ probability level.

SSR markers and bulked segregant analysis

Of the 851 SSR primer pairs screened on the parental and bulk DNA samples, 89 primers amplified polymorphic fragments (Table 2.10). The highest number of

polymorphic markers was detected on the A genome (44 markers) while the D genome had the least (17 markers). These polymorphic markers were screened on individual DNA samples of the resistant and susceptible bulks to select highly polymorphic markers. Three markers on the A (*Xcfa2141*, *Xcfa2163*, and *Xcfa2185*) and four markers on the B genome (*Xbarc75*, *Xcfd60.1*, *Xcfd60.2*, and *Xgwm132*) were identified as highly polymorphic. No polymorphic microsatellite primer was detected on genome D. The seven markers for the A and B genomes were used to evaluate the mapping population (Table 2.10).

Table 2.10. Screening SSR markers of different genomes on parental lines, resistant and susceptible bulks, and individuals of the bulks to select polymorphic markers to map a 'Brio'/'TC 67' recombinant inbred line populatione[a].

Genome	Markers screened on parental lines and bulks	Polymorphic markers on parental lines and bulks	Markers screened on the individuals of the bulks	Highly polymorphic markers on the individuals of the bulks [a]	Markers used to genotype the mapping population
Genome A	323	44	44	3	3
Genome B	319	28	28	4	4
Genome D	209	17	17	0	0
Total	**851**	**89**	**89**	**7**	**7**

[a] The markers for which the fragments of the individual DNA samples were similar to the fragments of the bulks (similarity ≥ 90%), were used to genotype the entire mapping population.

QTL mapping

The three polymorphic markers on the A genome were grouped and ordered by MAPMAKER/EXP to make a linkage map belonging to chromosome 5A (Figure 2.6). The length of the linkage map was determined to be 10.8 cM, calculated using the

Kosambi mapping function of the MAPMAKER/EXP software. Thus it was determined that the SSR markers were located close together on a small part of the chromosome.

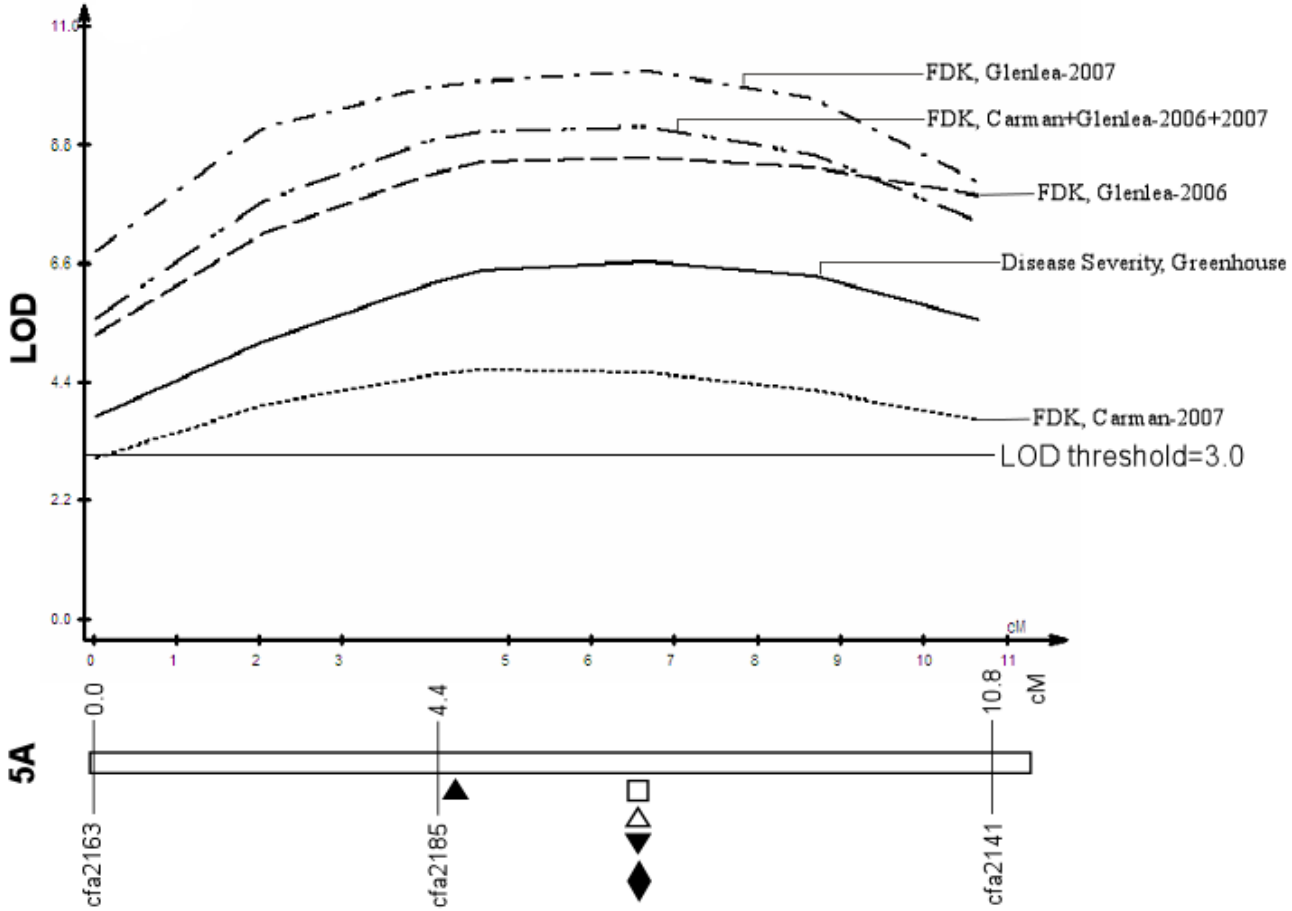

Figure 2.6. Linkage map and LOD curves after interval mapping (IM) analysis of fusarium head blight resistance on chromosome 5A on 230 recombinant inbred lines from the cross 'Brio'/'TC 67'.

Genetic distances are shown in centimorgan (cM) on the upper side of the linkage group and QTL positions for FHB severity (greenhouse) and FDK (field) on the lower side. □ = QTL for disease severity (greenhouse), △ = QTL for FDK, Glenlea-2006, ▲ = QTL for FDK, Carman-2007, ▼= QTL for FDK, Glenlea-2007, and ◆ = QTL for FDK, Carman+Glenlea-2006+2007.

Interval mapping with QTL Cartographer detected a major QTL on chromosome 5AL that was associated with both reduced disease severity and FDK under greenhouse and field conditions, respectively (Figure 2.6). This QTL explained 14.4% of phenotypic variation for severity (type II resistance) in the greenhouse and 19.2-23.0% for FDK (type IV resistance) across locations and years. Another genomic region on chromosome 5AL was also detected for FDK based on the single location-year data for Carman-2007 which explained 9.4% of phenotypic variation. As the position of this genomic region is very close to the other QTL and its effect was not consistent among locations and years, it is likely a function of phenotypic error. The consistent and major QTL detected in the present study was positioned at the interval of the markers *Xcfa2141* and *Xcfa2185* tending to *Xcfa2185* (Figure 2.6). The resistance allele for this QTL was derived from the resistant parent 'TC 67'.

Positive correlations were observed among the phenotypic data of the traits for which the major QTL on chromosome 5AL was detected. A correlation range of 0.33-0.42 ($P < 0.01$) was observed between disease spread data in the greenhouse and FDK field data. The correlation among FDK data associated to this QTL varied from 0.44 to 0.63 ($P < 0.01$).

One of the four polymorphic markers of the B genome (*Xcfd60.1*) showed a significant deviation from the expected segregation ratio of 1:1 in the mapping population according to a χ^2 test for fitness ($P < 0.001$). For the three remaining markers which were located individually on three different chromosomes, single marker analysis (SMA) was conducted with QTL Cartographer to determine if the markers were linked to a QTL. A regression analysis was also applied to estimate the R^2 values. The results showed that the marker *Xcfd60.2* was linked to unstable QTL(s) on chromosome 5BS which explained 8.0

and 5.06% of phenotypic variation for disease severity in Carman-2006 and disease index in 2006 over two locations, respectively. As the location of a QTL on a chromosome cannot be determined using SMA it is not known if there are two different QTLs on chromosome 5BS working separately for resistance to disease severity and index, or just one which confers resistance to both traits. It is likely that there is only one QTL for both traits linked to *Xcfd60.2* on 5BS because disease severity and index are very similar (both represent a combination of type I and type II resistances) and likely share an identical genetic basis. This QTL was derived from the moderately susceptible parent 'Brio'. This finding is not surprising as we observed transgressive segregation for all traits including disease severity and index in the mapping population.

For the remaining traits of FHB resistance, including disease incidence, severity, and DON accumulation no QTL were detected in the population. Neither was any QTL detected for plant height and number of days to anthesis in either the greenhouse or field experiments.

Discussion

FHB resistance

Phenotypic evaluation of genotypes is the first step in QTL mapping. Under natural conditions, FHB occurs unpredictably and the disease is not uniformly spread across the field (Buerstmayr et al. 2002). Therefore, artificial inoculation is essential for a reliable FHB resistance evaluation and to detect QTLs in a mapping population. We applied single-floret inoculation in the greenhouse and measured the percent of infected spikelets to determine the spread of the disease within a spike as an indicator of type II

resistance. Spray inoculation was applied in the field to measure the disease incidence (type I), severity (combined effect of type I and type II), DON accumulation (type III), and FDK development (type IV). Spray inoculation is a simple and reliable method to evaluate type I resistance (Yang et al. 2005b). On the other hand, the combined effects of type I and type II resistance can be evaluated using spray inoculation and may be described as field resistance (Schmolke et al. 2005; Somers et al. 2003).

There were high positive correlations among disease incidence, severity, and index (Table 2.5) which is evidence that these traits are controlled by similar genetic systems. Our results support the results of Steiner et al. (2004) who observed a high positive correlation between FHB severity and incidence but a weaker association of both traits with disease spread. Disease incidence is an indicator of type I resistance which is usually evaluated in spray- or naturally-inoculated plots or pots (Buerstmayr et al. 2009). As mentioned before, disease severity is used in spray-inoculated field trials to determine a combination of type I and type II resistance. Disease index is a combination of both disease incidence and severity as it is calculated using a formula involving both variables. So it is not surprising that these three disease traits are highly correlated and possibly controlled by the same QTLs. The correlation between FDK and DON or the correlation between either of them with the disease incidence, severity and index were poor (Table 2.5). This is evidence that resistance to FDK and DON accumulation is controlled by loci different from the resistance genes/QTLs controlling these three traits.

Correlation between agronomic traits and resistance to FHB

In the present study, plant height had significant negative correlations with FHB incidence, severity, and index following spray-inoculated field experiments. Taller lines

tended to be less diseased than shorter ones. This happened in spite of spray inoculation, providing the same amount of inoculum to plants independent of plant height. This phenomenon seems to be a common feature reported in several studies (Buerstmayr et al. 2000; Gervais et al. 2003; Häberle et al. 2009; Handa et al. 2008; Hilton et al. 1999; Klahr et al. 2007; Mesterházy 1995; Paillard et al. 2004; Schmolke et al. 2005; Schmolke et al. 2008; Somers et al. 2003; Srinivasachary et al. 2008; Steiner et al. 2004; Wilde et al. 2007). These observations support the hypothesis that semidwarf genotypes are more subject to infection by *Fusarium* due to higher moisture and humidity enhancing disease development (Klahr et al. 2007; Somers et al. 2003). The correlation of plant height and FHB resistance following spray inoculation as well as overlapping QTL regions suggests either linkage between loci or pleiotropy (Schmolke et al. 2005). However, our data did not support the presence of FHB resistance genes/QTLs linked to the genetic factors controlling plant height. The correlation coefficient between plant height and DON accumulation in this study was -0.31 while it was estimated as -0.50 by Somers et al. (2003). The correlation values between plant height and disease severity following single-floret inoculation in the greenhouse was positive but non-significant in our study as well as in the studies conducted by Somers et al. (2003) and Steiner et al. (2004). Plant height also did not correlate to FDK in our study (Table 2.7). In general, the correlation coefficients estimated in the present study are weaker than that reported in previous studies which can be attributed to differences in genetic backgrounds of the populations used, environmental conditions, or inoculation methods.

The correlations of number of days to anthesis with disease incidence, severity, and index were also significant and negative indicating that lines with a later heading date tended to be less diseased than early-maturing lines. A negative association between

heading date and FHB has been also reported in several studies (Gervais et al. 2003; Häberle et al. 2009; Paillard et al. 2004; Schmolke et al. 2005; Somers et al. 2003; Wilde et al. 2007). We estimated a correlation value of -0.33 to -0.41 between number of days to anthesis and DON accumulation which is similar to the results of Somers et al. (2003). The results mentioned here are different from those of Arthur (1891) who indicated that early-maturing lines are more resistant to FHB and from researchers who observed positive correlations between heading date and FHB (Klahr et al. 2007; Steiner et al. 2004). In the present study, the association between number of days to anthesis and disease severity under greenhouse conditions was significant and positive which supports the results of Steiner et al. (2004). Somers et al. (2003) also found a positive correlation between days to heading and disease spread but it was not significant. The correlation between number of days to anthesis and FDK in our study was significant and positive with a range of 0.25-0.27 (Table 2.7). It would appear that the correlation between number of days to anthesis and disease traits is somewhat contradictory which may be due to differences in genetic background, environmental variation, or methods used for evaluation. The genetic basis for heading date and FHB resistance may be different (Buerstmayr et al. 2000) but it is possible that late or early maturing lines escape infection by not being at anthesis when the optimal conditions are present for infection (Somers et al. 2003) or by slowing down disease spread when weather conditions are not optimal for disease development (Lin et al. 2006).

We observed a negative correlation between the presence of awns and FHB development which is different from the results of Mesterházy (1995) who stated that the presence of awns enhances FHB development. Based on the results of the present study awnedness is a strong morphological marker linked to resistance genes/QTLs for FDK.

The suppressor *B1* gene for awnedness is reportedly linked to FHB resistance genes/QTLs (Ban and Suenaga 2000; Gervais et al. 2003). Our results also showing an association between spike threshability and FHB development support those of Steiner et al. (2004).

QTL mapping and molecular markers

The highest heritability for disease traits in this study was estimated for disease spread in the greenhouse experiment (Table 2.4) as environmental effect is more controlled and genetic effect may be better expressed. The heritability of FDK within single location-years was also relatively high (Table 2.4). The heritability value of FDK across two locations in two years was the lowest of all (Table 2.4) because of the interaction effects of genotype, location, and year. In fact, the locations or years are random samples of disease hot spot locations or years in the target population of environments, which consists of disease-prone genotypes. Among the agronomic traits, number of days to anthesis in the greenhouse and field had heritability values of 0.998 and 0.959, respectively, and it was 0.925 for plant height in the greenhouse. We should be able to detect QTLs that explain more phenotypic variance within the greenhouse or single location-years because their heritabilities are high.

Molecular mapping of the 'Brio'/'TC 67' population showed lower SSR polymorphism than reported for other populations. This was possibly because 'TC 67' is an adapted spring wheat cultivar which is not widely different from the moderately susceptible parent 'Brio'. In addition, preliminary screening of the markers on resistant and susceptible parents and further screening on the individuals of the bulks possibly narrowed the screens resulting in a limited number of polymorphic markers. Despite high

correlations between agronomic traits and FHB resistance, neither specific QTLs for the agronomic traits nor overlapping QTLs for agronomic traits and FHB resistance were detected within the population. Although the population was genotyped with 851 markers, the map obtained was probably not complete. Nevertheless, interval mapping (IM) detected a major QTL for resistance to disease severity (greenhouse) and FDK (field) on chromosome 5AL.

The resistance alleles on chromosome 5AL in this investigation were from the resistant parent 'TC 67'. The 5A chromosome has been found to be involved in FHB resistance in widely diverse wheat germplasm. Quantitative trait loci on this chromosome have been identified for type I resistance in the populations derived from the crosses of 'DH181'/'AC Foremost' (Yang et al. 2005b) and 'Nanda2419'/'Wangshuibai' (Lin et al. 2006), for field resistance in the crosses of 'Sumai 3'/'Gamenya' (Xu et al. 2001) and 'Renan'/'Recital' (Gervais et al. 2003), and for disease severity in the populations from 'Frontana'/'Remus' (Steiner et al. 2004), 'Arina'/'Forno' (Paillard et al. 2004), 'Wangshuibai'/'Alondra'"s" (Jia et al. 2005), and 'Spark'/'Rialto' (Srinivasachary et al. 2008) under natural or spray-inoculated field conditions. The chromosome 5A was also shown to carry QTLs for type II resistance in populations from different backgrounds such as 'Fundulea 201R'/'Patterson' (Shen et al. 2003a), 'Strongfield'/'Blackbird' (Somers et al. 2006), 'Ernie'/'MO 94-317' (Liu et al. 2007), and 'Veery'/'CJ 9306' (Jiang et al. 2007a).

Buerstmayr et al. (2002, 2003 #54) detected a QTL for resistance to both disease spread and fungal penetration under field conditions on chromosome 5A (*Qfhs.ifa-5A*) in a 'CM-82036'/'Remus' DH population. Based on the results of experiments using different inoculation methods, Buerstmayr et al. (2002, 2003 #54) concluded that

Qfhs.ifa-5A may contribute mainly to type I resistance and to a lesser extent to type II resistance. Similar conclusions were drawn by Chen et al. (2006) using the evaluation of the 'W14'/'Pioneer Brand 2684' DH population.

In a DH population derived from 'Wuhan-1'/'Maringa' which later was corrected to'Wuhan-1'/'Nyu Bai' (McCartney et al. 2007), Somers et al. (2003) detected a QTL on chromosome 5AS for low DON content. This QTL was later validated in a population derived from the cross 'Veery'/'CJ 9306' (Jiang et al. 2007b). A QTL for low FDK on chromosome 5A was also reported in a population of 'Arina'/'Riband' (Draeger et al. 2007).

The effect of the 5AL QTL on disease severity and FDK in 'Brio'/'TC 67' can be attributed to the presence of two linked QTLs in one position or the presence of one pleiotropic QTL conferring resistance to disease severity and FDK. However, a correlation range of 0.33-0.42 observed between the phenotypic data of disease severity and FDK is not very high. This may be due to the environmental variation or different mechanisms controlling different FHB resistance expression (Shen et al. 2003a) or indirect evidence that the two traits are controlled by different loci (Somers et al. 2003). However, pleiotropic effects of many FHB-resistance QTLs have been mentioned before. In the study of a population of 'W14'/'Pioneer Brand 2684', Chen et al. (2006) detected a 5AS QTL which explained phenotypic variation for FHB incidence and severity, DON accumulation, and FDK. A QTL on chromosome 5A for reduced DON accumulation was reported in the cross of 'Wangshuibai'/'Annong 8455' which also showed effects on type II resistance (Ma et al. 2006b). Abate et al. (2008) detected a QTL on 5AS associated with both reduced DON and FDK in a population of wheat from the cross 'Ernie'/'MO 94-317' which was co-localized with a QTL for type II resistance in this population (Liu

et al. 2007). Finally, Yu et al. (2008) detected a QTL on the distal end of the 5AS chromosome in a population of 'Wangshuibai'/'Wheaton' which contributed to type I, type II, and type III resistance.

All the QTLs reported on chromosome 5A in the studies discussed above are at least 30 cM distant from the QTL reported in the present study. However, recently, Li et al. (2008) reported three genomic regions on 5A for low FDK in a population of wheat derived from 'Nanda 2419'/'Wangshuibai' one of which (*QFdk.nau-5A.3*) corresponds to the QTL detected in the present study.

Using single marker analysis (SMA), a QTL was detected on chromosome 5BS, with a low and inconsistent effect on disease severity and index (a combination of type I and type II resistances). This QTL was from the moderately susceptible parent 'Brio'. Results have shown that moderately susceptible cultivars may contain FHB resistance alleles that when combined with alleles from resistant cultivars can increase their level of resistance to FHB (Waldron et al. 1999). QTLs for resistance to FHB on chromosome 5B have been reported from a few studies. A QTL with a minor effect for type II resistance was identified on this chromosome from the crosses of 'Huapei 57-2'/'Patterson' (Bourdoncle and Ohm 2003) and 'Nanda 2419'/' Wangshuibai' (Lin et al. 2004). Paillard et al. (2004) identified a main QTL for resistance to disease severity on chromosome 5BL in a population of winter wheat 'Arina'/'Forno' cross under spray-inoculated field conditions. Jia et al. (2005) detected a QTL for disease severity on chromosome 5B in naturally infected trials in a 'Wangshuibai'/'Alondra'"s" DH population. Another QTL was identified on 5BL for disease severity under spray-inoculated field trials in a population of 'Cansas'/'Ritmo' (Klahr et al. 2007). There is evidence for the presence of

type II resistance QTLs with epistatic effects on chromosomes 5A (Ma et al. 2006a) or 5B (Yang et al. 2005b) without any main effect.

The major QTL on 5AL which is linked to *Xcfa2185* explained 14.4% of the phenotypic variation for disease severity (greenhouse), 19.2-23.0% for FDK single location-year data, and 19.7% for FDK combined data of two locations in two years (Table 2.11). On the other hand, the heritability values for these traits were 96, 90-92, and 67%, respectively (Table 2.4). Likewise, the minor QTL on 5BS which is linked to *Xcfd60.2* covered 8.0% of disease severity (Carman-2006) variation while the heritability value for the trait was 88%. Consequently, there are gaps between the amount of phenotypic variation covered by the genetic factors (markers) and the proportion of the phenotypic variation that is potentially due to genetic effects. It is likely that other QTLs and/or epistatic interactions have not yet been identified in this population. Especially minor QTLs may play an important role in this case. The undetected QTL in the present study may result from the limitation of the bulked segregant analysis strategy, as this technique may target only major effect QTLs, not minor effect QTLs (Michelmore et al. 1991). Furthermore, there may be a lack of available markers in locations associated with QTLs on a chromosome since the map does not cover 100% of the wheat genome.

The detection of transgressive segregation in disease spread and FDK as shown in Table 2.4, indicates that neither of the parents carry a full complement of resistance QTLs/genes. It also suggests that improvements in FHB resistance can be made by combining resistance genes from different sources (Somers et al. 2003).

In conclusion, the QTL detected on chromosome 5AL in 'TC 67' is a consistent QTL with major effects on type II (disease severity) and type IV (FDK) resistance. It can be classified among the QTLs with an intermediate effect on type II resistance compared

to the well-known 3BS QTL detected in Sumai 3 and its derivatives. This novel QTL provides an alternative for the currently known QTLs or may be combined with them to enhance the level of resistance to FHB in wheat cultivars. However, the positive association between FHB and hard threshability may limit the use of this QTL.

CHAPTER 3

MOLECULAR GENETIC DIVERSITY AND VARIATION FOR AGGRESSIVENESS AMONG *FUSARIUM GRAMINEARUM* ISOLATES FROM DIFFERENT SOURCES

Molecular genetic diversity and variation for aggressiveness among *Fusarium graminearum* isolates from different sources

Summary

Phylogenetic relationships among 58 isolates of putative *Fusarium graminearum* from Canada, Iran, and the International Maize and Wheat Improvement Centre (CIMMYT), Mexico were characterized using *Tri101* gene sequencing data. All Canadian and Iranian isolates clustered in one group and were identified as *F. graminearum* lineage 7 (= *F. graminearum sensu stricto*) within the *F. graminearum* clade while the isolates received from CIMMYT were placed in *F. graminearum* lineage 3 (= *Fusarium boothii*) within the *Fg* clade or *Fusarium cerealis*. The PCR assay based on the *Tri12* gene revealed the presence of the three trichothecene chemotypes of NIV, 3-ADON, and 15-ADON among the isolates tested with 15-ADON being the predominant chemotype. All *Fusarium boothii* isolates from CIMMYT were identified as 15-ADON chemotype, while all *F. cerealis* isolates were determined to be the NIV chemotype. While we did not find the NIV chemotype among the Canadian isolates, it was the predominant chemotype among the Iranian isolates. There was evidence of shift from the 15-ADON to more toxigenic 3-ADON chemotype among the Canadian isolates within the period of 1996-2004. High variation in aggressiveness was observed among and within the species tested with the isolates of *F. graminearum sensu stricto* being the most aggressive species, followed by *F. boothii* and *F. cerealis*. We observed association between chemotypes and aggressiveness with the observation that the NIV chemotypes had the lowest aggressiveness among all isolates, followed by the 15-ADON and 3-ADON chemotypes.

Introduction

Fusarium graminearum Schwabe [teleomorph: *Gibberella zeae* (Schwein.) Petch.] is the most dominant and widespread pathogen causing fusarium head blight (FHB) of small grain cereals worldwide. Fusarium head blight is one of the most destructive and economically important diseases of wheat, barley, and other small grains in many countries, and is particularly favoured by conditions of high humidity and warm temperatures. In addition to reducing grain yield and quality, FHB may result in grain contaminated with harmful mycotoxins such as deoxynivalenol (DON) and zearalenone (Bai and Shaner 1994; Desjardins et al. 1996; Marasas et al. 1984; McMullen et al. 1997; Miller et al. 1991; Parry et al. 1995; Snijders 1990b; Sutton 1982; Tuite et al. 1990).

FHB was first described over a century ago and was considered a major threat to wheat and barley during the early years of the 20th century (Dickson and Mains 1929). Since then epidemics have been sporadic, but have occurred during recent decades in many countries including in the USA and Canada (Bai and Shaner 1994; Ban 2001; Gilchrist et al. 1997; McMullen et al. 1997; Mesterházy 2003; Reis 1990; Snijders 1990b; Snijders 1990d; Sutton 1982).

The sexual stage of *F. graminearum*, *G. zeae*, is a homothallic ascomycete, as the alternative forms of the mating type (MAT) locus are juxtaposed at the same locus in *G. zeae* (Yun et al. 2000). Sexual reproduction in *G. zeae* can occur either by self-fertilization or outcrossing but the relative frequency of selfing and outcrossing in nature is not well known. Extensive sexual recombination should increase the level of variation within populations of *F. graminearum* (*G. zeae*) (Burdon 1993). *Fusarium graminearum* isolates demonstrate high variation in genotypic characteristics and phylogenetic profiles,

mycotoxin production and trichothecene chemotypes, pathogenicity/aggressiveness, vegetative compatibility groups (VCGs), and phenotypic features.

Historically, two naturally occurring and morphologically distinct populations within *F. graminearum* known as group 1 and group 2 were described by Purss (1969; 1971) and Francis and Burgess (1977) based on inability or ability of cultures to form perithecia, respectively (Francis and Burgess 1977). Subsequent analysis based on both morphological characteristics and DNA sequence data indicated that group 1 and group 2 were phylogenetically distinct, and consequently they were renamed as *Fusarium pseudograminearum* Aoki and O'Donnell (teleomorph: *Gibberella coronicola* Aoki and O'Donnell) and *Fusarium graminearum*, respectively (Aoki and O'Donnell 1999a, b).

Fusarium graminearum (*G. zeae*) was thought to be a single species spanning six continents until the genealogical concordance phylogenetic species recognition (GCPSR) approach (Taylor et al. 2000) was used to determine species limits among a global collection of *F. graminearum* isolates (O'Donnell et al. 2000; Ward et al. 2002). Using the GCPSR approach and phylogenetic analysis of DNA sequences of portions of nuclear genes from the isolates of *F. graminearum* collected from around the world, O'Donnell et al. (2000) detected seven phylogenetically distinct and biogeographically structured lineages. The *F. graminearum* species was named the *F. graminearum* clade or *Fg* clade and the lineages were designated species (O'Donnell et al. 2000). Using more isolates of *F. graminearum* six additional lineages (= species) were later discovered (O'Donnell et al. 2008; O'Donnell et al. 2004; Starkey et al. 2007; Ward et al. 2002; Yli-Mattila et al. 2009). So what previously was known as *F. graminearum* 'group 2' is now known to be a monophyletic species complex consisting of at least 13 distinct phylogenetic species. These lineages have been formally named, and the use of new species names is

recommended (O'Donnell et al. 2004). These species have different geographic distributions, differ in production of trichothecenes, and may differ in their ability to cause disease on particular crops (Cumagun et al. 2004; O'Donnell et al. 2000; O'Donnell et al. 2004).

The name *Fusarium graminearum* (lineage 7 within the *Fg* clade) with the teleomorph *G. zeae* was assigned to the principal causal agent of FHB in wheat and barley, and appears to have a cosmopolitan distribution (O'Donnell et al. 2004). It is the predominant species in the *Fg* clade found in Canada (K. O'Donnell, Pers. Comm.), USA (Burlakoti et al. 2008; Zeller et al. 2003, 2004), Argentina (Ramirez et al. 2007), and central Europe (Tóth et al. 2005). *Fusarium graminearum sensu stricto* isolates have also been detected from New Zealand (Monds et al. 2005) and several Asian countries, including China (Gale et al. 2002), Japan (Karugia et al. 2009), and Korea (Lee et al. 2009).

There are many reports dicussing the genetic diversity of *F. graminearum* in the literature (Akinsanmi et al. 2006; Burlakoti et al. 2008; Carter et al. 2000; Dusabenyagasani et al. 1999; Fernando et al. 2006; Gagkaeva and Yli-Mattila 2004; Gale et al. 2002; Karugia et al. 2009; Lee et al. 2009; Miedaner et al. 2001; Mishra et al. 2004; Ouellet and Seifert 1993; Qu et al. 2008; Ramirez et al. 2007; Schmale III et al. 2006; Tóth et al. 2005; Waalwijk et al. 2003; Walker et al. 2001; Zeller et al. 2003, 2004). These reports show high genetic variation within *F. graminearum* individual field populations, populations sampled across a large-scale geographical zone, or within collections of isolates. On the other hand, little or no population subdivision has been observed among the isolates of the pathogen sampled from fields separated by hundreds of kilometres (Fernando et al. 2006; Gale et al. 2002). By analysis of large numbers of *G.*

zeae isolates from different populations collected across USA, Zeller et al. (2003, 2004) concluded that a large, homogeneous, interbreeding population of the FHB pathogen, *F. graminearum sensu stricto*, is present over USA; genetic diversity results from a continuous recombination among inocula which is most likely from multiple origins over large geographical distances.

Fusarium species produce trichothecenes which are divided into two broad categories based on the presence (B-trichothecenes) or absence (A-trichothecenes) of a keto group at the C-8 position of the trichothecene ring (Ueno et al. 1973). All *Fg* clade species are B-trichothecene producers (Ward et al. 2002). They produce predominantly either deoxynivalenol (DON) or its C-4 oxygenated derivative, nivalenol (NIV). Miller et al. (1991) described the following strain-specific profiles of trichothecene metabolites (chemotypes) within the *F. graminearum* species complex and related species: *i*) DON chemotype which produces DON and/or its acetylated derivatives, and is subdivided into 3-ADON chemotypes (DON and 3-ADON producers) and 15-ADON chemotypes (DON and 15-ADON producers), and *ii*) NIV chemotypes which produce nivalenol and/or its diacetylated derivatives. DON-producing isolates of *F. graminearum* appear to occur more frequently than NIV-producing isolates in different parts of the world (Abbas et al. 1986; Abramson et al. 1993; Alvarez et al. 2009; Gale et al. 2007; Guo et al. 2008; Jennings et al. 2004; Mirocha et al. 1989; Pineiro et al. 1996; Ramirez et al. 2006; Scoz et al. 2009; Tóth et al. 2005), and the 15-ADON chemotype is more prevalent than the 3-ADON chemotype in many countries (Abbas et al. 1986; Abramson et al. 1993; Alvarez et al. 2009; Gale et al. 2007; Guo et al. 2008; Jennings et al. 2004; Ji et al. 2007; Mirocha et al. 1989; Moon et al. 1999; Pineiro et al. 1996; Scoz et al. 2009; Seo et al. 1996; Tóth et al. 2005). However, recently a significant shift from DON- to NIV-producing *F.*

graminearum in northwestern Europe has been reported (Waalwijk et al. 2003). There are also indications that the original 15-ADON chemotype is being replaced by the 3-ADON chemotype in North America (Ward et al. 2008).

The terms pathogenicity and aggressiveness are commonly used in the literature describing genetic resistance to fungal pathogens. There are differences in definitions and usages of these terms among authors who work with different pathogens and diseases but in general, pathogenicity is the ability of a pathogen to cause disease and aggressiveness is the amount of disease caused by an isolate of the pathogen (Trigiano et al. 2008).

DON produced by the pathogen during the infection period has been proposed as a virulence factor (Proctor et al. 1995). The aggressiveness of *F. graminearum* isolates also depends on their DON-producing capacity (Mesterházy 2002; Miedaner et al. 2000). DON-nonproducing isolates of *F. graminearum* caused a low level of disease severity in plants (Desjardins et al. 1996; Eudes et al. 2001; Nicholson et al. 1998). Bai et al. (2001a) indicated that the DON-nonproducing isolates still could infect wheat spikes but could not spread beyond the initial infection site, suggesting that DON is an aggressiveness factor, rather than a pathogenicity factor (Harris et al. 1999; Proctor et al. 1995). There are also several reports indicating that DON-producing isolates are more aggressive than NIV-producing isolates (Cumagun et al. 2004; Desjardins et al. 2004; Goswami and Kistler 2005; Logrieco et al. 1990; Miedaner et al. 2000; Muthomi et al. 2000). Goswami et al. (2005) also observed a significant correlation between the amount of the dominant trichothecene (either DON and its acetylated forms or NIV) produced by the *Fg* clade species and the level of aggressiveness on wheat.

High variation in pathogenicity and aggressiveness has been found among *F. graminearum* isolates from different geographical regions (Akinsanmi et al. 2004; Bai

and Shaner 1996; Cullen et al. 1982; Cumagun et al. 2004; Mesterházy 1984; Miedaner et al. 1996, 2000 #224, 1996 #225; Muthomi et al. 2000; Walker et al. 2001; Wu et al. 2005). A significant variation for aggressiveness was observed within the individual field populations of *F. graminearum* from Germany and among the isolates from a world collection (Miedaner et al. 2001). Gilbert et al. (2001) observed high variation in aggressiveness among Canadian isolates of *F. graminearum* in single-floret- and spray-inoculated experiments. All *F. graminearum* isolates from central Europe were found to be highly pathogenic in *in vitro* aggressiveness tests (Tóth et al. 2005). There are other reports indicating variation in aggressiveness among the isolates of *F. graminearum* (Cumagun et al. 2004; Goswami and Kistler 2005; Xue et al. 2004).

There is evidence that advanced wheat lines/cultivars representing a resistant reaction to FHB at the International Maize and Wheat Improvement Centre (CIMMYT), Mexico do not demonstrate the same reaction in other locations, *e.g.* Canada and USA (J. Gilbert, Pers. Comm.). The difference in the reaction of wheat lines/cultivars to FHB may be attributed to pathogen isolates, environmental conditions, and/or the interaction of both. The first step in clarifying the problem is to define the pathogen profile to see if there are differences between *Fusarium* isolates used at CIMMYT wheat nurseries and isolates used to assess FHB resistance in other wheat growing areas. Understanding the genetic profile and diversity of the pathogen may provide insights into the evolutionary and epidemiological potential of the pathogen, and may lead to an improvement in our strategies for control of the pathogen and management of the disease(s) caused by it. The objectives of this study were: *a*) to elucidate the phylogenetic relationships among the putative isolates of *F. graminearum* from Canada, Mexico, and Iran based on trichothecene 3-*O*-acetyltransferase (*Tri101*) gene sequencing data, *b*) to determine the

trichothecene chemotypes of the isolates, *c*) to assess the variation in aggressiveness among the isolates, and *d*) to determine if there is an association between phylogenetic structure and/or chemotypes with aggressiveness.

Materials and methods

Fusarium isolates

Fifty eight *Fusarium* isolates from Canada, Iran, and CIMMYT, Mexico along with seven reference isolates representing seven species within the *Fg* clade (O'Donnell et al. 2000) received from NCAUR-ARS-USDA (Peoria, IL) were used in this study (Table 3.1). Among the experimental isolates, 20 from Canada and 15 from CIMMYT had originally been isolated from *Fusarium*-infected wheat, barley, or maize and had morphologically been identified as *F. graminearum*. The 23 Iranian isolates of the pathogen were isolated from FHB-infected wheat spikes collected from Iran. For identification, the isolates were cultured on PDA and carnation leaf agar (CLA) and incubated for 10-14 days under alternating temperatures of 25 C day/20 C night (Nelson et al. 1983). Cultural and morphological characteristics were used to identify the fungal isolates (Nelson et al. 1983). For mid-term storage, all isolates were first grown on circles of sterile filter paper (Whatman® filter paper No. 3) placed on PDA in 9 cm Petri dishes. After the filter paper was colonized, it was peeled from the agar under aseptic conditions and allowed to dry for several days in a biohazard hood. Subsequently, the colonized paper was cut into 3 mm^2 pieces and stored at -20°C in small Eppendorf® tubes to create a stock supply from which future cultures were grown for all experiments.

For the specific purpose of phylogenetic analysis, the sequencing data of the *Tri101* gene of the 11 currently designated *Fusarium* spp. within the *Fg* clade were downloaded from GenBank (Table 3.1).

Mycelium production and DNA extraction

Mycelial disks of *F. graminearum* isolates on PDA were transferred to 125 ml flasks containing 60 ml Yeast-Malt broth culture media (0.3% yeast extract, 0.3% malt extract, 0.5% peptone, and 2% dextrose) and were grown at 25 C on a rotary shaker (200 rpm) for 3-4 days (O'Donnell 1992). The mycelium was harvested as follows: mycelium suspension was poured into 50 ml tubes and centrifuged at 3500 x *g* at 25 C for 10 min at an Allegra^{TM} 6R centrifuge (Beckman Coulter, Brea, CA, USA), the supernatant was discarded and 10 ml sterile distilled water added to the mycelium pellet. This was centrifuged for another 10 min and the supernatant was again discarded. The mycelia were blotted briefly between sterile paper towels. The harvested mycelia were lyophilized for two days in smaller tubes and stored for further use.

DNA was extracted using the modified CTAB miniprep method (Gardes and Bruns 1993): 300 µl of CTAB extraction buffer (1.0 M Tris-HCl pH = 8.0, 5.0 M NaCl, 0.5 M EDTA pH = 8.0, 1.1% CTAB) and 33 µl of 20% SDS were added to 50 mg of lyophilized and pulverized mycelium, mixed slowly, and incubated at 65 C for ≈ 1 h, mixing every 20 min. After cooling the samples at room temperature, 300 µl of chloroform-isomyl alcohol 24:1 was added to each sample, gently shaken for 20 min, and then spun for 20 min at 4000 x *g* in an Allegra^{TM} 25R centrifuge (Beckman Coulter, Brea, CA, USA). The supernatant (250 µl) was removed and DNA was precipitated by adding 160 µl of isopropanol to each sample. The samples were gently shaken (up and down) for

2 min then spun for 20 min at 4000 x *g* to pellet the DNA. The supernatants were aspirated from the samples and the pellets were gently washed by adding 500 µl of 70% ethanol making sure the pellets were released from the bottom of the tubes. This step was repeated once. The pellets were completely air-dried under a fume hood over night and then resuspended in 100 µl of 0.1x TE buffer (1 M Tris-HCl pH = 7.5, and 0.5 M EDTA pH = 7.5) with RNAse. DNA samples were diluted to 10 ng/µl by adding appropriate amounts of 0.1x TE buffer to use in PCR reactions (see below).

DNA amplification and sequencing

The *Tri101* gene was amplified as two overlapping fragments with the primer pairs AT1 and AT2 (Table 3.2) designed by Dr. Kerry O'Donnell (Pers. Comm.). The PCR reaction mixture typically contained 1x PCR buffer, 2 mM $MgSO_4$, 0.8 mM of each dNTP (Invitrogen™, Carlsbad, CA, USA), 0.3 pmol/µl of each primer (Invitrogen™), 0.4x BSA, 0.02 unit/µl Hi Fi Platinum® *Taq* DNA polymerase (Perkin-Elmer, Foster City, CA, USA), and 10 ng DNA in a reaction volume of 49 µl. PCR products were amplified in a PTC-200 thermal cycler (MJ Research, Waltham, MA, USA) with the following program: 1) an initial denaturing step of 2 min at 94 C, 2) 35 cycles of 15 s at 94 C for DNA denaturation, 45 s at 60 C for primer annealing, and 1 min at 68 C for primer extension, 3) a final extension of 10 min at 68 C, and 4) hold the program at 15 C.

Table 3.1. List of *Fusarium* isolates used for genetic diversity and variation for aggressiveness showing with their identification code, host, geographic origin, and year of collection.

Serial number[a]	Identification code	Host	Geographic origin	Year
1	DAOM 170785	Maize	Ottawa, Ontario, Canada	1998
2	DAOM 177406	Wheat	Chatham, Ontario, Canada	1998
3	DAOM 177408	Wheat	Chatham, Ontario, Canada	1998
4	DAOM 177409	Wheat	Chatham, Ontario, Canada	1998
5	DAOM 178148	Wheat	Chatham, Ontario, Canada	1998
6	DAOM 178149	Barley	Petrolia, Ontario, Canada	1998
7	DAOM 180376	Maize	Ottawa, Ontario, Canada	1998
8	DAOM 180377	Maize	Ottawa, Ontario, Canada	1998
9	DAOM 180378	Maize	Ottawa, Ontario, Canada	1998
10	DAOM 180379	Maize	Ottawa, Ontario, Canada	1998
11	DAOM 192130	Wheat	St. Jean, Manitoba, Canada	1998
12	DAOM 192131	Wheat	St. Jean, Manitoba, Canada	1998
13	DAOM 213295	Wheat	Burdett, Alberta, Canada	1998
14	EMMB 19/03	Wheat	Plum Coulee, Manitoba, Canada	2003
15	J & R SL 12	Wheat	Swan Lake, Manitoba, Canada	1996
16	MSDS 3/03	Wheat	Beausejour, Manitoba, Canada	2003
17	40/04	Wheat	Somerset, Manitoba, Canada	2004
18	71/04	Wheat	Gretna, Manitoba, Canada	2004
19	98/04	Wheat	Anola, Manitoba, Canada	2004
20	136/04	Wheat	Elkhorn, Manitoba, Canada	2004
21	IR-1	Wheat	Sari, Mazandaran, Iran	2005
22	IR-2	Wheat	Sari, Mazandaran, Iran	2005
23	IR-3	Wheat	Sari, Mazandaran, Iran	2005
24	IR-4	Wheat	Behshahr, Mazandaran, Iran	2005
25	IR-5	Wheat	Behshahr, Mazandaran, Iran	2005
26	IR-6A	Wheat	Behshahr, Mazandaran, Iran	2005
27	IR-6B	Wheat	Behshahr, Mazandaran, Iran	2005
28	IR-7A	Wheat	Aliabad, Golestan, Iran	2005
29	IR-7B	Wheat	Aliabad, Golestan, Iran	2005

Table 3.1. List of *Fusarium* isolates used for ... (Continued).

Serial number[a]	Identification code	Host	Geographic origin	Year
30	IR-8	Wheat	Aliabad, Golestan, Iran	2005
31	IR-9A	Wheat	Aliabad, Golestan, Iran	2005
32	IR-9B	Wheat	Aliabad, Golestan, Iran	2005
33	IR-10	Wheat	Azadshahr, Golestan, Iran	2005
34	IR-12	Wheat	Azadshahr, Golestan, Iran	2005
35	IR-13	Wheat	Moghan, Ardabil, Iran	2005
36	IR-14	Wheat	Moghan, Ardabil, Iran	2005
37	IR-16	Wheat	Moghan, Ardabil, Iran	2005
38	IR-18A	Wheat	Moghan, Ardabil, Iran	2005
39	IR-18B	Wheat	Moghan, Ardabil, Iran	2005
40	IR-21	Wheat	Moghan, Ardabil, Iran	2005
41	IR-23	Wheat	Moghan, Ardabil, Iran	2005
42	IR-24A	Wheat	Moghan, Ardabil, Iran	2005
43	IR-24B	Wheat	Moghan, Ardabil, Iran	2005
44	CM-1	Wheat	Toluca, Edo de México, México	1995
45	CM-2	Wheat	Toluca, Edo de México, México	1995
46	CM-3	Wheat	Toluca, Edo de México, México	1995
47	CM-4	Wheat	Toluca, Edo de México, México	1995
48	CM-5	Wheat	Toluca, Edo de México, México	1995
49	CM-6	Wheat	Toluca, Edo de México, México	1995
50	CM-7	Wheat	Toluca, Edo de México, México	1995
51	CM-8	Wheat	El Tigre, Jalisco, México	1997
52	CM-9	Wheat	Jesús María, Jalisco, México	1997
53	CM-10	Wheat	Tepatitlan, Jalisco, México	1997
54	CM-11	Wheat	Tepatitlan, Jalisco, México	1997
55	CM-12	Wheat	Tepatitlan, Jalisco, México	1997
56	CM-13	Wheat	Tepatitlan, Jalisco, México	1997
57	CM-14	Wheat	Patzcuaro, Michoacan, México	1997
58	CM-15	Wheat	Patzcuaro, Michoacan, México	1997

Table 3.1. List of *Fusarium* isolates used for ... (Continued).

Serial number[a]	Identification code	Host	Geographic origin	Year
59	NRRL 28585	Herbaceous vine	Brazil	Unknown
60	NRRL 28436	Sweet potato	New Caledonia	Unknown
61	NRRL 29105	Maize ear	Kaski, Nepal	Unknown
62	NRRL 26754	*Acacia mearnsii*	South Africa	Unknown
63	NRRL 26156	Wheat	Shanghai, China	Unknown
64	NRRL 28063	Maize stalk	Michigan, USA	Unknown
65	NRRL 29306	Maize	New Zealand	Unknown
66	NRRL 29148	Grape ivy	Pennsylvania, USA	Unknown
67	NRRL 31238	Unknown	Unknown	Unknown
68	NRRL 36905	Wheat	Minnesota, USA	Unknown
69	NRRL 37605	Wheat	Ipolydamásd, Hungary	Unknown

[a] The isolates 1-20 which had morphologically been determined as *Fusarium graminearum* were received from Cereal Research Centre, Winnipeg, Manitoba, Canada, isolates 21-43 were isolated from wheat spikes collected from Iran, and isolates 44-58 which also had morphologically been determined as *F. graminearum* were received from the International Maize and Wheat Improvement Centre (CIMMYT), Mexico. The isolates 59-65 representing seven species within the *Fg* clade were received from NCAUR-ARS-USDA (Peoria, IL) to use as reference isolates. For sequencing purpose, the sequences of the isolates 59-69 representing 11 species within the *Fg* clade were downloaded from GenBank using Blast search to use as reference sequences. The accession numbers of the isolates 59-69 were AF212586, AF212582, AF212593, AF212595, AF212599, AF212605, AY225882, AF212589, AY452813, DQ452409, and DQ452412, respectively.

Following purification of the amplified DNA with a MultiScreen® PCR plate (Millipore Corporation, Billerica, MA, USA), cycle sequencing was conducted in a PTC-200 thermal cycler with BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) using the following program: 1) an initial denaturing step of 5 min at 92 C, 2) 60 cycles of 10 s at 92 C for DNA denaturation, 5 s at 55 C for

primer annealing, and 4 min at 60 C for primer extension, 3) a final extension of 10 min at 60 C, and 4) hold the program at 4 C. Three primers, AT3, AT4, and AT6 were used to sequence *Tri101* gene but as they did not fully sequence the gene we designed four new primers to cover the gaps in the sequences: F140, F158, F171, and R184 (Table 3.2). All sequencing reaction mixtures were run on an ABI PRISM® 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions.

Table 3.2. List of primers used for *Tri101* gene amplification and/or sequencing in *Fusarium* isolates[a].

Primer name	Sequence	Forward /reverse
PCR primers:		
AT1	AAAATGGCTTTCAAGATACAGC	Forward
AT2	C(A/G)TA(C/T)TGCGC(A/G)TA(A/G)TTGGTCCA	Reverse
Sequencing primers:		
AT3	TTGATGCTCGACCGGCAATGG	Forward
AT4	GTTGTGGTAGGTCATGTTTTG	Reverse
AT6	ATCCATAGCACCGTGCTGTCC	Reverse
F140	GACGTACCTGCACAACAAC	Forward
F158	AGAGTCTTGGTAGCAGCATC	Forward
F171	CGGAGGTCTTTCACTACAAC	Forward
R184	GTCAGGGATACGTTGGACT	Reverse

[a] AT1, AT2, AT3, AT4, and AT6 primers were kindly designed by K. O'Donnell, NCAUR, ARS, USDA (Peoria, IL).

The sequencing data of the *Tri101* gene of the following isolates representing 11 species of the *Fg* clade (O'Donnell et al. 2000) from GenBank were also included as reference sequences in the study: NRRL 28585 (*F. austroamericanum*), NRRL 28436 (*F. meridionale*), NRRL 29105 (*F. boothii*), NRRL 26754 (*F. acasiae-mearnsii*), NRRL 26156 (*F. asiaticum*), NRRL 28063 (*F. graminearum*), NRRL 29306 (*F. cortaderiae*),

NRRL 29148 (*F. mesoamericanum*), NRRL 31238 (*F. brasilicum*), 36905 (*F. gerlachii*), and 37605 (*F. vorosii*). Furthermore, sequences of a *Fusarium pseudograminearum* isolate (NRRL 28338) were used as the outgroup in these analyses (Table 3.1).

Phylogenetic analysis

DNA sequences were processed and assembled using SOOMOS 0.6 (Agriculture and Agri-Food Canada) and sequence multiple alignments were conducted using MEGA 4. Phylogenetic analysis was conducted using PAUP* v. 4.0b10 to estimate the genetic diversity and evolutionary relationships of the isolates from the aligned sequences (Swofford 2003). Maximum parsimony (MP) searches were conducted using the heuristic search option with 1000 random addition sequences and the tree bisection-reconnection (TBR) method of branch swapping. Bootstrap analysis was performed with 500 pseudoreplicates and 70% consensus levels to assess relative support for internal nodes and clade stability under parsimony frameworks.

Determination of trichothecene chemotypes

Trichothecene chemotypes were determined by multiplex PCR assays based on the *Tri12* gene. The primers used for the amplification of the *Tri12* gene included 12CON (5´-CATGAGCATGGTGATGTC-3´), 12NF (5´-TCTCCTCGTTGTATCTGG-3´), 12-15F (5´-TACAGCGGTCGCAACTTC-3´), and 12-3F (5´-CTTTGGCAAGCCCGTGCA-3´). PCR was performed in 10 µl volume with the following reaction mixture: 1x GeneAmp® PCR buffer II (Applied Biosystems, Foster City, CA, USA), 2 mM $MgCl_2$, 0.16 mM of each dNTP, 0.2 µmol/µl of each primer, 0.04 unit/µl Ampli*Taq*® DNA polymerase (Applied Biosystems, Foster City, CA, USA), and 20 ng DNA was amplified

in a PTC-200 thermal cycler (MJ Research, Waltham, MA, USA) with the following program: 1) an initial step of 2 min at 94 C, 2) 30 cycles of 30 s at 94 C, 30 s at 52 C, and 1 min at 72 C, 3) a final extension of 7 min at 72 C, and 4) hold the program at 15 C. PCR products were resolved on 1.2% (wt/vol) agarose gel and scored relative to a 100-bp DNA size ladder (Invitrogen^{TM}, Carlsbad, CA, USA). The *Tri12* multiplex PCR produced amplicons of approximately 840 bp, 670 bp, and 410 bp corresponding with NIV, 15-ADON, and 3-ADON chemotypes, respectively (Figure 3.4).

Inoculum production and aggressiveness tests

A method used by Afshari-Azad (Afshari-Azad 1992) was modified as follows and used for inoculum production: 2.5 g of blended straw from wheat and barley was added to 125 ml tap water in a 250 ml flask, and autoclaved two times with 24 h interval. A small plug of PDA containing the fungal isolate was added to the mixture, and the culture was shaken for 96 h at 120 rpm at 25-30 C. The culture was passed through a cheese cloth and the suspension was diluted to 5 x 10^4 macroconidia/ml to use in inoculations. The isolates listed in Table 3.1 along with the following seven reference isolates representing seven species within the *Fg* clade (O'Donnell et al. 2000) were used individually for inoculum production and inoculations: NRRL 28585 (*F. austroamericanum*), NRRL 28436 (*F. meridionale*), NRRL 29105 (*F. boothii*), NRRL 26754 (*F. acasiae-mearnsii*), NRRL 26156 (*F. asiaticum*), NRRL 28063 (*F. graminearum*), and NRRL 29306 (*F. cortaderiae*).

Figure 3.1. Use of glassine bags to cover the single-floret-inoculated spikes in the greenhouse.

The susceptible wheat cultivar 'Roblin' was used for inoculations to measure disease spread caused by the isolates and to compare aggressiveness. Plants were grown in plastic pots (16 x 13 x 13 cm^3) containing Sunshine Mix No. 4 Agregate Plus (Sun Gro Horticulture Canada Ltd., Seba Beach, AB, Canada) in the greenhouse under a 16-h photoperiod and fertilized with NPK (20:20:20) all purpose fertilizer (Plant-Prod®, Brampton, ON, Canada) weekly. Plants were inoculated with macroconidia of *Fusarium* isolates when each spike reached 50% anthesis. Using a micropipette, 10 µl of the inoculum was injected into a single floret located 1/3 from the top of the spike, inoculated spikes were covered with glassine bags (Seedburo Equipment Co., Chicago, IL, USA) for 48 h to provide constant high humidity (Figure 3.1). Three replications (pots) and at least

four spikes per pot were used for inoculation by each isolate. Disease spread was rated 21 days after inoculation by counting the number of spikelets showing disease symptoms and calculating the percent of FHB-infected spikelets per spike as an indicator of aggressiveness (Snijders and Perkowski 1990)..

Statistical analysis

Average percent FHB values over spikes were calculated for each pot (replicate) and percentage data were arcsine-transformed prior to analysis. SAS® 9.2 (SAS Institute Inc., Raleigh, North Carolina) were used for data analysis and to determine the association of morphological and developmental traits with disease-related features.

Results

Identification of the pathogen isolates

A total of 23 isolates were obtained from the 24 FHB-infected wheat samples from Iran which all were identified as *F. graminearum* based on cultural and morphological characteristics (Nelson et al. 1983). Rate of growth in all isolates was rapid, aerial mycelium was present in the cultures with a white colour, and the colour of the colonies on the underside of the Petri plates was shades of carmine red (Figures 3.2A and B). Microconidia were absent and macroconidia were produced from monophialidic conidiophores (Figure 3.2C). Macroconidia were 3-7 septate, thick-walled, straight to moderately sickle-shaped, ventral surface almost straight and dorsal surface smoothly arched, with a cone-shaped apical cell or constricted as a snout and a foot-shaped basal cell (Figure 3.2D).

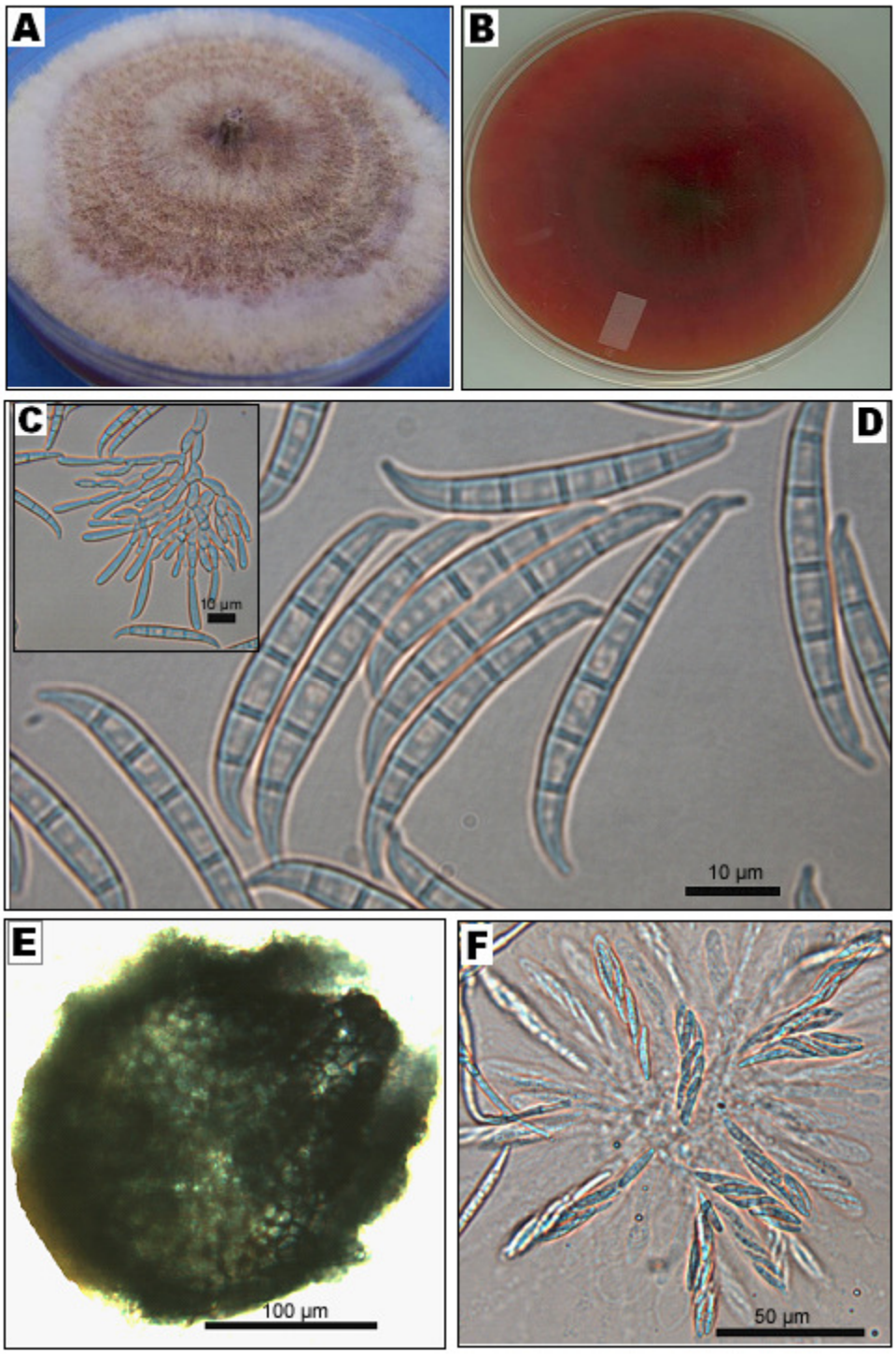

Figure 3.2. *Fusarium graminearum* cultural and morphological characteristics.

(A) Colony picture from the upper side, (B) Colony picture from the upper, (C) monophialidic conidiophores, (D) macroconidia, (E) perithecium, and (F) asci containing ascospores.

Perithecia, a distinguishing character of the sexual state (*G. zeae*), were produced on culture media (PDA or CLA) after 1-2 months at temperatures of 25-30 C. They were dark purple pear-shaped fungal bodies with an ostiole at the top and full of asci (Figure 3.2E). Asci were clavate with a short stipe and a thin wall usually containing 8 ascospores (Figure 3.2F). Ascospores were hyaline or very light brown, curved, fusoid with rounded ends, and were 0-3 septate.

Molecular phylogenetic analysis

The length of the *Tri101* gene used to make the sequence data set in this study was 1350 bp. Results of maximum parsimony analysis showed 1236 constant characters, 65 parsimony-uninformative variable characters, and 49 parsimony-informative characters in the sequences. Results also showed 300 most-parsimonious trees to demonstrate and describe the results.

Analyses of the sequences including the experimental and the reference isolates detected two distinct clades (Figure 3.3). All Canadian, Iranian, and seven Mexican isolates along with the 11 reference isolates of the *Fg* clade clustered together (*Fg* clade) while the remaining eight isolates from Mexico formed a different cluster; both clusters had a bootstrap (BP) value of 100%. Canadian and Iranian isolates formed a distinct cluster within the *Fg* clade along with the lineage 7 (= *F. graminearum*) reference isolate (BP = 89%) while seven isolates of the pathogen from Mexico clustered with the lineage 3 reference isolate, *Fusarium boothii* (BP = 100%). The eight Mexican isolates were originally received from CIMMYT as *F. graminearum* isolates, so they were put in the present study to determine the species based on DNA sequencing data. However, they were determined to be *Fusarium cerealis* (Cook) Scc. (= *Fusarium crookwellense*

Burgess, Nelson and Toussoun) using traditional taxonomy. Sequencing data from the present study supported isolates of *F. graminearum sensu stricto* and *F. boothii* being single species (BP = 89% and 100%, respectively).

The isolates which clustered with *F. graminearum sensu stricto* showed polymorphism and six Canadian isolates (DAOM 177408, DAOM 178148, DAOM 178149, DAOM 192130, DAOM 192131, and DAOM 213295) along with *F. graminearum sensu stricto* reference isolate formed a monophyletic subgroup in the cluster (BP = 73%). However, the isolates that clustered with *F. boothii* species and the isolates of *Fusarium cerealis* cluster were completely uniform (BP = 100% for each group).

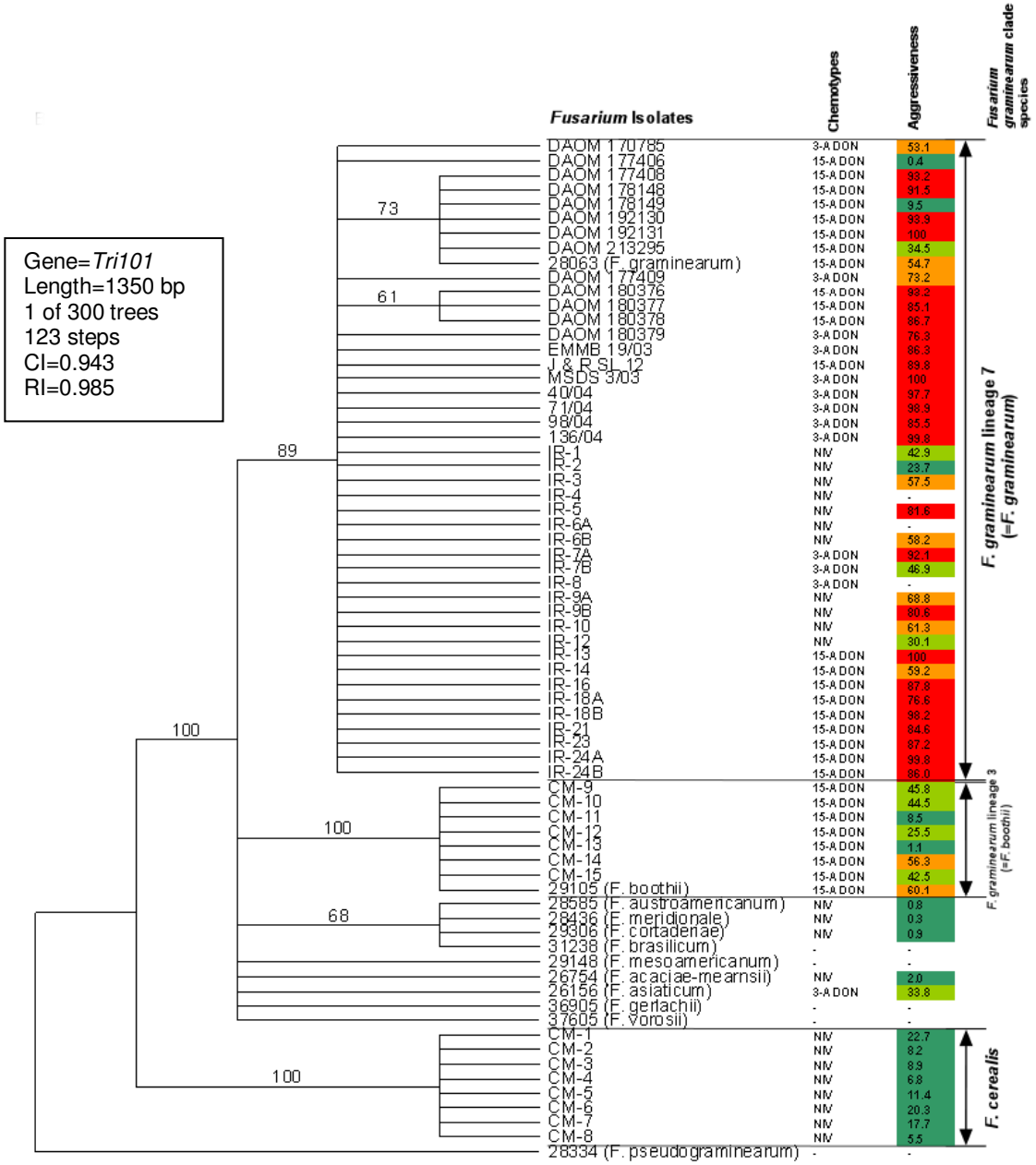

Figure 3.3. One of 300 most-parsimonious phylograms generated from the *Tri101* gene sequencing data using PAUP* v. 4.0b10 along with chemotypes and aggressiveness values of *Fusarium* isolates. The isolate 28334 (*F. pseudograminearum*) was used to root the tree. Bootstrap values of ≥ 50% from 500 parsimony replications are shown above the internodes. The values for consistency index (CI) and retention index (RI) are indicated in the top left box. Colour coding is used to differentiate aggressiveness measured as percent infected spikelets: Dark green = 0.0-25%, Light green = 25.1-50.0%, Orange = 50.1-75%, and Red = 75.1-100%. Aggressiveness values are back-transformed from least squares means of arcsine-transformed data.

Trichothecene chemotypes

The PCR assay based on *Tri12* gene showed the 840, 670, and 410 bp PCR products indicating the presence of NIV, 15-ADON, and 3-ADON chemotypes, respectively, among the isolates tested (Figure 3.4). The majority of the experimental isolates (27/58) were of the 15-ADON chemotype, followed by NIV (19/58) and 3-ADON (12/58) chemotypes (Figure 3.3 and Table 3.3). The majority of the isolates of *F. graminearum sensu stricto* and all isolates of *F. boothii* along with their reference isolates of NRRL 28063 and NRRL 29105 were determined to be the 15-ADON chemotype (Figure 3.3 and Table 3.3). The 3-ADON chemotype was detected only among a group of *F. graminearum sensu stricto* isolates (18.5%) along with a reference isolate NRRL 26156 (*F. asiaticum*) (Figure 3.3 and Table 3.3). All isolates of *F. cerealis* which is not a species within the *Fg* clade, a group of *F. graminearum sensu stricto* isolates (16.9%) and the reference isolates of NRRL 28585 (*F. austroamericanum*), NRRL 28436 (*F. meridionale*), NRRL 26754 (*F. acaciae-mearnsii*), and NRRL 29306 (*F. cortaderiae*) were determined to be of the NIV chemotype (Figure 3.3 and Table 3.3).

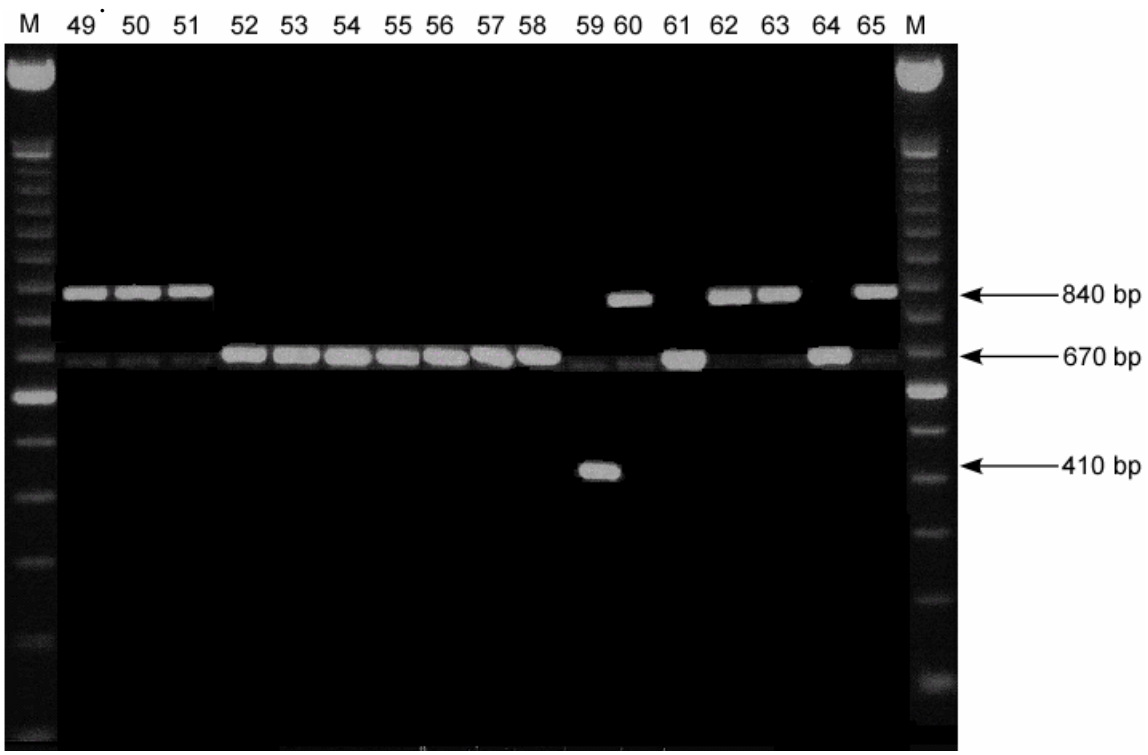

Figure 3.4. Amplification products of *Tri12* gene for *Fusarium* isolates produced by multiplex PCR using the primers 12CON, 12NF, 12-15F, and 12-3F specific to trichothecene chemotypes NIV, 15-ADON, and 3-ADON, respectively.

The amplification fragments of 840, 670, and 410 bp correspond with NIV, 15-ADON, and 3-ADON chemotypes, respectively. The lane M show a 100-bp ladder and the lanes 49-65 represent the following *Fusarium* isolates: CM-6, CM-7, CM-8, CM-9, CM-10, CM-11, CM-12, CM-13, CM-14, CM-15, NRRL 26156, NRRL 26754, NRRL 28063, NRRL 28436, NRRL 28585, NRRL 29105, and NRRL 29306.

No NIV chemotype was detected among *F. graminearum sensu stricto* isolates from Canada while the majority of the isolates received from Iran were of the NIV chemotype (Table 3.3). The majority of the isolates collected across Canada before 1998 were of the 15-ADON chemotype while recently collected isolates (after 2004) were

identified as 3-ADON producers (Table 3.1 and Figure 3.3). Among the Iranian isolates, the three chemotypes of NIV, 3-ADON, and 15-ADON were detected in the northern parts of the country including Sari, Behshahar, Aliabad, and Azadshahr while 15-ADON was the only chemotype detected among the *Fusarium* isolates collected from northwestern parts, *i.e.* Moghan (Table 3.1 and Figure 3.3).

Table 3.3. Distribution of trichothecene chemotypes among *Fusarium* isolates collected from Canada, Iran, and CIMMYT, Mexico based on *Tri12* gene[a].

Fusarium species	**Chemotypes**					
	NIV		**15-ADON**		**3-ADON**	
F. graminearum sensu stricto (Canada)	0	(0.0)	11	(19.0)	9	(15.5)
F. graminearum sensu stricto (Iran)	11	(19.0)	9	(15.5)	3	(5.2)
Subtotal (*F. graminearum sensu stricto* isolates)	**11**		**20**		**12**	
F. boothii	0	(0.0)	7	(12.1)	0	(0.0)
F. cerealis	8	(13.8)	0	(0.0)	0	(0.0)
Total (all isolates)	**19**	**(32.8)**	**27**	**(46.6)**	**12**	**(20.7)**

[a] Trichothecene chemotypes were determined by amplification of *Tri12* gene using a multiplex PCR conducted by 12CON, 12NF, 12-15F, and 12-3F primers.

[b] Values in the parenthesis represent percents.

Aggressiveness

Three isolates, IR-4, IR-6A, and IR-8, failed to sporulate and were not tested for aggressiveness. All other experimental isolates infected the susceptible cultivar 'Roblin' and caused FHB disease spread ranging from 0.4 to 100% (Figures 3.3 and 3.5). The Iranian isolate of IR-13 and two Canadian isolates of DAOM 192131 and MSDS 3/03 were the most aggressive isolates while another Canadian isolate, DAOM 177406, was the least aggressive. We conclude that there is a high variation in aggressiveness among

the isolates collected from different sources. The highest variation in aggressiveness was observed among the Canadian isolates ranging from 0.4-100% and the least variation among the CIMMYT isolates ranging from 1.1-56.3%. Range of aggressiveness among Iranian isolates varied from 23.7-100%. The frequency of the isolates with aggressiveness > 50.0% was higher than that of isolates with aggressiveness < 50.0%.

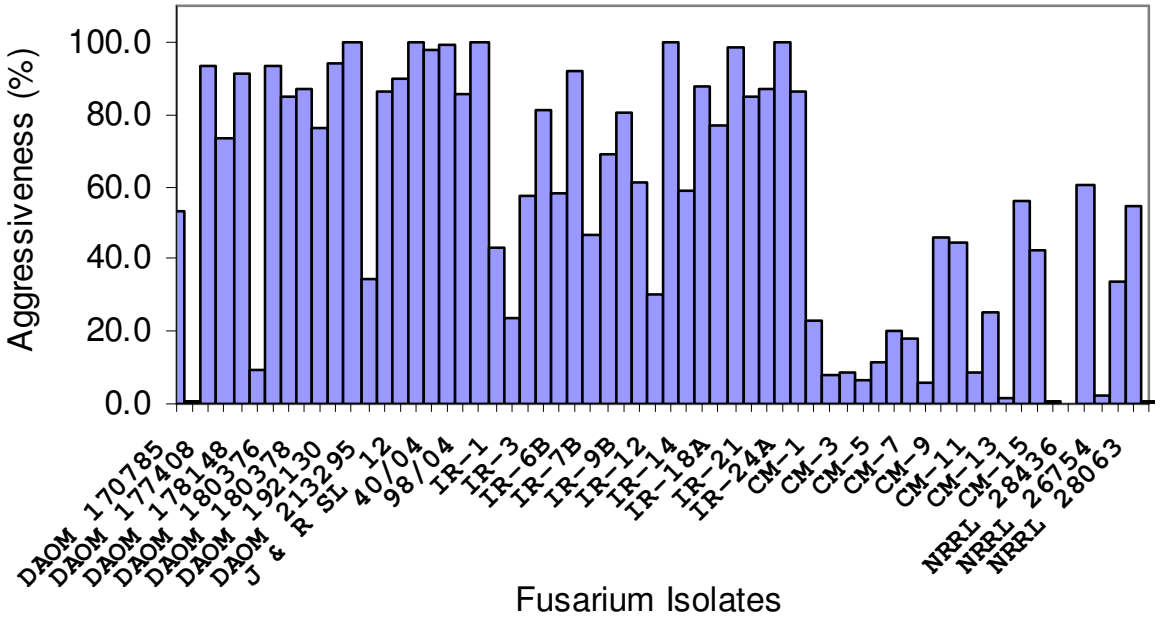

Figure 3.5. Comparison of aggressiveness of *Fusarium* isolates collected from Canada, Iran, and Mexico on the susceptible cultivar 'Roblin' measured as disease spread 21 days after inoculation using single-floret injection.

Aggressiveness values are back-transformed from least squares means of arcsine-transformed data.

Association between pathogen profile and aggressiveness

High variation in aggressiveness was observed among and within the phylogenetically determined *Fusarium* species in the *Fg* clade. Aggressiveness among the

isolates of *F. graminearum* lineage 7 in the *Fg* clade (= *Fusarium graminearum sensu stricto*) ranged from 0.4-100% with a mean of 74.3%, while it was 1.1-56.3% among the isolates of *F. graminearum* lineage 3 (= *F. boothii*) with a mean of 32.0%. Mean aggressiveness of *Fusarium graminearum sensu stricto* isolates was more than twice that of *F. boothii* isolates. On the other hand, aggressiveness of the reference isolate NRRL 28063 (*Fusarium graminearum sensu stricto*) was lower than that of the reference isolate NRRL 29105 (*F. boothii*) with aggressiveness values of 54.7% and 60.1%, respectively. Among the rest of the reference isolates tested, NRRL 26156 (*F. asiaticum*) had an aggressiveness value of 33.8% but the isolates 26754 (*F. acaciae-mearnsii*), NRRL 29306 (*F. cortaderiae*), and NRRL 28585 (*F. austroamericanum*), and NRRL 28436 (*F. meridionale*) were among the least aggressive isolates with disease aggressiveness ≤ 2%. Isolates of *F. cerealis*, which is not a species within the *F. graminearum* clade, had a mean aggressiveness of 12.7%.

Association between trichothecene chemotypes and aggressiveness

The NIV isolates had the lowest mean level of aggressiveness (35.7%) while the 3-ADON chemotypes had the highest mean (82.7%). The 15-ADON chemotypes had an intermediate mean value of 66.0%. If the reference isolates with significantly lower values of aggressiveness are not considered the pattern of aggressiveness for the chemotypes still remains the same. This is true even if the CIMMYT isolates which also had significantly lower values for aggressiveness are removed from the analysis.

Discussion

All Canadian and Iranian isolates were identified as *F. graminearum* lineage 7 (= *F. graminearum sensu stricto*) within the *Fg* clade while the *Fusarium* isolates obtained from CIMMYT, Mexico, were divided into two clusters: a distinct cluster which was *F. graminearum* lineage 3 (= *F. boothii*) within the *Fg* clade and another cluster which was identified as *F. cerealis* (Figure 3.3). *Fusarium graminearum sensu stricto* is a cosmopolitan species reported from different parts of the world (Burlakoti et al. 2008; Gale et al. 2002; Karugia et al. 2009; Lee et al. 2009; Monds et al. 2005; O'Donnell et al. 2004; Ramirez et al. 2007; Suga et al. 2008; Tóth et al. 2005; Zeller et al. 2003, 2004) but the endemic area of *F. boothii* is problematic given its distribution in Africa, Mexico, and Mesoamerica (O'Donnell et al. 2004). Following an earlier report of an Iranian isolate from corn (NRRL 13383) being identified as *F. graminearum sensu stricto* (O'Donnell et al. 2000; O'Donnell et al. 2004; Starkey et al. 2007; Ward et al. 2002), we report that *F. graminearum sensu stricto* within the *Fg* clade is the principal pathogen of FHB in Iran.

Our results showed the presence of the 15-ADON chemotype among the isolates of both *F. graminearum sensu stricto* and *F. boothii* species within the *Fg* clade (Figure 3.3 and Table 3.3). The 3-ADON chemotype was also detected among the isolates of *F. graminearum sensu stricto* and in the reference isolate of NRRL 26156 (*F. asiaticum*) (Figure 3.3). In addition, all isolates of *F. cerealis*, some isolates of *F. graminearum sensu stricto,* and the reference isolates of NRRL 28585 (*F. austroamericanum*), NRRL 28436 (*F. meridionale*), NRRL 26754 (*F. acaciae-mearnsii*), and NRRL 29306 (*F. cortaderiae*) were identified as the NIV chemotype (Figure 3.3). We conclude that NIV, 3ADON, and 15ADON chemotypes have multiple independent evolutionary origins

which supports the conclusion that trichothecene chemotypes are not well correlated with the evolutionary relationships of the *Fg* clade (O'Donnell et al. 2000; Ward et al. 2002). This finding also indicates that mycotoxin production in the *Fg* clade is not species-specific. Ward et al. (2002) showed that each of the trichothecene chemotypes had a single evolutionary origin in the ancestor of extant species within the *Fg* clade, and that polymorphism within these virulence-associated genes has persisted through multiple speciation events in these fungi. They concluded that the polyphyletic distribution of trichothecene chemotypes relative to the *Fg* clade is the result of non-phylogenetic sorting of ancestral polymorphism into descendant species and the sharing of ancestral polymorphism among extant species which is referred to as transspecies evolution (Ward et al. 2002).

All isolates of *F. graminearum sensu stricto* collected from Canada were determined to be DON producers and the majority of them were identified as the 15-ADON chemotype. All isolates of *F. boothii* received from CIMMYT were also identified as the 15-ADON chemotype. In contrast, the NIV chemotype was predominant among the isolates of Iran which is in agreement with the results of Haratian et al. (2008). Goswami et al. (2005) also determined the Iranian *F. graminearum* isolate NRRL 13383 isolated from corn to be a NIV chemotype. Other studies have also reported a correlation between mycotoxin chemotype and geographic origin (Desjardins et al. 2000; Jennings et al. 2004; Ji et al. 2007; Lee et al. 2001; Miller et al. 1991; Zhang et al. 2007). Such ecological differences in chemotype distribution may contribute to establish regional differences in grain contamination (Ramirez et al. 2006). While most Canadian isolates collected earlier than 1998 were determined to be 15-ADON producer, all isolates collected after 2004 were found to be of the 3-ADON chemotype which may be

considered as evidence that the dominant 15-ADON FHB pathogen is being replaced by the more toxigenic population of *F. graminearum sensu stricto* with 3-ADON chemotype in North America (Ward et al. 2008). While the eastern provinces of Prince Edward island and Quebec in Canada had a significantly higher frequency of the 3-ADON chemotype than the western provinces, the frequency of the 3-ADON chemotype in western provinces increased significantly between the 1998 and 2004 (Ward et al. 2008).

In the present study, we observed high variation in aggressiveness among and within the species with the isolates of *F. graminearum sensu stricto* being the most aggressive, followed by *F. boothii* and *F. cerealis* (Figure 3.5). In an investigation on the isolates of *Fusarium* representing eight species of the *Fg* clade and three lineages of *F. culmorum*, Tóth et al. (2008) found that *F. boothii* was among the least pathogenic species to wheat while *F. graminearum sensu stricto* isolates were the most aggressive. In a study of comparative aggressiveness of eight *Fusarium* spp. including *F. graminearum*, *Fusarium acuminatum* Ellis and Everhart, *Fusarium avenaceum* (Corda ex Fries) Sacc., *F. crookwellense*, *Fusarium culmorum* (W. G. Smith) Sacc., *Fusarium equiseti* (Corda) Sacc., *Fusarium poae* (Peck) Wollenw., and *Fusarium sporotrichioides* Sherb., Xue et al. (2004) observed the most rapid and severe disease development was caused by *F. graminearum*, followed by *F. crookwellense*. Gilbert et al. (2001) observed high variation in aggressiveness among the isolates of *F. graminearum* collected from different parts of Canada using single-floret- and spray-inoculated experiments. Values of disease spread in the reference isolates of NRRL 28063 (*F. graminearum*) and NRRL 29105 (*F. boothii*) did not support the difference observed for aggressiveness between the isolates of the two species in this study. It is not surprising to expect such a result as only one or a few isolates may not well represent the true characteristics of a species (*e.g.* aggressiveness)

even though DNA sequences may clearly show differences. NIV chemotypes had the lowest aggressiveness in the present study which confirms several earlier reports (Cumagun et al. 2004; Desjardins et al. 2004; Goswami and Kistler 2005; Logrieco et al. 1990; Miedaner et al. 2000; Muthomi et al. 2000). Variability in aggressiveness among the isolates of a species in some cases may cause difficulties in diagnosing the disease in the field and prevent the timely application of control measures (Goswami and Kistler 2005). The existence of high variability in the pathogen also emphasizes the need for breeders to include a wide range of isolates in their screening for selection of disease resistant varieties (Goswami and Kistler 2005).

The present study clearly showed differences among *Fusarium* isolates used in the CIMMYT wheat breeding program and the isolates from elsewhere, *i.e.* Canada and Iran. In contrast to Canada and Iran where FHB pathogen isolates were identified as *F. graminearum sensu stricto*, the CIMMYT isolates belonged to the less aggressive *F. boothii* within the *Fg* clade or to *F. cerealis*. These differences in pathogen isolates may explain why advanced wheat lines/cultivars which demonstrate a resistant reaction at CIMMYT may not express the same reaction in Canada, USA, or other parts of the world. The results of the further study which was conducted to better understand host-pathogen interaction using representative isolates of the pathogen and wheat genotypes from Canada, Iran, and CIMMYT is presented in Chapter 4.

CHAPTER 4

HOST-PATHOGEN INTERACTIONS BETWEEN WHEAT GENOTYPES AND *FUSARIUM* ISOLATES FROM DIFFERENT SOURCES

Host-pathogen interactions between wheat genotypes and *Fusarium* isolates from different sources

Summary

Fusarium head blight (FHB) is a devastating disease of wheat and other small grain cereals in humid and semi-humid areas worldwide. The interactions between *Fusarium* isolates and wheat genotypes from Canada, Iran, and the International Maize and Wheat Improvement Centre (CIMMYT), Mexico were investigated in the present study by inoculating the representative isolates of two species of *Fusarium graminearum sensu stricto* and *Fusarium boothii* within the *Fusarium graminearum* clade on wheat genotypes with different levels of resistance to FHB. The representative isolates of *F. boothii* used at CIMMYT produced the least disease on wheat genotypes tested regardless of the origin of the genotypes while *F. graminearum sensu stricto* isolates from Canada and Iran produced more severe FHB disease on the genotypes. We observed significant differences among the genotypes inoculated by single isolates of the pathogen and two of the more recent CIMMYT wheat genotypes, NG8675/NING8645 and SHA3/CBRD, were consistently among the most resistant genotypes to the disease regardless of the *Fusarium* species or isolates inoculated. Our results also showed significant interactions between the *Fusarium* isolates and wheat genotypes used in the present study.

Introduction

Fusarium head blight (FHB) is a devastating disease of wheat and other small grain cereals in humid and semi-humid areas worldwide. The risk of FHB is high when a susceptible cultivar is grown, the natural inoculum (conidia or ascospores on crop debris) is abundant, and the weather is warm and humid at flowering. Despite the range of species involved in the disease, *Fusarium graminearum* Schwabe [teleomorph: *Gibberella zeae* (Schwein.) Petch.] appears to be the predominant species worldwide. FHB can greatly reduce grain yield and quality, lower seed germination, and cause seedling blight. In addition, the infected grain may contain harmful levels of mycotoxins which are detrimental to livestock and a safety concern in human food (Bai and Shaner 1994).

Phylogenetic analysis using DNA sequences of nuclear genes of *F. graminearum*, revealed 13 biogeographically structured lineages (= species) within the *F. graminearum* clade (referred to as the *Fg* clade) (O'Donnell et al. 2000; O'Donnell et al. 2008; O'Donnell et al. 2004; Starkey et al. 2007; Ward et al. 2002; Yli-Mattila et al. 2009). These species have formally been named. *Fusarium graminearum* (lineage 7 in the *Fg* clade) was assigned to the major causal agent of FHB in wheat and barley (O'Donnell et al. 2004). It is the predominant species in the *Fg* clade found in Canada (K. O'Donnell, Pers. Comm.), USA (Burlakoti et al. 2008; Zeller et al. 2003, 2004), Argentina (Ramirez et al. 2007), and central Europe (Tóth et al. 2005). *Fusarium graminearum sensu stricto* isolates have also been detected from New Zealand (Monds et al. 2005) and several Asian countries (Gale et al. 2002; Karugia et al. 2009; Lee et al. 2009; Suga et al. 2008). The seven lineages within the *Fg* clade were also given the following names: [1] *Fusarium*

austroamericanum, [2] *Fusarium meridionale*, [3] *Fusarium boothii*, [4] *Fusarium mesoamericanum*, [5] *Fusarium acaciae-mearnsii*, [6] *Fusarium asiaticum*, and [8] *Fusarium cortaderiae*. The following names were given to rest of the species within the *Fg* clade without a lineage designation: *Fusarium brasilicum, Fusarium gerlachii*, *Fusarium vorosii*, *Fusarium aethiopicum*, and *Fusarium ussurianum.*

Large variation in aggressiveness and/or pathogenicity of *Fusarium graminearum* (*G. zeae*) isolates has been observed. Significant variation for aggressiveness was reported among the isolates of *F. graminearum* from a single field (Miedaner and Schilling 1996) and within the individual field populations from Germany and among the isolates from a world collection (Miedaner et al. 2001). Gilbert et al. (2001) observed high variation in aggressiveness among the Canadian isolates of *F. graminearum*. All *F. graminearum* isolates from central Europe were found to be highly pathogenic in *in vitro* aggressiveness tests (Tóth et al. 2005). Variation in aggressiveness among *F. graminearum* isolates has also been reported by other investigators (Cumagun et al. 2004; Goswami and Kistler 2005; Xue et al. 2004). Different isolates of *Fusarium* spp. may show variation in aggressiveness and there may be significant interactions between wheat cultivars and pathogen isolates. However, there is no evidence for stable pathogen races (Bai and Shaner 1996; Mesterházy 1984, 1988; Mesterházy 2003; Snijders and Van Eeuwijk 1991; Wang and Miller 1987).

The development of resistant cultivars is a key component in an effective strategy to disease control. High variation in resistance to FHB has been identified among wheat germplasm, even though complete resistance or immunity has not been reported. However, breeding for FHB resistance is difficult as the most resistant sources are of exotic origin with poor agronomic traits, the inheritance of resistance is complicated, and

screening of FHB resistance is environmentally biased, labour-intensive, and costly (Buerstmayr et al. 2002).

Five types of genetic resistance to FHB have been identified in wheat: resistance to initial infection (type I), resistance to fungal spread within plant tissues (type II) (Schroeder and Christensen 1963), resistance to toxin accumulation (type III), resistance to kernel infection (type IV), and tolerance (Mesterházy 1995; Miller et al. 1985; Wang and Miller 1988). It has also been recognized that resistance to FHB in wheat involves active and passive mechanisms (Mesterházy 1995).

Resistance to FHB in wheat is usually stable and resistant cultivars show consistent resistance to almost all isolates of *F. graminearum* worldwide. Based on the test of reaction of wheat cultivars to different species of *Fusarium*, Mesterházy (1981) concluded that resistance to certain isolates of *F. graminearum* as well as to other species of *Fusarium* was not strain-specific or species-specific in wheat cultivars. Van Eeuwijk et al. (1995) did not observe specific interactions between wheat cultivars and pathogen isolates from different geographic areas. It can be concluded that resistance to FHB is horizontal or non-specific in nature at least for the most prevalent species like *F. graminearum* and *Fusarium culmorum* (W. G. Smith) Sacc. (Mesterházy et al. 1999; Snijders and Van Eeuwijk 1991; Van Eeuwijk et al. 1995). The resistance genes present in the FHB resistance sources currently used in wheat are not expected to be overcome by new isolates of the pathogen in the near future. However, given the large genetic variability that exists in *Fusarium* spp. (Bowden and Leslie 1999), use of at least a few different resistance genes in a wheat breeding would be a wise approach (Buerstmayr et al. 2009).

Observations show that advanced wheat lines/cultivars representing a high level of FHB resistance at the International Maize and Wheat Improvement Centre (CIMMYT), Mexico do not retain their resistance in other regions, *e.g.* Canada and USA (J. Gilbert, Pers. Comm.). The objective of the present study was to investigate the interactions between *Fusarium* isolates and wheat genotypes from Canada, Iran, and CIMMYT, Mexico to better understand the wheat-*Fusarium* pathosystem and to clarify the nature of the difference in reactions between wheat genotypes at CIMMYT and other geographic zones.

Materials and methods

Field experiments and wheat genotypes used

A total of 63 wheat lines/cultivars obtained from Canada, Iran, and CIMMYT, Mexico were evaluated for resistance to FHB in two locations (Carman and Glenlea, Manitoba, Canada) in 2006 and 2007. In addition, 38 FHB-resistant wheat lines were received from CIMMYT and evaluated in Carman in 2008. The experimental design in all experiments was a randomized complete block design with three replicates. Plots consisted of 1 m (Carman) or 1.5 m (Glenlea) length rows with 30 cm row spacing and sowing density was ≈ 5 g of seed per plot. A mixture of highly aggressive isolates of *F. graminearum* (J. Gilbert, Pers. Comm.) stored at Cereal Research Centre (CRC), Winnipeg, Manitoba, was used for the inoculum production and inoculations. Plots were spray-inoculated with an aqueous solution of macroconidia at 5 x 10^4 macroconidia/ml when 50% of the plants had reached anthesis. Nurseries were mist-irrigated (Carman) or sprinkler-irrigated (Glenlea) for 1 h after inoculation. In Carman the mist system operated

for a further 12 hours for 5 min in each hour. Three weeks after inoculation, the genotypes were scored for disease severity according to a 0-100% scale for visually infected spikelets on a whole-plot basis. Based on the results of field evaluations, five genotypes of wheat with differential levels of resistance to FHB were selected from each of Canada, Iran, and Mexico to use in host-pathogen interaction studies in the greenhouse (Table 4.1).

Fusarium isolates

A total of 20, 23, and 15 isolates morphologically assigned to *F. graminearum* from Canada, Iran, and CIMMYT, respectively, were used in the present study. Using the *Tri101* gene sequencing data, the isolates were phylogenetically analyzed and clustered to different lineages (= species). The isolates were characterized for aggressiveness by inoculating them on the susceptible wheat cultivar 'Roblin'. A detailed description of the identification of the *Fusarium* isolates, *Tri101* gene sequencing, phylogenetic analysis, and aggressiveness tests are shown in Chapter 3.

The two most aggressive isolates of the *Fg* clade lineage 7 (= *Fusarium graminearum*) from both Canada and Iran and two isolates of the *Fg* clade lineage 3 (= *Fusarium boothii*) from CIMMYT were selected and used in the present study (Table 4.2).

Table 4.1. Fusarium head blight severity following spray inoculation of wheat genotypes from Canada, Iran, and CIMMYT (Mexico).

Number	Name/cross	Selection history	Disease severity[a]	Origin
1	AC VISTA	-	74.58	Canada
2	ROBLIN	-	73.27	Canada
3	KANATA	-	55.83	Canada
4	93FHB37	-	40.83	Canada
5	5602 HR	-	35.42	Canada
6	N-83-5	ATTILA50Y//ATTILA/BCN	87.26	Iran
7	N-81-8	TINAMOU	79.16	Iran
8	N-82-14	WEAVER/WL3926//SW89.3064	67.43	Iran
9	N-83-6	PR1/BAGULA"S"//NANJING82149/KAUZ	48.36	Iran
10	N-82-13	SW89.3064/STAR	47.50	Iran
11	CS/LE.RA//CS/3/PVN	CIGM81.1282-3B-3B-0M	100.00	CIMMYT, Mexico
12	CHUM18//JUP/BJY	CM91046-7Y-0M-0Y-4M-8Y-0B-0FC-2FUS-0Y-1SCM	83.78	CIMMYT, Mexico
13	MILAN/DUCULA	CMSS93B01075S-74Y-010M-010Y-010M-8Y-0M-2SJ-0Y	56.67	CIMMYT, Mexico
14	SHA3/CBRD	-0SHG-2GH-0FGR-0FGR	10.67	CIMMYT, Mexico
15	NG8675/NING8645	-3SCM	7.33	CIMMYT, Mexico

[a] Based on least squares means (LS means) of combined data of two locations in two years for genotypes 1-10 and LS means of one location in one year for the genotypes 11-15.

Table 4.2. Fusarium head blight severity following single-floret inoculation of the cultivar 'Roblin' by *Fusarium* isolates from Canada, Iran, and CIMMYT (Mexico) under controlled conditions.

Isolate	Description	Species[a]	Disease severity[b]	Origin
1	MSDS #3/03	*Fg* clade lineage 7[c]	100.00	Beausejour, Manitoba, Canada
2	DAOM 192131	*Fg* clade lineage 7	100.00	St. Jean, Manitoba, Canada
3	IR-13	*Fg* clade lineage 7	100.00	Moghan, Ardabil, Iran
4	IR-24A	*Fg* clade lineage 7	97.73	Moghan, Ardabil, Iran
5	CIMMYT-14	*Fg* clade lineage 3[d]	48.77	CIMMYT, Mexico
6	CIMMYT-9	*Fg* clade lineage 3	46.80	CIMMYT, Mexico

[a] Identification of the species based on phylogenetic analysis of DNA sequencing data.

[b] Percent infected spikelets .

[c] *Fg* clade lineage 7 = *F. graminearum*.

[d] *Fg* clade lineage 3 = *F. boothii*.

Greenhouse experiments and data collection

Wheat lines/cultivars were inoculated using single-floret inoculation under greenhouse conditions of the Cereal Research Centre, Winnipeg, Manitoba in 2009. The experimental layout was a factorial design with randomized complete block design as basic design and three replications for each treatment. Experimental plots were 16 x 13 x 13 cm^3 pots. Greenhouse growing conditions were maintained with 16 h light (25 C) and 8 h dark (20 C) supplemented with incandescent high pressure sodium lights (OSRAM SYLVANIA LTD; Mississauga, ON, Canada). Wheat plants were treated with a combination of propiconazole and spinosad one month after seeding to control powdery mildew and thrips. When wheat genotypes reached 50% anthesis, they were inoculated by injecting a 10-µl droplet of conidial suspension (5 x 10^4 macroconidia/ml) into the floret in a spikelet positioned 1/3 of the spike from the top using a micropipette. At least five

spikes in each pot (replication) were inoculated and the spikes were covered with 20 x 5 cm^2 glassine bags (Seedburo Equipment Co.; Chicago, IL, USA) for 48 h to constant high humidity. Disease severity was scored as the percentage of diseased spikelets per spike 21 days after inoculation. A general view of the greenhouse experiments is shown in Figure 4.1.

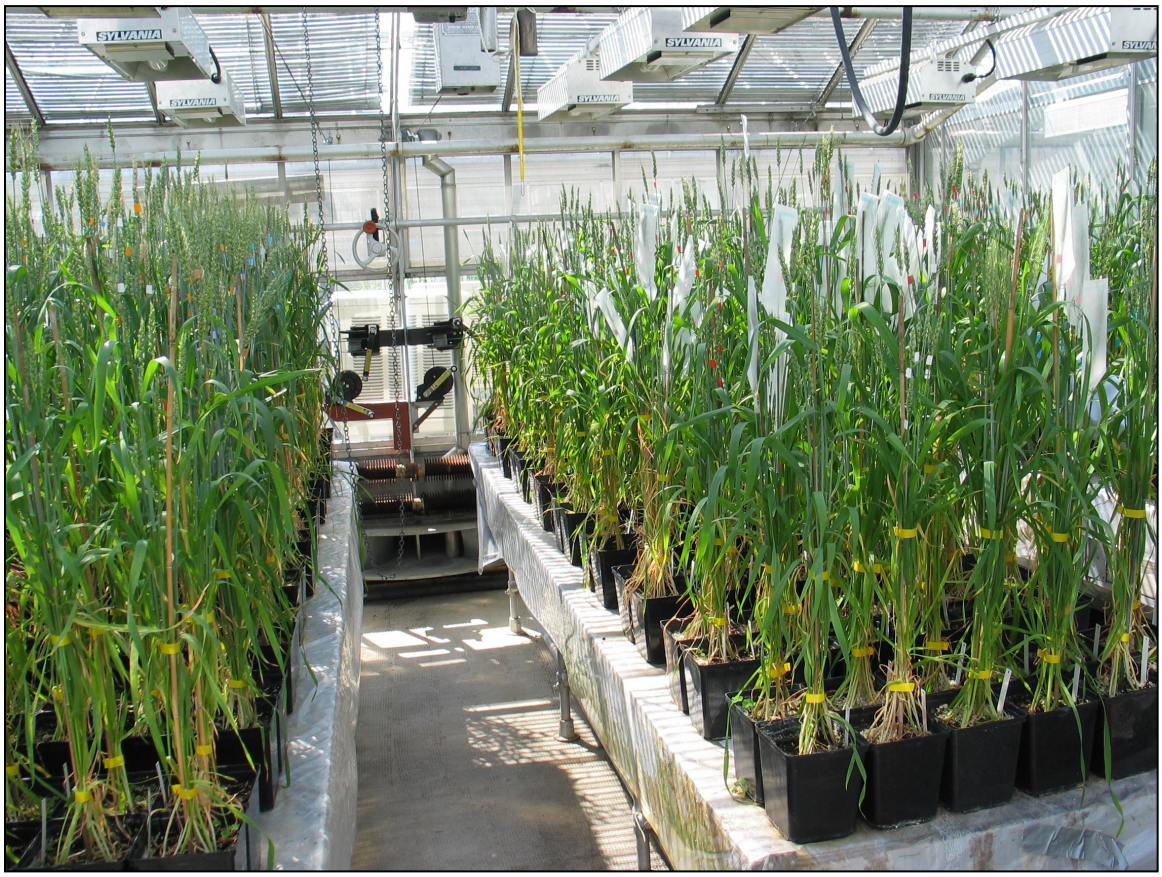

Figure 4.1. A general view of inoculations and experiments in the greenhouse.

Statistical analysis

Statistical analyses were performed using SAS® 9.2 (SAS Institute Inc., Raleigh, NC, USA). Before conducting the analysis of variance (ANOVA), data were tested for normality using PROC UNIVARIATE. If variables did not follow a normal distribution,

an arcsine transformation was applied. Analyses of variances were performed on uniform transformed data of each resistance trait using PROC MIXED. Genotype and isolates were considered fixed while block effects were considered random.

Results

High variation was observed in the FHB expressed by different *Fusarium* isolates on individual wheat genotypes and in the disease observed among different wheat genotypes caused by individual *Fusarium* isolates (Table 4.3). Among the wheat genotypes, the Iranian advanced wheat line N-81-8 (TINAMOU) showed the highest variation in reaction to *Fusarium* isolates with disease severity values of 5.87% and 99.43% caused by the Mexican isolate CIMMYT-14 (*F. boothii*) and the Iranian isolate IR-13 (*F. graminearum sensu stricto*), respectively. The Canadian wheat line 93FHB37 had the lowest range of reaction (2.71-18.5%) when inoculated with the six experimental *Fusarium* isolates, with the lowest reaction to CIMMYT-14 and the highest reaction to the Canadian isolate MSDS #3/03 (*F. graminearum sensu stricto*). Among the *Fusarium* isolates tested, the Canadian isolate DAOM 192131 (*F. graminearum sensu stricto*) caused the highest variation in FHB on wheat genotypes with the disease values of 4.44% and 99.51% on NG8675/NING8645 and MILAN/DUCULA, respectively. The Mexican isolate CIMMYT-14 (*F. boothii*) had the lowest variation with disease values ranging from 2.40% on N-82-13 to 32.39% on ROBLIN.

Analysis of variance of disease severity data collected from 15 wheat genotypes inoculated with six *Fusarium* isolates showed significant differences among the isolates

and among wheat genotypes ($P < 0.0001$) (Table 4.4). The interaction of isolate x genotype was also significant ($P < 0.0001$) as shown in Table 4.4.

Table 4.3. Disease severity on wheat genotypes following single-floret inoculation with *Fusarium* isolates under controlled conditions.

Genotype	Isolate					
	MSDS #3/03	DAOM 192131	IR-13	IR-24A	CIMMYT-14	CIMMYT-9
AC VISTA	96.15[a]	95.47	71.83	94.60	19.88	46.56
ROBLIN	96.59	84.78	69.41	91.33	32.39	23.22
KANATA	72.76	23.73	51.98	42.76	3.52	3.43
93FHB37	18.50	7.93	4.68	10.37	2.71	6.55
5602 HR	72.72	21.32	.	55.34	5.23	6.79
N-83-5	59.99	32.35	47.82	53.75	11.03	3.83
N-81-8	96.07	91.83	99.43	97.24	5.87	10.72
N-82-14	27.47	18.71	18.84	60.08	3.95	2.75
N-83-6	15.63	4.68	6.27	27.49	3.76	2.66
N-82-13	14.91	22.00	22.90	34.37	2.40	4.29
CS/LE.RA//CS/3/PVN	77.82	79.73	88.71	93.77	18.73	24.83
CHUM18//JUP/BJY	45.65	50.47	92.28	61.61	22.00	27.14
MILAN/DUCULA	98.84	99.51	97.17	98.12	6.51	12.73
SHA3/CBRD	22.67	8.42	9.28	10.78	2.68	2.96
NG8675/NING8645	24.11	4.44	12.76	15.88	2.51	2.28

[a] Values are back-transformed from least squares means of arcsine-transformed data.

Comparison of the least squares means of disease severity of the six *Fusarium* isolates inoculated on 15 genotypes of wheat under greenhouse conditions showed that the Iranian isolate IR-24A with the highest disease values was the most aggressive isolate, followed by the Canadian isolate MSDS #3/03. These two isolates both belonged to *F. graminearum sensu stricto*, grouped together in group A (Table 4.5). The isolates IR-13 and DAOM 192131 which again belonged to *F. graminearum sensu stricto* were grouped

together in group B (Table 4.5). Finally, the two Mexican isolates of CIMMYT-14 and CIMMYT-9, both members of *F. boothii*, with the lowest values of disease severity were placed in group C at the bottom of the table as the least aggressive isolates (Table 4.5).

Table 4.4. Analysis of variance of fusarium head blight disease severity data collected from the inoculation of 15 wheat genotypes by six *Fusarium* isolates under greenhouse conditions[a].

Sources of Variation	df	SS	MS	F Value	Pr > F
Isolate	5	80.1737	16.0347	217.96	< 0.0001
Genotype	14	116.2060	8.3004	112.78	< 0.0001
Isolate*Genotype	70	39.3189	0.5617	7.63	< 0.0001
Block	2	0.2788	0.1394	0.72	0.5067
Spike (Block)	12	2.3269	0.1939	2.63	0.5067
Residual	1220	89.7789	0.0736	-	-

[a] Arcsine square root transformed data were used for data analysis.

The Mexican wheat genotype MILAN/DUCULA was the most susceptible wheat line, followed by the genotypes AC VISTA (Canada), N-81-8 (Iran), ROBLIN (Canada), and CS/LE.RA//CS/3/PVN (Mexico), all together in group A (Table 4.6). On the other hand, four genotypes of NG8675/NING8645 (Mexico), N-83-6 (Iran), SHA3/CBRD (Mexico), and 93FHB37 (Canada) were among the most resistant genotypes (Table 4.6). The remaining genotypes showed intermediate reactions to FHB.

There were significant differences among the *Fusarium* isolates ($P < 0.001$) on all wheat genotypes except on 93FHB37, when disease severity data from the inoculation of the six *Fusarium* isolates on single wheat genotypes were used for the analysis of variance (data not shown). Similarly, significant differences were observed among the wheat genotypes ($P < 0.0001$) using analysis of variance of data from the inoculation of genotypes by individual isolates (data not shown).

Table 4.5. Comparison of least squares means of fusarium head blight severity and grouping of six *Fusarium* isolates inoculated on 15 genotypes of wheat under greenhouse conditions[a].

Isolate	Description	LS Means[b]	Standard Error	Letter Group[c]
4	IR-24A	59.29	0.0378	A
1	MSDS #3/03	59.25	0.0397	A
3	IR-13	49.43	0.0380	B
2	DAOM 192131	43.18	0.0380	B
6	CIMMYT-9	9.97	0.0384	C
5	CIMMYT-14	8.05	0.0383	C

[a] Least squares means were compared according to Tukey-Kramer method at $P < 0.05$.

[b] Values are back-transformed from Arcsine transformed data.

[c] Values with the same letter are not significantly different at $P < 0.05$.

The least squares means of disease severity caused by the six *Fusarium* isolates were compared on individual genotypes. In general, a similar pattern was observed for aggressiveness of *Fusarium* isolates on wheat genotypes: the Canadian and Iranian isolates, as *F. graminearum sensu stricto*, were more aggressive and the Mexican *F. boothii* isolates were less so (Table 4.7). The Canadian isolate MSDS #3/03 was the most aggressive isolate on 8 out of 13 genotypes (≈ 62%). In contrast, the Mexican isolate CIMMYT-14 was the least aggressive isolate on 10 genotypes (≈ 77%). We observed that the Mexican isolates were the least aggressive on all wheat genotypes, except 93FHB37 on which the Iranian isolate IR-13 was less aggressive than CIMMYT-9.

Table 4.6. Comparison of least squares means of fusarium head blight severity and grouping of 15 genotypes of wheat inoculated by six *Fusarium* isolates under greenhouse conditions[a].

Genotype	Name/cross	LS Means[b]	Standard Error	Letter Group[c]
13	MILAN/DUCULA	77.27	0.0897	A
1	AC VISTA	75.10	0.0867	A
7	N-81-8	73.09	0.0908	A
2	ROBLIN	69.58	0.0867	A
11	CS/LE.RA//CS/3/PVN	66.45	0.0906	A
12	CHUM18//JUP/BJY	50.81	0.0888	B
6	N-83-5	32.09	0.0867	C
5	5602 HR	29.60	0.0867	CD
3	KANATA	29.17	0.0867	CD
8	N-82-14	19.00	0.0877	DE
10	N-82-13	14.60	0.0901	EF
15	NG8675/NING8645	8.95	0.0908	F
9	N-83-6	8.69	0.0877	F
14	SHA3/CBRD	8.48	0.0867	F
4	93FHB37	7.99	0.0908	F

[a] Least squares means were compared according to Tukey-Kramer method at $P < 0.05$.

[b] Values are back-transformed from Arcsine transformed data.

[c] Values with the same letter are not significantly different at $P < 0.05$.

The least squares means of disease severity data of the experimental wheat genotypes were also compared based on reaction to individual isolates. Different patterns were observed for the reaction of the genotypes to *Fusarium* isolates but there were genotypes that always showed higher levels of disease and those with lower disease values to all isolates (Table 4.8). AC VISTA was among the five most susceptible genotypes to all isolates. On the other side, SHA/CBRD and NG8675/NING8645 were among the five most resistant genotypes to all *Fusarium* isolates.

Discussion

In the present study, aggressiveness of six *Fusarium* isolates originating from Canada, Iran, and CIMMYT, Mexico, was compared by inoculating them on 15 wheat genotypes from the same countries with differential levels of resistance to FHB to characterize differences between the Mexican isolates and the isolates received from other regions and to determine their host-pathogen interactions.

The two isolates of *F. boothii* received from CIMMYT, Mexico caused the least disease on almost all wheat genotypes with the least variation (Table 4.3) and means of FHB (Table 4.5) among the genotypes. On the other hand, the isolates of *F. graminearum sensu stricto* had higher mean disease values and variation on wheat genotypes with significant differences among the isolates (Tables 4.3 and 4.5). Low aggressiveness/pathogenicity or variation in *Fusarium* isolates can be attributed to the species of *Fusarium* or to the isolates of a FHB causal agent such as *F. graminearum* or *F. culmorum* (Bai and Shaner 1996; Mesterházy 1977; Mesterházy 1978, 1988; Snijders and Van Eeuwijk 1991). High variation in pathogenicity and aggressiveness has been observed among *F. graminearum* isolates from different geographical zones (Akinsanmi et al. 2004; Bai and Shaner 1996; Cullen et al. 1982; Cumagun et al. 2004; Gilbert et al. 2001; Goswami and Kistler 2005; Mesterházy 1978, 1984, 1988; Miedaner et al. 1996; Miedaner et al. 2000; Miedaner and Schilling 1996; Miedaner et al. 2001; Muthomi et al. 2000; Walker et al. 2001; Wu et al. 2005; Xue et al. 2004). Furthermore, isolates belonging to the *Fg* clade showed high levels of strain- and lineage-specific variation in their aggressiveness on susceptible wheat cultivars (Goswami and Kistler 2002; Goswami and Kistler 2005; Sanyal et al. 2000).

The two wheat genotypes, NG8675/NING8645 and SHA3/CBRD, consistently were among the five most resistant genotypes to disease severity regardless of the *Fusarium* species and isolates even though they showed lower disease values and consequently expressed more resistance when inoculated with *F. boothii* (Table 4.5). These lines may be valuable sources of stable type II resistance for wheat breeding programs. The occurrence of certain wheat genotypes with good resistance to all isolates of the two species tested is evidence that resistance to FHB does not have a strain-specific or species-specific basis. No strain-specific or species-specific resistance has been identified in wheat against FHB in the previous studies (Mesterházy 1981, 1987; Mesterházy 1997b). It is assumed that resistance to FHB has a horizontal or non-specific nature at least for the most prevalent species such as *F. graminearum* and *F. culmorum* (Mesterházy 1977; Mesterházy et al. 1999; Snijders and Van Eeuwijk 1991; Van Eeuwijk et al. 1995). However, pathogen-induced signal transduction pathways have been identified in wheat which are highly specific for particular pathogen strains and play a role in the wheat–*F. graminearum* interaction (Golkari et al. 2007).

Table 4.7. Comparison of least squares means and grouping of six *Fusarium* isolates based on the reaction of individual wheat genotypes under greenhouse conditions[a, b, c].

AC VISTA	ROBLIN	KANATA	93FHB37	5602 HR	N-83-5	N-81-8	N-82-14	N-83-6	N-82-13	CS/LE.RA//CS/3/PVN	CHUM18//JUP/BJY	MILAN/DUCULA	SHA3/CBRD	NG8675/NING8645
1 A	1 A	1 A	1 A	1 A	1 A	3 A	4 A	4 A	4 A	4 A	3 A	2 A	1 A	1 A
2 A	4 A	3 AB	4 AB	4 AB	4 AB	4 A	1 B	1 AB	3 AB	3 A	4 AB	1 A	4 B	2 A
4 A	2 A	4 BC	2 AB	3 BC	3 AB	1 A	3 BC	3 BC	2 AB	2 A	2 B	4 A	3 BC	4 A
3 B	3 AB	2 C	6 AB	2 CD	2 B	2 A	2 BC	2 C	1 BC	1 A	1 B	3 A	2 BC	3 B
6 BC	5 BC	5 D	3 AB	6 D	5 C	6 B	5 C	5 C	6 CD	6 B	6 B	6 B	6 C	6 BC
5 C	6 C	6 D	5 B	5 D	6 C	5 B	6 C	6 C	5 D	5 B	5 B	5 B	5 C	5 C

[a] Arcsine square root transformed data were used for data analysis and least squares means were compared according to Tukey-Kramer method at $P < 0.05$.

[b] Red, green, and yellow colours in the table represent Canadian, Iranian, and Mexican isolates, respectively: 1 = MSDS #3/03, 2 = DAOM 192131 , 3 = IR-13, 4 = IR-24A, 5 = CIMMYT-14, and 6 = CIMMYT-9.

[c] Isolates with the same letter in each column are not significantly different at $P < 0.05$.

Table 4.8. Comparison of least squares means and grouping of 15 wheat genotypes based on their reaction to individual *Fusarium* isolates under greenhouse conditions[a, b, c, d].

MSDS #3/03		DAOM 192131		IR-13		IR-24A		CIMMYT-14		CIMMYT-9	
13	A	13	A	7	A	13	A	2	A	1	A
2	A	1	AB	13	A	7	A	12	AB	12	AB
1	A	7	AB	12	AB	1	A	1	ABC	11	AB
7	A	2	AB	11	AB	11	A	11	ABCD	2	AB
11	AB	11	BC	1	BC	2	AB	6	BCDE	13	BC
3	AB	12	CD	2	BC	12	BC	13	BCDE	7	BC
5	AB	6	DE	3	CD	8	C	7	BCDE	5	BC
6	BC	3	DEF	6	CD	5	C	5	CDE	4	BC
12	BCD	10	DEF	10	DE	6	C	8	DE	10	C
8	CD	5	DEF	8	E	3	CD	9	DE	6	C
15	CD	8	EF	15	E	10	CDE	3	E	3	C
14	CD	14	EF	14	E	9	CDE	4	E	14	C
4	CD	4	EF	9	E	15	DE	14	E	8	C
9	D	9	F	4	E	14	DE	15	E	9	C
10	D	15	F	.	.	4	E	10	E	15	C

[a] Arcsine square root transformed data were used for data analysis and least squares means were compared according to Tukey-Kramer method at $P < 0.05$.

[b] Numbers 1-15 indicate the experimental wheat genotypes: 1 = AC VISTA, 2 = ROBLIN, 3 = KANATA, 4 = 93FHB37, 5 = 5602 HR, 6 = N-83-5, 7 = N-81-8, 8 = N-82-14, 9 = N-83-6, 10 = N-82-13, 11 = CS/LE.RA//CS/3/PVN, 12 = CHUM18//JUP/BJY, 13 = MILAN/DUCULA, 14 = SHA3/CBRD, and 15 = NG8675/NING8645.

[c] Red, green, and yellow colours in the table are representing Canadian, Iranian, and Mexican isolates, respectively.

[d] Genotypes with the same letter in each column are not significantly different at $P < 0.05$.

There were interactions between the isolates of the pathogen and wheat genotypes in the present study. In a 3-year study of FHB resistance, Mesterházy (1984) also found significant isolate x genotype interactions each year between 11 isolates of *F. graminearum* and two wheat genotypes. In a study of *F. culmorum* in wheat, a significant genotype x pathogen strain interaction was observed (Snijders 1987). Furthermore, Mesterházy (1988) observed significant interactions for the isolate x genotype using two isolates of *F. graminearum* and two isolates of *F. culmorum* inoculated on 21 wheat genotypes. Such isolate x genotype interactions were also reported by other investigators (Bai and Shaner 1996; Tóth et al. 2008).

It has been observed that advanced wheat lines/cultivars showing resistance to FHB at CIMMYT do not always show the same level of resistance in other regions (J. Gilbert, Pers. Comm.). Our results clearly showed the difference between the aggressiveness of *Fusarium* isolates used at CIMMYT *Fusarium* nurseries and those used in other regions, *e.g.* Canada and Iran, on different wheat genotypes. The *Fusarium* isolates used at CIMMYT *Fusarium* nurseries belong to *F. boothii* and *F. cerealis* (see Chapter 3) which are among the least aggressive *Fusarium* species (Tóth et al. 2008). It is also possible that an additional decrease in aggressiveness occurred for the isolates stored at CIMMYT before we received them. However, all wheat genotypes used in the present study developed less FHB following inoculation by CIMMYT isolates compared to the Canadian and Iranian isolates (Table 4.3).

CHAPTER 5

GENERAL DISCUSSION AND CONCLUSIONS

General discussion and conclusions

This dissertation has contributed new information towards the genetic analysis of resistance to fusarium head blight (FHB) in wheat as follows:

- Identified QTLs for resistance to FHB in a mapping population developed from the cross of a *Triticum timopheevii* derived FHB-resistant line, 'TC 67', and a moderately susceptible bread wheat cultivar, 'Brio'. The association between agronomic traits and resistance to FHB was also investigated.
- Determined phylogenetic lineages (= species) within the *Fusarium graminearum* clade (*Fg* clade) for *Fusarium* isolates from Canada, Iran, and CIMMYT, Mexico using *Tri101* gene sequencing data.
- Determined trichothecene chemotypes of the isolates based on *Tri12* gene multiplex PCR. The isolates were also investigated for aggressiveness patterns and variation.
- Clarified the host-pathogen interactions for *Fusarium* isolates and wheat genotypes from Canada, Iran, and CIMMYT, Mexico.

Development of and use of resistant wheat cultivars is the most practical and economic approach for control of FHB (Yang et al. 2005b). Research on FHB resistance as well as breeding efforts have mainly focused on introgressing resistance from Chinese sources. The 3BS QTL from the resistant Chinese line 'Sumai 3' and its derivatives, which confers resistance to disease spread within the spike, is widely used in wheat breeding programs. To avoid complete dependence on limited sources of resistance, finding new and different sources of resistance is a critical goal. *Triticum timopheevii* is a source of FHB resistance which is genetically more related to common and durum wheat

than other wild relatives. The FHB-resistant wheat line 'TC 67' derived from *T. timopheevii* most probably has a genetic basis of FHB resistance different from that found in Chinese sources.

We used a 'Brio'/'TC 67' derived population to map FHB resistance QTLs and to study the association between FHB resistance and agronomic traits. Using interval mapping (IM), a QTL was detected on chromosome 5AL derived from the resistant parent 'TC 67'. This QTL which is positioned between the markers *Xcfa2141* and *Xcfa2185* is a consistent QTL with major effects on type II (disease spread) and type IV (FDK) resistance. It is not evident whether one QTL with pleiotropic effects or two different QTLs at this region control the resistance to disease spread and FDK. Using single marker analysis (SMA), another QTL was detected on chromosome 5BS in the mapping population with a low and inconsistent effect on disease severity and FHB index under field conditions. This QTL was derived from the moderately susceptible parent 'Brio'. Our results showed gaps between the phenotypic variation that is potentially due to genetic effects (heritability values) and the amount of phenotypic variation covered by the QTLs. Therefore, it is possible that other QTLs especially minor QTLs and/or their epistatic interactions have not yet been identified in this population.

Alien relatives of wheat are one of the most important sources of FHB resistance which can be used to introgress and pyramid resistance QTLs/genes in wheat to enhance the level of resistance to the disease. This is the first report of QTLs on chromosomes 5AL and 5BS for FHB resistance from a population of wheat with a *T. timopheevii* background. Furthermore, we report for the first time a major QTL for both type II resistance and low FDK. The 'Brio'/'TC 67' population, especially the lines carrying the

major QTL detected in this study along with the SSR locus closely linked to it, provides germplasm for breeding FHB-resistant wheat varieties.

The association between agronomic traits and resistance to FHB was also investigated in the 'Brio'/'TC 67' derived population. Both plant height and number of days to anthesis had significant negative correlations with disease incidence, severity, index, and DON following spray inoculation under field conditions. So, the 5BS QTL for disease severity and index may be linked to these traits which is undesirable in wheat breeding as taller and late-maturing genotypes usually are not selected for commercial purposes. Furtunately, significant positive correlations were estimated for the association of number of days to anthesis with FDK and type II resistance which may be evidence of linkage of the 5AL QTL for low FDK and type II with early-maturity. This association may be due in part to the fact that kernels were already developing by the time infection occurred and were less severely affected by the disease than late-maturing genotypes in which kernel development had not begun. We observed correlations between spike threshability and both FDK and disease severity, *i.e.* genotypes with tough glumes were more resistant to the disease. This association indicates that there may be a linkage between the 5AL QTL detected in the present study and tough glumes which must be considered. Some correlations between agronomic traits and FHB were not strong. In general, the resistance found in alien species is usually associated with undesirable charactersitics which are not easy to remove from the genome (Bai and Shaner 2004) and may hinder introgression of FHB resistance QTLs/genes from alien sources to wheat lines. Our results showed a strong, consistent, and negative correlation between the presence of awns and FHB traits including disease incidence, disease spread, DON, and FDK. In contrast to our results, previous reports show that awned genotypes with a short

peduncle and a compact spike are more susceptible to FHB (Hilton et al. 1999; Mesterházy 1995; Parry et al. 1995; Rudd et al. 2001), even though there are exceptions.

The selection of pathogen isolates is important for *Fusarium* nurseries and screening FHB-resistant lines/cultivars and is the first step to adopting appropriate management strategies for disease control in wheat and other small grains. There is evidence that wheat genotypes displaying a resistant reaction to FHB at CIMMYT showed a more susceptible reaction in other locations (J. Gilbert, Pers. Comm.). To examine the profile of the pathogen from different locations, *Fusarium* isolates from Canada, Iran, and CIMMYT were investigated for phylogenetic features, trichothecene chemotypes, and aggressiveness.

We characterized the phylogenetic relationships among 58 isolates of putative *F. graminearum* using *Tri101* gene sequencing data. All Canadian and Iranian isolates clustered in one group and were identified as *F. graminearum* lineage 7 (= *F. graminearum sensu stricto*) within the *Fg* clade while the isolates received from CIMMYT were placed in *Fusarium boothii* within the *Fg* clade or were identified as *Fusarium cerealis*. This investigation characterized the *Fusarium* populations from three geographical zones and revealed large differences between the pathogens used in CIMMYT (Mexico) wheat nurseries and the isolates collected from Canada and Iran. This novel finding is important for testing wheat genotypes to detect their reaction to the disease in FHB nurseries, breeding wheat for resistance to FHB, and disease control measures. Previous reports showed that *F. graminearum sensu stricto* has a cosmopolitan distribution while *F. boothii* is endemic to Africa, Mexico, and Mesoamerica (O'Donnell et al. 2004).

Our results revealed the presence of the three chemotypes of NIV, 3-ADON, and 15-ADON among the isolates tested with 15-ADON as the predominant chemotype. Differences in chemotype production were observed among *Fusarium* isolates originating from different geographical zones: while the Iranian isolates were determined to be 3-ADON, 15-ADON, or NIV producers, the Canadian and Mexican isolates did not produce NIV. Both 3-ADON and 15-ADON chemotypes were found among the Canadian isolates while the Mexican isolates produced 15-ADON and NIV. This finding is evidence for the association of trichothecene chemotypes with geographical zones which has been observed in other studies (Desjardins et al. 2000; Jennings et al. 2004; Ji et al. 2007; Lee et al. 2001; Miller et al. 1991; Zhang et al. 2007) and may influence disease control practices in different locations. All *F. boothii* isolates from CIMMYT were identified as 15-ADON producers while all isolates of *F. cerealis* were determined to be the NIV chemotype. The presence of the 15-ADON chemotype among the isolates of different species supports the conclusion that trichothecene chemotypes have multiple evolutionary origins which are different from those of the species (O'Donnell et al. 2000; Ward et al. 2002). This finding also indicates that mycotoxin production within the *Fg* clade is not species-specific. There has been a shift from the dominant 15-ADON chemotype to the highly toxigenic 3-ADON chemotype in North America including in Canada (Ward et al. 2008) which was also confirmed among the Canadian isolates collected in the present study. Those collected in 1998 were uniformly a 15-ADON chemotype, but by 2004 more isolates produced 3-ADON. Replacing 15-ADON by 3_ADON may have negative consequences for wheat production and health in Canada as 3-ADON appears to be more toxigenic on wheat. However, these results may be modified by analysis of pathogen populations using larger sample sizes.

High variation in aggressiveness was observed among and within the species tested with the isolates of *F. graminearum sensu stricto* being the most aggressive species, followed by *F. boothii* and *F. cerealis*. Similar observations were made by Tóth et al. (2008). We conclude that aggressiveness is basically a species-specific trait. The possible negative effects of unsuitable long-term storage (*e.g.* lab bench vs -20 C) on aggressiveness of *Fusarium* isolates at CIMMYT should also be considered. Previous reports have shown that aggressiveness of *F. graminearum* isolates depends on their DON-producing capacity (Mesterházy 2002; Miedaner et al. 2000) and DON-producing isolates are more aggressive than NIV-producing isolates on plants (Cumagun et al. 2004; Desjardins et al. 2004; Goswami and Kistler 2005; Logrieco et al. 1990; Miedaner et al. 2000; Muthomi et al. 2000). This was confirmed in the present study by observing that NIV chemotypes had the lowest aggressiveness among all isolates.

As FHB is a significant threat to cereal production worldwide, information on the global distribution of FHB pathogen diversity is critical to identifying and implementing pathogen control strategies, and developing plant germplasm with broad resistance to a diverse complex of FHB pathogens.

We conclude that the inoculum used at CIMMYT FHB nurseries is originally from the less aggressive *F. boothii* or *F. cerealis* isolates while the highly aggressive *F. graminearum sensu stricto* prevails elsewhere and is used for wheat screening. Therefore, it is possible that the inoculum used at CIMMYT failed as a strong screening tool leading to selection of wheat genotypes that were not resistant to *F. graminearum sensu stricto*.

In spite of high variation in aggressiveness among the isolates of *Fusarium* species, there is no evidence for stable pathogen races (Bai and Shaner 1996; Mesterházy 1984, 1988; Mesterházy 2003; Snijders and Van Eeuwijk 1991; Wang and Miller 1987).

On the other hand, resistance to FHB in wheat is usually stable, and resistant genotypes demonstrate a consistent reaction to different species and isolates of *Fusarium* species. Therefore, it appears that resistance to FHB is horizontal or non-specific (Mesterházy 1977; Mesterházy 1981, 1987; Mesterházy 1997a; Mesterházy et al. 1999; Snijders and Van Eeuwijk 1991; Van Eeuwijk et al. 1995). For the final part of the present study we investigated host-pathogen interactions of *Fusarium* isolates and wheat genotypes from Canada, Iran, and CIMMYT by inoculating representative isolates of *F. graminearum sensu stricto* and *F. boothii* on wheat genotypes with different levels of resistance to FHB. The representative isolates of *F. boothii* used at CIMMYT produced the least disease on all wheat genotypes tested except one while *F. graminearum sensu stricto* isolates from Canada and Iran had higher FHB values on wheat genotypes. The CIMMYT isolates resulted in low disease values on wheat genotypes leading to expression of resistant reactions in wheat regardless of the origin of the genotypes. We observed significant differences among the genotypes inoculated by single isolates of the pathogen and two of the more recent CIMMYT wheat genotypes, NG8675/NING8645 and SHA3/CBRD, consistently were among the most resistant genotypes to disease spread regardless of the *Fusarium* species or isolates inoculated. Our results also showed significant interactions between the *Fusarium* isolates and wheat genotypes used in the present study which confirms previous reports (Bai and Shaner 1996; Mesterházy 1984, 1988; Snijders 1987; Tóth et al. 2008).

REFERENCES

Abate, Z.A., Liu, S., and McKendry, A.L. 2008. Quantitative trait loci associated with deoxynivalenol content and kernel quality in the soft red winter wheat 'Ernie'. Crop Sci. **48**: 1408-1418.

Abbas, H.K., Mirocha, C.J., and Tuite, J. 1986. Natural occurrence of deoxynivalenol, 15-acetyl-deoxynivalenol, and zearalenone in refusal factor corn stored since 1972. Appl. Environ. Microbiol. **51**: 841-843.

Abramson, D., Clear, R.M., and Smith, D.M. 1993. Trichothecene production by *Fusarium* ssp. isolated from Manitoba grain. Can. J. Plant Pathol. **15**: 147-152.

Afshari-Azad, H. 1992. Produktion extracellularer, hydrolytischer Enzyme von *Pseudocercosporella herpotrichoides* (Fron) Deighton, *Fusarium culmorum* (W. G. Smith) Sacc. sowie *Rhizoctonia cerealis in vitro* bzw. in planta und ihre Beziehung zur Pathogenese. Georg-August-Universitaet Göttingen, Göttingen, Germany. p. 125 pp.

Akinsanmi, O.A., Backhouse, D., Simpfendorfer, S., and Chakraborty, S. 2006. Genetic diversity of Australian *Fusarium graminearum* and *F. pseudograminearum*. Plant Pathol. **55**: 494-504.

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

Alexander, N.J., Hohn, T.M., and McCormick, S.P. 1998. The *TRI11* gene of *Fusarium sporotrichioides* encodes a cytochrome P-450 monooxygenase required for C-15 hydroxylation in trichothecene biosynthesis. Appl. Environ. Microbiol. **64**: 221-225.

Ali, S., and Francl, L. 2001. Progression of *Fusarium* species on wheat leaves from seedling to adult stages in North Dakota. *In* Proceedings of 2001 National Fusarium Head Blight Forum. *Edited by* S. M. Canty, J. Lewis, L. Siler, and R. W. Ward, Erlanger, KY, USA. p. 99.

Alvarez, C.L., Azcarate, M.P., and Pinto, V.F. 2009. Toxigenic potential of *Fusarium graminearum* sensu stricto isolates from wheat in Argentina. Int. J. Food Microbiol. **135**: 131-135.

Andersen, A.L. 1948. The development of *Gibberella zeae* head blight of wheat. Phytopathology **38**: 595-611.

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

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

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

Aoki, T., and O'Donnell, K. 1999b. Morphological characterization of *Gibberella coronicola* sp. nov., obtained through mating experiments of *Fusarium pseudograminearum*. Mycoscience **40**: 443-453.

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

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

Atanasoff, D. 1924. Fusarium blight of the cereal crops. Mededelingen van de Landbouwhogeschool **27**: 1-132.

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

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

Bai, G.-H., Chen, L.F., and Shaner, G.E. 2003a. Breeding for resistance to *Fusarium* head blight of wheat in China. *In* Fusarium Head Blight of Wheat and Barley. *Edited by* K. J. Leonard, and W. R. Bushnell. APS Press, St Paul, MN, USA. pp. 296-317.

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

Bai, G.-H., Guo, P., and Kolb, F.L. 2003b. Genetic relationships among head blight resistant cultivars of wheat assessed on the basis of molecular markers. Crop Sci. **43**: 498-507.

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

Bai, G.-H., Plattner, R., Desjardins, A.E., and Kolb, F.L. 2001b. Resistance to Fusarium head blight and deoxynivalenol accumulation in wheat. Plant Breed. **120**: 1-6.

Bai, G.-H., Plattner, R., Shaner, G.E., and Kolb, F.L. 2000a. A QTL for deoxynivalenol tolerance in wheat. Phytopathology **90**: S4 (Abstr.).

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

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

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

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

Bai, G.-H., Shaner, G.E., and Ohm, H.W. 1993. Inheritance of resistance to *Fusarium graminearum* in eight wheat cultivars. Phytopathology **83**: 1414 (Abstr.).

Bai, G.-H., Xiao, Q.P., and Mei, J.F. 1989a. Studies on the inheritance of scab resistance in six wheat varieties. Acta Agric. Shanghai **5**: 17-23.

Bai, G.-H., Zhou, C.-F., Ge, Y.-F., Qian, C.-M., Chen, Z.-D., and Yao, G.-C. 1989b. A study on scab-resistance in new wheat cultivars and advanced lines. Jiangsu Agric. Sci. **7**: 20-22.

Bai, G.-H., Zhou, C.-F., Qian, C.-M., Xia, S.-S., and Ge, Y.-F. 1989c. An analysis on combining ability of resistance to scab and other characters in eight wheat cultivars. Jiangsu Agric. Sci. **1** 79-83 (Suppl.).

Bai, G.-H., Zhou, C.-F., Qian, C.-M., Xia, S.-S., Ge, Y.-F., and Chen, Z.-D. 1990. Genetic analysis on resistant genes to scab extension in wheat cultivars. *In* Advances in Researches on Inheritance to Diseases in Major Crops. *Edited by* L. -H. Zhu. Jiangsu Science-Tech. Publishing House, Nanjing, China (in Chinese). pp. 171-177.

Ban, T. 1997. Evaluation of resistance to *Fusarium* head blight in indigenous Japanese species of *Agropyron* (*Elymus*). Euphytica **97**: 39-44.

Ban, T. 2000. Review-study on the genetics of resistance to *Fusarium* head blight caused by *Fusarium graminearum* in wheat. *In* Proceedings of the International Symposium on Wheat Improvement for Scab Resistance,. Suzhou and Nanjing, China. pp. 82-93.

Ban, T. 2001. Studies on the genetics of resistance to Fusarium head blight caused by *Fusarium graminearum* Schwabe in wheat (*Triticum aestivum* L.). Bull. Kyushu Natl. Agric. Exp. Stn. **38**: 27-78.

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

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

Basten, C.J., Weir, B.S., and Zeng, Z.-B. 1997. QTL cartographer: A reference manual and

tutorial for QTL mapping Dept. of Statistics, North Carolina State Univ. , Raleigh, NC, USA.

Beavis, W.D. 1998. QTL analysis: Power, precision and accuracy. *In* Molecular Dissection of Complex Traits. *Edited by* A. H. Paterson. CRC Press, Boca Raton, FL, USA. pp. 145-161.

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

Bennett, F.T. 1933. *Fusarium* species on British cereals. Ann. Appl. Biol. **20**: 272-290.

Bennett, J.W., and Klich, M. 2003. Mycotoxins. Clin. Microbiol. Rev. **16**: 497-516.

Blaney, B.J., and Dodman, R.L. 1988. Production of the mycotoxins zearalenone 4-deoxynivalenol and nivalenol by isolates of *Fusarium graminearum* groups 1 and 2 from cereals in Queensland Australia. Aust. J. Agric. Res. **39**: 21-29.

Bourdoncle, W., and Ohm, H.W. 2003. Quantitative trait loci for resistance to Fusarium head blight in recombinant inbred wheat lines from the cross Huapei 57-2/Patterson. Euphytica **131**: 131-136.

Bowden, J.L., and Leslie, J.F. 1994. Diversity of *Gibberella zeae* at small spatial scales. Phytopathology **84**: 1140 (Abstr.).

Bowden, R.L., and Leslie, J.F. 1992. Nitrate-nonutilizing mutants of *Gibberella zeae* (*Fusarium graminearum*) and their use in determining vegetative compatibility. Exp. Mycol. **16**: 308-315.

Bowden, R.L., and Leslie, J.F. 1999. Sexual recombination in *Gibberella zeae*. Phytopathology **89**: 182-188.

Bowden, R.L., Leslie, J.F., Lee, J., and Lee, Y.-W. 2006. Cross fertility of lineages in *Fusarium graminearum* (*Gibberella zeae*). *In* The Global Fusarium Iniatiative for International Collaboration, A Strategic Planning Workshop. *Edited by* T. Ban, J. M. Lewis, and E. E. Phipps. CIMMYT, Mexico D.F., Mexico. pp. 54-60.

Breseghello, F., and Sorrells, M.E. 2006. Association mapping of kernel size and milling quality in wheat (*Triticum aestivum* L.) cultivars. Genetics **172**: 1165-1177.

Brown, D.W., McCormick, S.P., Alexander, N.J., Proctor, R.H., and Desjardins, A.E. 2001. A genetic and biochemical approach to study trichothecene diversity in *Fusarium sporotrichioides* and *Fusarium graminearum*. Fungal Genet. Biol. **32**: 121-133.

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

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

Buerstmayr, H., Lemmens, M., Grausgruber, H., and Ruekenbauer, P. 1996. Scab resistance of international wheat germplasm. Cereal Res. Commun. **24**: 195-202.

Buerstmayr, H., Lemmens, M., Hartl, L., Doldi, L., Steiner, B., Stierschneider, M., and Ruckenbauer, P. 2002. Molecular mapping of QTLs for Fusarium head blight resistance in spring wheat. I. Resistance to fungal spread (Type II resistance). Theor. Appl. Genet. **104**: 84-91.

Buerstmayr, H., Lemmens, M., Schmolke, M., Zimmermann, G., Hartl, L., Mascher, F., Trottet, M., Gosman, N.E., and Nicholson, P. 2008. Multi-environment evaluation of level and stability of FHB resistance among parental lines and selected offspring derived from several European winter wheat mapping populations. Plant Breed. **127**: 325-332.

Buerstmayr, H., Steiner, B., Hartl, L., Griesser, M., Angerer, N., Lengauer, D., Miedaner, T., Schneider, B., and Lemmens, M. 2003a. Molecular mapping of QTLs for Fusarium

head blight resistance in spring wheat. II. Resistance to fungal penetration and spread. Theor. Appl. Genet. **107**: 503-508.

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

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

Burdon, J.J. 1993. The structure of pathogen populations in natural plant communities. Ann. Rev. Phytopathology **31**: 305-323.

Burgess, L.W., and Griffin, D.M. 1968. The recovery of *Gibberella zeae* from wheat straws. Aust. J. Exp. Agric. Anim. Husb. **8**: 364-370.

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

Burlakoti, R.R., Ali, S., Secor, G.A., Neate, S.M., McMullen, M.P., and Adhikari, T.B. 2008. Genetic relationships among populations of *Gibberella zeae* from barley, wheat, potato, and sugar beet in the upper Midwest of the United States. Phytopathology **98**: 969-976.

Bushnell, W.R., Hazen, B.E., and Pritsch, C. 2003. Histology and physiology of *Fusarium* head blight. *In* Fusarium Head Blight of Wheat and Barley. *Edited by* K. J. Leonard, and W. R. Bushnell. APS Press, St Paul, MN, USA. pp. 44-83.

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

Caldwell, R.W. 1968. Zearalenone (RAL) production among *Fusarium* species. Purdue Univ., West Lafayette, IN, USA.

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

Cao, W., Fedak, G., Armstrong, K., Xue, A., and Savard, M.E. 2009. Registration of Spring Wheat Germplasm TC 67 Resistant to Fusarium Head Blight. Journal of Plant Registrations **3**: 104-106.

Caron, D. 1993. Les Fusarioses. *In* Maladies des blés et orges. ITCF. pp. 30-39.

Caron, D. 2000. Fusarioses des épis. Sait-on prévoir leur développement? Perspect. Agricoles **253**: 56-62.

Carter, J.P., Rezanoor, H.N., Desjardins, A.E., and Nicholson, P. 2000. Variation in *Fusarium graminearum* isolates from Nepal associated with their host of origin. Plant Pathol. **49**: 452-460.

Carter, J.P., Rezanoor, H.N., Holden, D., Desjardins, A.E., Plattner, R.D., and Nicholson, P. 2002. Variation in pathogenicity associated with the genetic diversity of *Fusarium graminearum*. Eur. J. Plant Pathol. **108**: 573-583.

Cassini, R. 1970. Facteurs favorables ou défavorables au développement des fusarioses et septorioses du blé. *In* Proceedings of the Meeting of Sections Cereals and Physiology, Dijon, France. pp. 271-279.

Champeil, A., Doré, T., and Fourbet, J.F. 2004. *Fusarium* head blight: epidemiological origin of the effects of cultural practices on head blight attacks and the production of mycotoxins by *Fusarium* in wheat grains. Plant Sci. **166**: 1389-1415.

Chen, C.-H. 1983. A study on the inheritance of scab-resistance in wheat. Acta Agric. Univ. Zhejiangensis **9**: 115-126.

Chen, J., Griffey, C.A., Maroof, M.A.S., Stromberg, E.L., Biyashev, R.M., Zhao, W., Chappell, M.R., Pridgen, T.H., Dong, Y., and Zeng, Z. 2006. Validation of two major quantitative trait loci for fusarium head blight resistance in Chinese wheat line W14. Plant Breed. **125**: 99-101.

Chen, P., Liu, W., Yuan, J., Wang, X., Zhou, B., Wang, S., Zhang, S., Feng, Y., Yang, B., Liu, G., Liu, D., Qi, L., Zhang, P., Friebe, B., and Gill, B.S. 2005. Development and characterization of wheat- *Leymus racemosus* translocation lines with resistance to *Fusarium* Head Blight. Theor. Appl. Genet. **111**: 941-948.

Chen, P.D., and Liu, D.J. 2000. Transfer of scab resistance genes from *Leymous racemosus*, *Roegneria ciliaris*, and *Roegneria kamoji* into common wheat. *In* Proceedings

of the International Symposium on Wheat Improvement for Scab Resistance, Suzhou and Nanjing, China. pp. 62-67.

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

Chen, Q., Eudes, F., Conner, R.L., Graf, R., Comeau, A., Collin, J., Ahmad, F., Zhou, R., Li, H., Zhao, Y., and Laroche, A. 2001. Molecular cytogenetic analysis of a durum wheat x *Thinopyrum distichum* hybrid used as a new source of resistance to Fusarium head blight in the greenhouse. Plant Breed. **120**: 375-380.

Chen, W.P., Chen, P.D., Liu, D.J., Kynast, R., Friebe, B., Velazhahan, R., Muthukrishnan, S., and Gill, B.S. 1999. Development of wheat scab symptoms is delayed in transgenic wheat plants that constitutively express a rice thaumatin-like protein gene. Theor. Appl. Genet. **99**: 755-760.

Chen, X., Faris, J.D., Hu, J., Stack, R.W., Adhikari, T., Elias, E.M., Kianian, S.F., and Cai, X. 2007a. Saturation and comparative mapping of a major Fusarium head blight resistance QTL in tetraploid wheat. Mol. Breed. **19**: 113-124.

Chen, Y., Wang, J.-X., Zhou, M.-G., Chen, C.-J., and Yuan, S.-K. 2007b. Vegetative compatibility of *Fusarium graminearum* isolates and genetic study on their carbendazim-resistance recombination in China. Phytopathology **97**: 1584-1589.

Cherewick, W.J., and Robinson, A.G. 1958. A rot of smutted inflorescence of cereals by *Fusarium poae* in association with the mite *Siteroptes graminum*. Phytopathology **48**: 232-234.

Chester, F.D. 1890. The scab of the wheat. Delaware Agric. Exp. Stn. Rep. 3.

Chongo, G., Gossen, B.D., Kutcher, H.R., Gilbert, J., Turkington, T.K., Fernandez, M.R., and McLaren, D. 2001. Reaction of seedling roots of 14 crop species to *Fusarium graminearum* from wheat heads. Can. J. Plant Pathol. **23**: 132-137.

Christensen, J.J., Stakman, E.C., and Immer, F.R. 1929. Susceptibility of wheat varieties and hybrids to fusarial head blight in Minnesota. Minn. Agric. Exp. Stn. Bull. 59.

Clarke, J., Thomas, J., Fedak, G., Somers, D.J., Gilbert, J., Pozniak, C., Fernandez, M., and Comeau, A. 2004. Progress in improvement of *Fusarium* resistance of durum wheat. *In* Proceedings of 2nd International Symposium on Fusarium Head Blight, Orlando, FL, USA. p. 43.

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

Clement, J.A., and Parry, D.W. 1998. Stem-base disease and fungal colonisation of winter wheat grown in compost inoculated with *Fusarium culmorum*, *F. graminearum* and *Microdochium nivale*. Eur. J. Plant Pathol. **104**: 323-330 (Abstr.).

Cooper, K.W. 1940. Relations of *Pedicolopsis graminum* and *Fusarium poae* to control of bud rot of carnations. Phytopathology **30**: 853-859.

Cullen, D., Caldwell, R.W., and Smalley, E.B. 1982. Cultural characteristics, pathogenicity, and zearalenone production by strains of *Gibberella zeae* isolated from corn. Phytopathology **72**: 1415-1418.

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

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

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

de Luna, L., Bujold, I., Carisse, O., and Paulitz, T.C. 2002. Ascospore gradients of *Gibberella zeae* from overwintered inoculum in wheat fields. Can. J. Plant Pathol. **24**: 457-464.

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

Del Ponte, E.M., Shah, D.A., and Bergstrom, G.C. 2002. Spatial patterns of Fusarium head blight in New York wheat fields in 2002. *In* Proceedings of 2002 National Fusarium

Head Blight Forum. *Edited by* S. M. Canty, J. Lewis, L. Siler, and R. W. Ward, Erlanger, KY, USA. p. 136.

Desjardins, A.E., Jarosz, A.M., Plattner, R.D., Alexander, N.J., Brown, D.W., and Jurgenson, J.E. 2004. Patterns of trichothecene production, genetic variability, and virulence to wheat of *Fusarium graminearum* from small holder farms in Nepal. J. Agric. Food Chem. **52**: 6341-6346.

Desjardins, A.E., Manandhar, G., Plattner, R.D., Maragos, C.M., Shrestha, K., and McCormick, S.P. 2000. Occurrence of *Fusarium* species and mycotoxins in Nepalese maize and wheat and the effect of traditional processing methods on mycotoxin levels. J. Agric. Food Chem. **48**: 1377-1383.

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

Detmers, F. 1892. Scab of wheat *(Fusisporium (Fusarium* Sacc.) *culmorum)*. Ohio Agric. Exp. Stn. Bull. **44**: 147-149.

Dexter, J.E., Clear, R.M., and Preston, K.R. 1996. Fusarium head blight: Effect on the milling and baking of some Canadian wheats. Cereal Chem. **73**: 695-701.

Dexter, J.E., Marchylo, B.A., Clear, R.M., and Clarke, J.M. 1997. Effect of fusarium head blight on semolina milling and pasta-making quality of durum wheat. Cereal Chem. **74**: 519-525.

Dickson, J.G. 1923. Influence of soil temperature and miosture on the development of the seedling blight of wheat and corn caused by *Gibberella saubinetii*. J. Agric. Res. **23**: 837-882.

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

Dickson, J.G., and Mains, E.B. 1929. Scab of wheat and barley and its control. USDA Farmers' Bull. No. 1599.

Dill-Macky, R. 1999. Residue management and fusarium head blight of wheat. *In* Proceedings of the 1st Canadian Workshop on Fusarium Head Blight. *Edited by* R. Clear, Winnipeg, MB, Canada. pp. 76-78.

Dill-Macky, R., and Jones, R.K. 2000. The effect of previous crop residues and tillage on Fusarium head blight of wheat. Plant Dis. **84**: 71-76.

Draeger, R., Gosman, N., Steed, A., Chandler, E., Thomsett, M., Srinivasachary, Schondelmaier, J., Buerstmayr, H., Lemmens, M., Schmolke, M., Mesterhazy, A., and Nicholson, P. 2007. Identification of QTLs for resistance to Fusarium head blight, DON accumulation and associated traits in the winter wheat variety Arina. Theor. Appl. Genet. **115**: 617-625.

Dufault, N., De Wolf, E., Lipps, P., and Madden, L. 2002a. Identification of environmental variables that affect perithecial development of *Gibberella zeae*. *In* Proceedings of 2002 National Fusarium Head Blight Forum. *Edited by* S. M. Canty, J. Lewis, L. Siler, and R. W. Ward, Erlanger, KY, USA. p. 141.

Dufault, N., De Wolf, E., Lipps, P., and Madden, L. 2002b. Relationship of temperature and moisture to *Gibberella zeae* perithecial development in a controlled environment. *In* Proceedings of 2002 National Fusarium Head Blight Forum. *Edited by* S. M. Canty, J. Lewis, L. Siler, and R. W. Ward, Erlanger, KY, USA. pp. 142-144.

Dusabenyagasani, M., Dostaler, D., and Hamelin, R.C. 1999. Genetic diversity among *Fusarium graminearum* strains from Ontario and Quebec. Can. J. Plant Pathol. **21**: 308-314.

Ehling, G., Cockburn, A., Snowdon, P., and Buschhaus, H. 1997. The significance of the Fusarium toxin deoxynivalenol (DON) for human and animal health. Cereal Res. Commun. **25**: 443-447

Engle, J.S., Lipps, P.E., Graham, T.L., and Boehm, M.J. 2004. Effects of choline, betaine, and wheat floral extracts on growth of *Fusarium graminearum*. Plant Dis. **88**: 175-180.

Eudes, F., Comeau, A., Rioux, S., and Collin, J. 2001. Impact of trichothecenes on Fusarium head blight (*Fusarium graminearum*) development in spring wheat (*Triticum aestivum*). Can. J. Plant Pathol. **23**: 318-322.

Eugenio, C.P. 1968. Factors influencing the biosynthesis of the fungal estrogen (F-2) and the effects of F-2 on perithecia formation by *Fusarium* species. Univ. Minnesota, St. Paul, MN, USA. p. 76

Faifer, G.C., De Miguel, M.S., and Godoy, H.M. 1990. Patterns of mycotoxin production by *Fusarium graminearum* isolated from Argentine wheat. Mycopathologia **109**: 165-170.

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

Fedak, G., Cao, W., Gilbert, J., Xue, A.G., Savard, M.E., and Voldeng, H.D. 2009. Two new sources of FHB resistance in bread wheat *In* 6th Canadian Workshop on Fusarium Head Blight. *Edited by* T. Ouellet, Don Leger, and J Thompson, Ottawa, Ontario, Canada p. 62.

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

Fedak, G., Han, F., Cao, W., Burvill, M., Kritenko, S., and Wang, L. 2003. Identification and characterization of novel sources of resistance to FHB. *In* Proceedings of 10th International Wheat Genetics Symposium, Paestum, Italy. pp. 354-356.

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

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

Fernando, W.G.D., Zhang, J.X., Dusabenyagasani, M., Guo, X.W., Ahmed, H., and McCallum, B. 2006. Genetic diversity of *Gibberella zeae* isolates from Manitoba. Plant Dis. **90**: 1337-1342.

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

Fuentes, R.G., Mickelson, H.R., Busch, R.H., Dill-Macky, R., Evans, C.K., Thompson, W.G., Wiersma, J.V., Xie, W., Dong, Y., and Anderson, J.A. 2005. Resource allocation and cultivar stability in breeding for Fusarium head blight resistance in spring wheat. Crop Sci. **45**: 1965-1972.

Gagkaeva, T.Y. 2003. Importance of Fusarium head blight in Russia and the search for new sources of genetic resistance in wheat and barley. *In* Proceedings of 2003 National Fusarium Head Blight Forum. *Edited by* S. M. Canty, J. Lewis, and R. W. Ward, Bloomington, MN, USA. pp. 219-222.

Gagkaeva, T.Y., and Yli-Mattila, T. 2004. Genetic diversity of *Fusarium graminearum* in Europe and Asia. Eur. J. Plant Pathol. **110**: 551-562.

Gale, L.R., Bryant, J.D., Calvo, S., Giese, H., Katan, T., O'Donnell, K., Suga, H., Taga, M., Usgaard, T.R., Ward, T.J., and Kistler, H.C. 2005. Chromosome complement of the fungal plant pathogen *Fusarium graminearum* based on genetic and physical mapping and cytological observations. Genetics **171**: 985-1001.

Gale, L.R., Chen, L.F., Hernick, C.A., Takamura, K., and Kistler, H.C. 2002. Population analysis of *Fusarium graminearum* from wheat fields in eastern China. Phytopathology **92**: 1315-1322.

Gale, L.R., Ward, T.J., Balmas, V., and Kistler, H.C. 2007. Population subdivision of *Fusarium graminearum* sensu stricto in the upper midwestern United States. Phytopathology **97**: 1434-1439.

Gang, G., Miedaner, T., Schuhmacher, U., Schollenberger, M., and Geiger, H.H. 1998. Deoxynivalenol and nivalenol production by *Fusarium culmorum* isolates differing in aggressiveness toward winter rye. Phytopathology **88**: 879-884.

Gardes, M., and Bruns, T.D. 1993. ITS primers with enhanced specificity for basidiomycetes - application to the identification of mycorrhizae and rusts. **2**: 113-118.

Garvin, D.F., Stack, R.W., and Hansen, J.M. 2003. Genetic analysis of extreme *Fusarium* head blight susceptibility conferred by a wild emmer chromosome. *In* Proceedings of 10th International Wheat Genetics Symposium, Paestum, Italy. pp. 1139-1141.

Garvin, D.F., Stack, R.W., and Hansen, J.M. 2009. Quantitative trait locus mapping of increased Fusarium head blight susceptibility associated with a wild emmer wheat chromosome. Phytopathology **99**: 447-452.

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

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

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

Gilbert, J., and Fernando, W.G.D. 2004. Epidemiology and biological control of *Gibberella zeae* / *Fusarium graminearum*. Can. J. Plant Pathol. **26**: 464-472.

Gilbert, J., Fernando, W.G.D., and Ahmed, H. 2003. Colonization of field stubble by *Fusarium graminearum*. *In* Proceedings of IXth International Fusarium Workshop, Sydney, Australia. p. 35.

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

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

Gilchrist, L., Rajaram, S., Mujeeb-kazi, A., van Ginkel, M., Vivar, H., and Pfeiffer, W. 1997. *Fusarium* scab screening program at CIMMYT. *In* Fusarium Head Scab: Global Status and Future Prospects. *Edited by* H. J. Dubin, L. Gilchrist, J. Reeves, and A. McNab. CIMMYT, Mexico D.F., Mexico. pp. 7-12.

Gilsinger, J., Kong, L., Shen, X., and Ohm, H. 2005. DNA markers associated with low Fusarium head blight incidence and narrow flower opening in wheat. Theor. Appl. Genet. **110**: 1218-1225.

Gladysz, C., Lemmens, M., and Buerstmayr, H. 2004. QTL mapping of novel resistance sources in tetraploid wheat. *In* Proceedings of 2nd International Symposium on Fusarium Head Blight, Orlando, FL, USA. p. 63.

Golkari, S., Gilbert, J., Prashar, S., and Procunier, J.D. 2007. Microarray analysis of *Fusarium graminearum*-induced wheat genes: identification of organ-specific and differentially expressed genes. Plant Biotechnol. J. **5**: 38-49.

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

Gordon, W.L. 1959. The occurrence *of Fusarium* species in Canada. VI. Taxonomy and geographic distribution *of Fusarium* species on plants, insects and fungi. Can. J. Bot. **37**: 257-290.

Gosman, N., Bayles, R., Jennings, P., Kirby, J., and Nicholson, P. 2007. Evaluation and characterization of resistance to fusarium head blight caused by *Fusarium culmorum* in UK winter wheat cultivars. Plant Pathol. **56**: 264-276.

Goswami, R.S., and Kistler, H.C. 2002. Assessment of the differential ability of *Fusarium graminearum* to spread on wheat and rice. *In* Proceedings of 2002 National Fusarium Head Blight Forum. *Edited by* S. M. Canty, J Lewis, L. Siler, and R Ward, Erlanger, KY, USA. p. 163.

Goswami, R.S., and Kistler, H.C. 2005. Pathogenicity and in planta mycotoxin accumulation among members of the *Fusarium graminearum* species complex on wheat and rice. Phytopathology **95**: 1397-1404.

Guo, P.G., Bai, G.H., and Shaner, G.E. 2003. AFLP and STS tagging of a major QTL for Fusarium head blight resistance in wheat. Theor. Appl. Genet. **106**: 1011-1017.

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

Gupta, P.K., Balyan, H.S., Edwards, K.J., Isaac, P., Korzun, V., Roeder, M., Gautier, M.F., Joudrier, P., Schlatter, A.R., Dubcovsky, J., de la Pena, R.C., Khairallah, M., Penner, G., Hayden, M.J., Sharp, P., Keller, B., Wang, R.C.C., Hardouin, J.P., Jack, P., and Leroy, P. 2002. Genetic mapping of 66 new microsatellite (SSR) loci in bread wheat. Theoretical & Applied Genetics **105**: 413-422.

Gupta, P.K., Rustgi, S., and Kulwal, P.L. 2005. Linkage disequilibrium and association studies in higher plants: Present status and future prospects. Plant Mol. Biol. **57**: 461-485.

Guyomarc'h, H., Sourdille, P., Charmet, G., Edwards, K.J., and Bernard, M. 2002. Characterisation of polymorphic microsatellite markers from Aegilops tauschii and transferability to the D-genome of bread wheat. Theoretical & Applied Genetics **104**: 1164-1172.

Häberle, J., Holzapfel, J., Schweizer, G., and Hartl, L. 2009. A major QTL for resistance against Fusarium head blight in European winter wheat. Theor. Appl. Genet. **119**: 325-332.

Häberle, J., Schmolke, M., Schweizer, G., Korzun, V., Ebmeyer, E., Zimmermann, G., and Hartl, L. 2007. Effects of two major fusarium head blight resistance QTL verified in a winter wheat backcross population. Crop Sci. **47**: 1823-1831.

Hallauer, A.R., and Miranda, J.B. 1981. Quantitative genetics in maize breeding. Iowa State Univ. Press, Ames, IA, USA.

Han, F., and Fedak, G. 2003. Molecular characterization of partial amphiploids from *Triticum durum* x tetraploid *Thinopyrum elongatum* as novel sources of resistance to wheat Fusariumhead blight. *In* Proceedings of 10th International Wheat Genetics Symposium, Paestum, Italy. pp. 1148-1150.

Handa, H., Namiki, N., Xu, D., and Ban, T. 2008. Dissecting of the FHB resistance QTL on the short arm of wheat chromosome 2D using a comparative genomic approach: from QTL to candidate gene. Mol. Breed. **22**: 71-84.

Hanson, E.W., Ausemus, E.R., and Stakman, E.C. 1950. Varietal resistance of spring wheats to Fusarial head blight. Phytopathology **40**: 902-914.

Haratian, M., Sharifnabi, B., Alizadeh, A., and Safaie, N. 2008. PCR analysis of the *Tri13* gene to determine the genetic potential of *Fusarium graminearum* isolates from Iran to produce nivalenol and deoxynivalenol. Mycopathologia **166**: 109-116.

Harris, L.J., Desjardins, A.E., Plattner, R.D., Nicholson, P., Butler, G., Young, J.C., Weston, G., Proctor, R.H., and Hohn, T.M. 1999. Possible role of trichothecene mycotoxins in virulence of *Fusarium graminearum* on maize. Plant Dis. **83**: 954-960.

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

Hoffer, G.N., Johnson, A.G., and Atanasoff, D. 1918. Corn root rot and wheat scab. J. Agric. Res. **14**: 611-612.

Hörberg, H.M. 2002. Patterns of splash dispersed conidia of *Fusarium poae* and Fusarium *culmorum*. Eur. J. Plant Pathol. **108**: 73-80.

Hutcheon, J.A., and Jordan, V.W.L. 1992. Fungicide timing and performance for *Fusarium* control in wheat. *In* Proceedings of the Brighton Crop Protection Conference-Pests and Diseases, Vol. 2. BCPC Publication, Farnham, UK. pp. 633-638.

Ichinoe, M., Kurata, H., Sugiura, Y., and Ueno, Y. 1983. Chemotaxonomy of *Gibberella zeae* with special reference to production of trichothecenes and zearalenone. Appl. Environ. Microbiol. **46**: 1364-1369.

Inch, S., Fernando, W.G.D., Gilbert, J., and Tekauz, A. 2000. Temporal aspects of ascospore and macroconidia release by *Gibberella zeae* and *Fusarium graminearum* Can. J. Plant Pathol. **22**: 186.

Inch, S., and Gilbert, J. 2003a. The incidence of *Fusarium* species recovered from inflorescences of wild grasses in southern Manitoba. Can. J. Plant Pathol. **25**: 379-383.

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

Ittu, M., Saulescu, N.N., Hagima, I., Ittu, G., and Mustatea, P. 2000. Association of Fusarium head blight resistance with gliadin loci in a winter wheat cross. Crop Sci. **40**: 62-67.

Jauhar, P.P., and Peterson, T.S. 1998. Wild relatives of wheat as sources of Fusarium head blight resistance. *In* Proceedings of the 1998 National Fusarium Head Blight Forum. *Edited by* P. Hart, R. W. Ward, R. Bafus, and K. Bedford, East Lansing, MI, USA. pp. 179-181.

Jauhar, P.P., and Peterson, T.S. 2001. Hybrids between durum wheat and *Thinopyrum junceiforme*: Prospects for breeding for scab resistance. Euphytica **118**: 127-136.

Jenczmionka, N.J., Maier, F.J., Loesch, A.P., and Schaefer, W. 2003. Mating, conidiation and pathogenicity of *Fusarium graminearum*, the main causal agent of the head-blight disease of wheat, are regulated by the MAP kinase gpmk1. Curr. Genet. **43**: 87-95.

Jenkinson, P., and Parry, D.W. 1994a. Isolation of *Fusarium* species from common broad-leaved weeds and their pathogenicity to winter wheat. Mycol. Res. **98**: 776-780.

Jenkinson, P., and Parry, D.W. 1994b. Splash dispersal of conidia of *Fusarium culmorum* and *Fusarium avenaceum*. Mycol. Res. **98**: 506-510.

Jennings, P., Coates, M.E., Walsh, K., Turner, J.A., and Nicholson, P. 2004. Determination of deoxynivalenol- and nivalenol-producing chemotypes of *Fusarium graminearum* isolated from wheat crops in England and Wales. Plant Pathol. **53**: 643-652.

Ji, L., Cao, K., Hu, T., and Wang, S. 2007. Determination of deoxynivalenol and nivalenol chemotypes of *Fusarium graminearum* isolates from China by PCR assay. J. Phytopathology **155**: 505-512.

Jia, G., Chen, P., Qin, G., Bai, G., Wang, X., Wang, S., Zhou, B., Zhang, S., and Liu, D. 2005. QTLs for Fusarium head blight response in a wheat DH population of Wangshuibai/Alondra's'. Euphytica **146**: 183-191.

Jiang, G.-L., Dong, Y., Shi, J., and Ward, R.W. 2007a. QTL analysis of resistance to Fusarium head blight in the novel wheat germplasm CJ 9306. II. Resistance to deoxynivalenol accumulation and grain yield loss. Theor. Appl. Genet. **115**: 1043-1052.

Jiang, G.-L., Shi, J., and Ward, R.W. 2007b. QTL analysis of resistance to Fusarium head blight in the novel wheat germplasm CJ 9306. I. Resistance to fungal spread. Theor. Appl. Genet. **116**: 3-13.

Johnson, A.G., Dickson, J.G., and Johan, H. 1920. An epidemic of *Fusarium* blight (scab) of wheat and other cereals. Phytopathology **10**: 51 (Abstr.).

Jordan, V.W.L., and Fielding, E.C. 1988. *Fusarium* spp. on wheat. Long Ashton Res. Stn. Rep. for 1987, Publ. 23.

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

Kang, Z., and Buchenauer, H. 2002. Studies on the infection process of *Fusarium culmorum* in wheat spikes: Degradation of host cell wall components and localization of trichothecene toxins in infected tissue. Eur. J. Plant Pathol. **108**: 653-660.

Karugia, G.W., Suga, H., Gale, L.R., Nakajima, T., Tomimura, K., and Hyakumachi, M. 2009. Population structure of the *Fusarium graminearum* species complex from a single Japanese wheat field sampled in two consecutive years. Plant Dis. **93**: 170-174.

Kephart, K. 1991. Climatic conditions and regional differences. *In* Soft Red Winter Wheat Quality: Issues for Producers, Merchants, and Millers. VPI Coop. Ext. Publ. 448-051. *Edited by* E Jones. Virginia Polytechnic Institute, Blacksburg, VA, USA. pp. 5-10.

Khonga, E.B., and Sutton, J.C. 1988. Inoculum production and survival of *Gibberella zeae* in maize and wheat residues. Can. J. Plant Pathol. **10**: 232-239.

Kim, J.C., Kang, H.J., Lee, D.H., Lee, Y.W., and Yoshizawa, T. 1993. Natural occurrence of Fusarium mycotoxins (trichothecenes and zearalenone) in barley and corn in Korea. Appl. Environ. Microbiol. **59**: 3798-3802.

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

Klahr, A., Zimmermann, G., Wenzel, G., and Mohler, V. 2007. Effects of environment, disease progress, plant height and heading date on the detection of QTLs for resistance to Fusarium head blight in an European winter wheat cross. Euphytica **154**: 17-28.

Koehler, B., Dickson, J.G., and Holbert, J.R. 1924. Wheat scab and corn root rot caused by *Gibberella saubinetti* in relation to crop successions. J. Agric. Res. **27**: 861-880.

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

Kosambi, D. 1944. The estimation of map distances from recombination values. Ann. Eugenic. **12**: 172-175.

Krupinsky, J.M., Bailey, K.L., McMullen, M.P., Gossen, B.D., and Turkington, T.K. 2002. Managing plant disease risk in diversified cropping systems. Agron. J. **94**: 198-209.

Kumar, S., Stack, R.W., Friesen, T.L., and Faris, J.D. 2007. Identification of a novel fusarium head blight resistance quantitative trait locus on chromosome 7A in tetraploid wheat. Phytopathology **97**: 592-597.

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

Lander, E.S., Green, P., Abrahamson, J., Barlow, A., Daly, M.J., Lincoln, S.E., and Newburg, L. 1987. Mapmaker an Interactive Computer Package for Constructing Primary Genetic Linkage Maps of Experimental and Natural Populations. Genomics **1**: 174-181.

Lee, J., Chang, I.-Y., Kim, H., Yun, S.-H., Leslie, J.F., and Lee, Y.-W. 2009. Genetic diversity and fitness of *Fusarium graminearum* populations from rice in Korea. Appl. Environ. Microbiol. **75**: 3289-3295.

Lee, T., Han, Y.-K., Kim, K.-H., Yun, S.-H., and Lee, Y.-W. 2002. *Tri13* and *Tri7* determine deoxynivalenol- and nivalenol-producing chemotypes of *Gibberella zeae*. Appl. Environ. Microbiol. **68**: 2148-2154.

Lee, T., Oh, D.-W., Kim, H.-S., Lee, J., Kim, Y.-H., Yun, S.-H., and Lee, Y.-W. 2001. Identification of deoxynivalenol- and nivalenol-producing chemotypes of *Gibberella zeae* by using PCR. Appl. Environ. Microbiol. **67**: 2966-2972.

Lemmens, M., Scholz, U., Berthiller, F., Dall'Asta, C., Koutnik, A., Schuhmacher, R., Adam, G., Buerstmayr, H., Mesterhazy, A., Krska, R., and Ruckenbauer, P. 2005. The ability to detoxify the mycotoxin deoxynivalenol colocalizes with a major quantitative trait locus for fusarium head blight resistance in wheat. MPMI **18**: 1318-1324.

Leslie, J.F. 1993. Fungal vegetative compatibility. Ann. Rev. Phytopathology **31**: 127-150.

Leslie, J.F., and Klein, K.K. 1996. Female fertility and mating type effects on effective population size and evolution in filamentous fungi. Genetics **144**: 557-567.

Lewellen, R.T., Sharp, E.L., and Hehn, E.R. 1967. Major and minor genes in wheat for resistance to *Puccinia striiformis* and their responses to temperature changes. Can. J. Bot. **45**: 2155-2172.

Li, C., Zhu, H., Zhang, C., Lin, F., Xue, S., Cao, Y., Zhang, Z., Zhang, L., and Ma, Z. 2008. Mapping QTLs associated with Fusarium-damaged kernels in the Nanda 2419 x Wangshuibai population. Euphytica **163**: 185-191.

Li, W.L., Faris, J.D., Muthukrishnan, S., Liu, D.J., Chen, P.D., and Gill, B.S. 2001. Isolation and characterization of novel cDNA clones of acidic chitinases and beta-1,3-glucanases from wheat spikes infected by *Fusarium graminearum*. Theor. Appl. Genet. **102**: 353-362.

Li, X.Y., and Wu, Z.S. 1994. Studies on the relationship between choline concentration in flowering spikes and resistance to scab among bread wheat varieties. Acta Agron. Sin. **20**: 176-185.

Li, Y.-F., and Yu, Y.-J. 1988. Monosomic analysis for scab resistance index in wheat cultivar "WZHHS". J. Huazhong Agric. Univ. **7**: 327-331.

Liao, Y.-C., and Yu, Y.-J. 1985. Genetic analysis on scab-resistance in local wheat cultivar Wang Shuibai. J. Huazhong Agric. Univ. **4**: 6-14.

Lin, F., Kong, Z.X., Zhu, H.L., Xue, S.L., Wu, J.Z., Tian, D.G., Wei, J.B., Zhang, C.Q., and Ma, Z.Q. 2004. Mapping QTL associated with resistance to *Fusarium* head blight in the Nanda2419 x Wangshuibai population. I. Type II resistance. Theor. Appl. Genet. **109**: 1504-1511.

Lin, F., Xue, S.L., Zhang, Z.Z., Zhang, C.Q., Kong, Z.X., Yao, G.Q., Tian, D.G., Zhu, H.L., Li, C.J., Cao, Y., Wei, J.B., Luo, Q.Y., and Ma, Z.Q. 2006. Mapping QTL associated with resistance to Fusarium head blight in the Nanda2419 x Wangshuibai population. II: Type I resistance. Theor. Appl. Genet. **112**: 528-535.

Lin, Y., Yang, Z., and Wu, Z. 1992. Genetic analysis of resistance to scab (*Gibberella zeae*) in wheat varieties from different regions. Acta Agric. Shanghai **8**: 31-36.

Liu, D.-J., Weng, Y.-Q., Chen, P.-D., and Wang, Y.-N. 1989. Gene transfer of scab resistance from *Roegneria kamoji* and *Elymus giganteus* to common wheat. Jiangsu Agric. Sci. **1**: 97-100 (Suppl.).

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

Liu, S., and Anderson, J.A. 2003a. Marker assisted evaluation of Fusarium head blight resistant wheat germplasm. Crop Sci. **43**: 760-766.

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

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

Liu, W.X., Chen, P.D., and Liu, D.J. 2000. Radiation-induced *Triticum aestivum-Leymous racemosus* translocations and their molecular cytogenetic analysis. *In* Proceedings of the International Symposium on Wheat Improvement for Scab Resistance, Suzhou and Nanjing, China. pp. 73-76

Liu, Z.-Z., and Wang, Z.-Y. 1990. Improved scab-resistance in China: Sources of resistance and problems. *In* Wheat for the Nontraditional Warm Areas, Proceedings of

International Conference. *Edited by* D. A. Saunders. CIMMYT, Mexico D.F., Mexico. pp. 178-188.

Liu, Z.-Z., Wang, Z.-Y., Huang, D.-C., Zhao, W.-J., Huang, X.-M., Yao, Q.-H., Sun, X.-J., and Yang, Y.-M. 1991. Generality of scab-resistance transgression in wheat and utilization of scab resistance genetic resources. Acta Agric. Shanghai **7**: 65-70.

Logrieco, A., Bottalico, A., and Altomare, C. 1988. Chemotaxonomic observations on zearalenone and trichothecene production by *Gibberella zeae* from cereals in southern Italy. Mycologia **80**: 892-895.

Logrieco, A., Manka, M., Altomare, C., and Bottalico, A. 1990. Pathogenicity of *Fusarium graminearum* chemotypes towards corn wheat triticale and rye. J. Phytopathology **130**: 197-204.

Lu, W.Z., Chen, S.H., and Wang, Y.Z. 2001. Research on Wheat Scab. Sci. Publ. House, Beijing, China.

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

Ma, H.X., Zhang, K.M., Gao, L., Bai, G.H., Chen, H.G., Cai, Z.X., and Lu, W.Z. 2006b. Quantitative trait loci for resistance to fusarium head blight and deoxynivalenol accumulation in Wangshuibai wheat under field conditions. Plant Pathol. **55**: 739-745.

Maclnnes, J., and Fogelman, R. 1923. Wheat scab in Minnesota. Univ. Minnesota Agric. Exp. Stn. Tech. Bull. No. 18. 32 pp.

Mains, E.B., Vestal, C.M., and Curtis, P.B. 1929. Scab of small grains and feeding trouble in Indiana in 1928. Proc. Indiana Acad. Sci. **39**: 101-110.

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

Marasas, W.F.O., Nelson, P.E., and Toussoun, T.A. 1984. Toxigenic *Fusarium* Species, Identity and Mycotoxicology. Penn. State Univ. Press, University Park, PA, USA.

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

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

Martin, R.A. 1988. Use of a high-through-put jet sampler for monitoring viable airborne propagules of *Fusarium* in wheat. Can. J. Plant Pathol. **10**: 359-360.

Martinelli, J., Bocchese, C., Gale, L.R., Xie, W., K., O.D., and Kistler, H.C. 2001. Soybean is a host for *Fusarium graminearum*. *In* Proceedings of 2001 National Fusarium Head Blight Forum. *Edited by* S. M. Canty, J. Lewis, L. Siler, and R. W. Ward, Erlanger, KY, USA. p. 136.

Mathre, D.E. 1982. Compendium of Barley Diseases. APS Press, St. Paul, MN, USA.

Matsui, K., Yoshida, M., Ban, T., Komatsuda, T., and Kawada, N. 2002. Role of male-sterile cytoplasm in resistance to barley yellow mosaic virus and *Fusarium* head blight in barley. Plant Breed. **121**: 237-240.

McCallum, B.D., Tekauz, A., and Gilbert, J. 2001. Vegetative compatibility among *Fusarium graminearum* (*Gibberella zeae*) isolates from barley spikes in southern Manitoba. Can. J. Plant Pathol. **23**: 83-87.

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

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

McDonald, B.A., and Linde, C. 2002. The population genetics of plant pathogens and breeding strategies for durable resistance. Euphytica **124**: 163-180.

McDonald, B.A., and McDermott, J.M. 1993. Population genetics of plant pathogenic fungi. BioScience **43**: 311-319.

McKay, R., and Loughnane, J.B. 1945. Observations on *Gibberella saubinetii* (Mont.) Sacc on cereals in Ireland in 1943 and 1944. Scientific Proceedings of the Royal Dublin Society **24**: 9-18.

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

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

Melchinger, E., Utz, H.F., and Schoen, C.C. 2004. QTL analyses of complex traits with cross validation, bootstrapping and other biometric methods. Euphytica **137**: 1-11.

Mergoum, M., Frohberg, R.C., Miller, J.D., and Stack, R.W. 2005. Registration of 'Steele-ND' wheat. Crop Sci. **45**: 1163-1164.

Mergoum, M., Frohberg, R.C., Miller, J.D., Stack, R.W., Olsona, T., Friesen, T.L., and Rasmussen, J.B. 2006. Registration of 'Glenn' wheat. Crop Sci. **46**: 473-474.

Mesterházy, A. 1977. Reaction of winter wheat varieties to four *Fusarium* species. Phytopathology Z. **90**: 104-112.

Mesterházy, A. 1978. Comparative analysis of artificial inoculation methods with *Fusarium s*pp. on winter wheat varieties. Phytopathology Z. **93**: 12-25.

Mesterházy, A. 1981. The role of aggressiveness of *Fusarium graminearum* isolates in the inoculation tests on wheat in seedling state. Acta Phytopathol. Acad. Sci. Hun. **16**: 281-292.

Mesterházy, A. 1984. A laboratory method to predict pathogenicity of *Fusarium graminearum* in the field and resistance of wheat to scab. Acta Phytopathol. Acad. Sci. Hun. **19**: 205-218.

Mesterházy, A. 1987. Selection of head blight resistant wheats through improved seedling resistance. Plant Breed. **98**: 25-36.

Mesterházy, A. 1988. Expression of resistance of wheat to *Fusarium graminearum* and *Fusarium culmorum* under various experimental conditions. J. Phytopathology **123**: 304-310.

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

Mesterházy, A. 1997a. Breeding for resistance to *Fusarium* head blight of wheat. *In* Fusarium Head Scab: Global Status and Future Prospects. *Edited by* H. J. Dubin, L. Gilchrist, J. Reeves, and A. McNab. CIMMYT, Mexico D.F., Mexico. pp. 79-85.

Mesterházy, A. 1997b. Methodology of resistance testing and breeding against Fusarium head blight in wheat and results of the selection. Cereal Res. Commun. **25**: 631-637.

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

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

Mesterházy, A., Bartok, T., Mirocha, C.G., and Komoroczy, R. 1999. Nature of wheat resistance to *Fusarium* head blight and the role of deoxynivalenol for breeding. Plant Breed. **118**: 97-110.

Michelmore, R.W., Paran, I., and Kesseli, R.V. 1991. Identification of markers linked to disease-resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. PNAS **88**: 9828-9832.

Miedaner, T., Cumagun, C.J.R., and Chakraborty, S. 2008. Population genetics of three important head blight pathogens *Fusarium graminearum*, *F. pseudograminearum* and *F. culmorum*. J. Phytopathology **156**: 129-139.

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

Miedaner, T., Reinbrecht, C., and Schilling, A.G. 2000. Association among aggressiveness, fungal colonization, and mycotoxin production of 26 isolates of *Fusarium graminearum* in winter rye head blight. Z. Pflanzenk. Pflanzen. **107**: 124-134.

Miedaner, T., and Schilling, A.G. 1996. Genetic variation of aggressiveness in individual field populations of *Fusarium graminearum* and *Fusarium culmorum* tested on young plants of winter rye. Eur. J. Plant Pathol. **102**: 823-830.

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

Miedaner, T., Wilde, F., Steiner, B., Buerstmayr, H., Korzun, V., and Ebmeyer, E. 2006. Stacking quantitative trait loci (QTL) for *Fusarium* head blight resistance from non-

adapted sources in an European elite spring wheat background and assessing their effects on deoxynivalenol (DON) content and disease severity. Theor. Appl. Genet. **112**: 562-569.

Millar, C.S., and Colhoun, J. 1969. *Fusarium* diseases of cereals. IV. Observations on *Fusarium nivale* on wheat. Trans. Br. Mycol. Soc. **52**: 57-66.

Miller, J.D., and Arnison, P.G. 1986. Degradation of deoxynivalenol by suspension cultures of the fusarium head blight resistant wheat cultivar Frontana. Can. J. Plant Pathol. **8**: 147-150.

Miller, J.D., Culley, J., Fraser, K., Hubbard, S., Meloche, F., Ouellet, T., Seaman, W.L., Seifert, K.A., Turkington, K., and Voldeng, H. 1998a. Effect of tillage practice on fusarium head blight of wheat. Can. J. Plant Pathol. **20**: 95-103.

Miller, J.D., Greenhalgh, R., Wang, Y., and Lu, M. 1991. Trichothecene chemotypes of three *Fusarium* species. Mycologia **83**: 121-130.

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

Miller, J.D., Taylor, A., and Greenhalgh, R. 1983. Production of deoxynivalenol and related compounds in liquid culture by *Fusarium graminearum*. Can. J. Microbiol. **29**: 1171-1178.

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

Miller, S.S., Chabot, D.M.P., Ouellet, T., Harris, L.J., and Fedak, G. 2004. Use of a *Fusarium graminearum* strain transformed with green fluorescent protein to study infection in wheat (*Triticum aestivum*). Can. J. Plant Pathol. **26**: 453-463.

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

Mishra, P.K., Tewari, J.P., Clear, R.M., and Turkington, T.K. 2004. Molecular genetic variation and geographical structuring in *Fusarium graminearum*. Ann. Appl. Biol. **145**: 299-307.

Monds, R.D., Cromey, M.G., Lauren, D.R., di Menna, M., and Marshall, J. 2005. *Fusarium graminearum*, *F. cortaderiae* and *F. pseudograminearum* in New Zealand: molecular phylogenetic analysis, mycotoxin chemotypes and co-existence of species. Mycol. Res. **109**: 410-420.

Mongrain, D., Couture, L., and Comeau, A. 2000. Natural occurrence of *Fusarium graminearum* on adult wheat midge and transmission to wheat spikes. Cereal Res. Commun. **28**: 173-180.

Moon, J., Lee, Y., and Lee, Y. 1999. Vegetative compatibility groups in *Fusarium graminearum* isolates from corn and barley in Korea. Plant Pathol. J. **15**: 53-56.

Mujeeb-Kazi, A., Bernard, M., Bekele, G.T., and Mirand, J.L. 1983. Incorporation of alien genetic information from *Elymus giganteus* into *Triticum aestivum*. *In* Proceedings of 6th International Wheat Genetics Symposium. *Edited by* S. Sakamoto, Maruzen, Kyoto, Japan. pp. 223-231.

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

Naef, A., and Defago, G. 2006. Population structure of plant-pathogenic *Fusarium* species in overwintered stalk residues from Bt-transformed and non-transformed maize crops. Eur. J. Plant Pathol. **116**: 129-143.

Najaphy, A., Toorchi, M., Mohammadi, S.A., Chalmers, K.J., Moghaddam, M., Torabi, M., and Aharizad, S. 2006. Identification of Fusarium head blight resistance QTLs in a wheat population using SSR markers. Biotechnology **5**: 222-227.

Nakagawa, M.O. 1955. Study on the resistance of wheat varieties to *Gibberella saubinetii*. II. Genetic factors affecting resistance to *Gibberella saubinetii*. Jpn. J. Breed. **5**: 15-22.

Nakagawa, M.O., Watanabe, S., Gocho, H., and Nishio, K. 1966. Studies on the occurrence forecast of *Gibberella zeae* on wheat varieties caused by climatic factors. Bull. Tokai-Kinki Natl. Agric. Exp. Stn. **15**: 55-67.

Naseri, B., Alizadeh, A., Saidi, A., and Safaee, N. 2000. Population diversity of *Fusarium graminearum* based on vegetative compatibility groups (VCGs) and its relationship to virulence of isolates. Iran. J. Plant Pathol. **36**: 77-80.

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

Nicholson, P., Simpson, D.R., Weston, G., Rezanoor, H.N., Lees, A.K., Parry, D.W., and Joyce, D. 1998. Detection and quantification of *Fusarium culmorum* and *Fusarium graminearum* in cereals using PCR assays. Physiol. Mol. Plant Pathol. **53**: 17-37.

Nightingale, M.J., Marchylo, B.A., Clear, R.M., Dexter, J.E., and Preston, K.R. 1999. Fusarium head blight: Effect of fungal proteases on wheat storage proteins. Cereal Chem. **76**: 150-158.

O'Donnell, K. 1992. Ribosomal DNA Internal transcribed spacers are highly divergent in the phytopathogenic ascomycete Fusarium sambucinum (*Gibberella pulicaris*). Curr. Genet. **22**: 213-220.

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

O'Donnell, K., Ward, T.J., Aberra, D., Kistler, H.C., Aoki, T., Orwig, N., Kimura, M., Bjornstad, A., and Klemsdal, S.S. 2008. Multilocus genotyping and molecular phylogenetics resolve a novel head blight pathogen within the *Fusarium graminearum*

species complex from Ethiopia. Fungal Genet. Biol. **45**: 1514-1522. doi:http://dx.doi.org/10.1016/j.fgb.2008.09.002.

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

Obst, A., Lepschy-Von Gleissenthall, J., and Beck, R. 1997. On the etiology of Fusarium head blight of wheat in South Germany - preceding crops, weather conditions for inoculum production and head infection, proneness of the crop to infection and mycotoxin production. Cereal Res. Commun. **25**: 699-703.

Okubara, P.A., Blechl, A.E., McCormick, S.P., Alexander, N.J., Dill-Macky, R., and Hohn, T.M. 2002. Engineering deoxynivalenol metabolism in wheat through the expression of a fungal trichothecene acetyltransferase gene. Theor. Appl. Genet. **106**: 74-83.

Oliver, R.E., Cai, X., Xu, S.S., Chen, X., and Stack, R.W. 2005. Wheat-alien species derivatives: A novel source of resistance to fusarium head blight in wheat. Crop Sci. **45**: 1353-1360.

Oliver, R.E., Cal, X., Friesen, T.L., Halley, S., Stack, R.W., and Xu, S.S. 2008. Evaluation of fusarium head blight resistance in tetraploid wheat (*Triticum turgidum* L.). Crop Sci. **48**: 213-222.

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

Olivera, P., Steffenson, B., and Anikster, Y. 2003. Reaction of *Aegilops sharonensis* to Fusarium head blight. *In* Proceedings of 2003 National Fusarium Head Blight Forum. *Edited by* S. M. Canty, J. Lewis, and R. W. Ward, Bloomington, MN, USA. p. 226.

Osborne, L., Jin, Y., Rosolen, F., and Hannoun, M.J. 2002. FHB inoculum distribution on wheat plants within the canopy. *In* Proceedings of 2002 National Fusarium Head Blight Forum. *Edited by* S. M. Canty, J. Lewis, L. Siler, and R. W. Ward, Erlanger, KY, USA. p. 175.

Otto, C.D., Kianian, S.F., Elias, E.M., Stack, R.W., and Joppa, L.R. 2002. Genetic dissection of a major Fusarium head blight QTL in tetraploid wheat. Plant Mol. Biol. **48**: 625-632.

Ouellet, T., and Seifert, K.A. 1993. Genetic characterization of *Fusarium graminearum* strains using RAPD and PCR amplification. Phytopathology **83**: 1003-1007.

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

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

Parry, D.W., Pettitt, T.R., Jenkinson, P., and Lees, A.K. 1994. The cereal *Fusarium* complex. *In* Ecology of Plant Pathogens. *Edited by* P. Blakeman, and B. Williamson. CAB Intemational, Wallingford, UK. pp. 301-320.

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

Paulitz, T.C., and Seaman, W.L. 1994. Temporal analysis of ascospore release by *Gibberella zeae* in artificially-inoculated field plots. Phytopathology **84**: 1070-1071 (Abstr.).

Peplow, A.W., Tag, A.G., Garifullina, G.F., and Beremand, M.N. 2003. Identification of new genes positively regulated by *Tri10* and a regulatory network for trichothecene mycotoxin production. Appl. Environ. Microbiol. **69**: 2731-2736.

Perkowski, J., Kiecana, I., Schumacher, U., Mueller, H.M., Chelkowski, J., and Golinski, P. 1997. Head infection and accumulation of *Fusarium* toxins in kernels of 12 barley genotypes inoculated with *Fusarium graminearum* isolates of two chemotypes. Eur. J. Plant Pathol. **103**: 85-90.

Pestsova, E., Ganal, M.W., and Roeder, M.S. 2000. Isolation and mapping of microsatellite markers specific for the D genome of bread wheat. Genome **43**: 689-697.

Pineiro, M.S., Scott, P.M., and Kanhere, S.R. 1996. Mycotoxin producing potential of *Fusarium graminearum* isolates from Uruguayan barley. Mycopathologia **132**: 167-172.

Pritsch, C., Muehlbauer, G.J., Bushnell, W.R., Somers, D.A., and Vance, C.P. 2000. Fungal development and induction of defense response genes during early infection of wheat spikes by *Fusarium graminearum*. MPMI **13**: 159-169.

Pritsch, C., Vance, C.P., Bushnell, W.R., Somers, D.A., Hohn, T.M., and Muehlbauer, G.J. 2001. Systemic expression of defense response genes in wheat spikes as a response to *Fusarium graminearum* infection. Physiol. Mol. Plant Pathol. **58**: 1-12.

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

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

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

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

Qu, B., Li, H.P., Zhang, J.B., Xu, Y.B., Huang, T., Wu, A.B., Zhao, C.S., Carter, J., Nicholson, P., and Liao, Y.C. 2008. Geographic distribution and genetic diversity of *Fusarium graminearum* and *F. asiaticum* on wheat spikes throughout China. Plant Pathol. **57**: 15-24.

Ramirez, M.L., Reynoso, M.M., Farnochi, M.C., and Chulze, S. 2006. Vegetative compatibility and mycotoxin chemotypes among *Fusarium graminearum* (*Gibberella zeae*) isolates from wheat in Argentina. Eur. J. Plant Pathol. **115**: 139-148.

Ramirez, M.L., Reynoso, M.M., Farnochi, M.C., Torres, A.M., Leslie, J.F., and Chulze, S.N. 2007. Population genetic structure of *Gibberella zeae* isolated from wheat in Argentina. Food Additives and Contaminants **24**: 1115-1120.

Rapilly, F., Lemaire, J.M., and Cassini, R. 1973. Les Fusarioses. *In* Les maladies des cereals-Principales maladies cryptogamiques. . INRA-ITCF. p. 119.

Reis, E.M. 1990. Integrated disease management-The changing concepts of controlling head blight and spot blotch. *In* Wheat for the Nontraditional Warm Areas, Proceedings of International Conference. *Edited by* D. A. Saunders. CIMMYT, Mexico D.F., Mexico. pp. 165-177.

Röder, M.S., Korzun, V., Wendehake, K., Plaschke, J., Tixier, M.-H., Leroy, P., and Ganal, M.W. 1998. A microsatellite map of wheat. Genetics **149**: 2007-2023.

Rostoks, N., Ramsay, L., MacKenzie, K., Cardle, L., Bhat, P.R., Roose, M.L., Svensson, J.T., Stein, N., Varshney, R.K., Marshall, D.F., Grainer, A., Close, T.J., and Waugh, R. 2006. Recent history of artificial outcrossing facilitates whole-genome association mapping in elite inbred crop varieties. PNAS **103**: 18656-18661.

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

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

Sanyal, R., Xie, W., and Kistler, H.C. 2000. Measuring differences in the ability of strains of *Fusarium graminearum* to spread within wheat heads *In* Proceedings of 2000 National Fusarium Head Blight Forum Erlanger, KY, USA. p. 173.

Schmale III, D.G., Leslie, J.F., Zeller, K.A., Saleh, A.A., Shields, E.J., and Bergstrom, G.C. 2006. Genetic structure of atmospheric populations of *Gibberella zeae*. Phytopathology **96**: 1021-1026.

Schmale III, D.G., Shah, D.A., and Bergstrom, G.C. 2005. Spatial patterns of viable spore deposition of *Gibberella zeae* in wheat fields. Phytopathology **95**: 472-479.

Schmale III, D.G., Shields, E.J., and Bergstrom, G.C. 2002. Airborne populations of *Gibberella zeae*: Spatial and temporal dynamics of spore deposition in a localized Fusarium head blight epidemic. *In* Proceedings of 2002 National Fusarium Head Blight Forum. *Edited by* S. M. Canty, J. Lewis, L. Siler, and R. W. Ward, Erlanger, KY, USA. p. 178.

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

Schmolke, M., Zimmermann, G., Schweizer, G., Miedaner, T., Korzun, V., Ebmeyer, E., and Hartl, L. 2008. Molecular mapping of quantitative trait loci for field resistance to Fusarium head blight in a European winter wheat population. Plant Breed. **127**: 459-464.

Schön, C.C., Utz, H.F., Groh, S., Truberg, B., Openshaw, S., and Melchinger, A.E. 2004. Quantitative trait locus mapping based on resampling in a vast maize testcross experiment and its relevance to quantitative genetics for complex traits. Genetics **167**: 485-498.

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

Schuelke, M. 2000. An economic method for the fluorescent labelingof PCR fragments. Nat. Biotechnol. **18**: 233-234.

Scoz, L.B., Astolfi, P., Reartes, D.S., Schmale, D.G., III, Moraes, M.G., and Del Ponte, E.M. 2009. Trichothecene mycotoxin genotypes of *Fusarium graminearum* sensu stricto and *Fusarium meridionale* in wheat from southern Brazil. Plant Pathol. **58**: 344-351.

Semagn, K., Skinnes, H., Bjornstad, A., Maroy, A.G., and Tarkegne, Y. 2007. Quantitative trait loci controlling Fusarium head blight resistance and low deoxynivalenol content in hexaploid wheat population from 'Arina' and NK93604. Crop Sci. **47**: 294-303.

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

Shaner, G.E. 2002. Resistance in hexaploid wheat to Fusarium head blight. *In* Proceedings of 2002 National Fusarium Head Blight Forum. *Edited by* S. M. Canty, J. Lewis, L. Siler, and R. W. Ward, Erlanger, KY, USA. pp. 208-211.

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

Shen, X., Kong, L., and Ohm, H.W. 2004. Fusarium head blight resistance in hexaploid wheat (*Triticum aestivum*)-*Lophopyrum* genetic lines and tagging of the alien chromatin by PCR markers. Theor. Appl. Genet. **108**: 808-813.

Shen, X., and Ohm, H. 2007. Molecular mapping of *Thinopyrum*-derived Fusarium head blight resistance in common wheat. Mol. Breed. **20**: 131-140.

Shen, X., Zhou, M., Lu, W., and Ohm, H. 2003b. Detection of Fusarium head blight resistance QTL in a wheat population using bulked segregant analysis. Theor. Appl. Genet. **106**: 1041-1047.

Shurtleff, M.C. 1980. Compendium of Corn Diseases. APS Press, St. Paul, MN, USA.

Sinah, R.C., Savard, M., and Lau, R. 1995. Production of monoclonal antibodies for the specific detection of deoxynivalenol and 15-Acetyldeoxynivalenol by ELISA. J. Agric. Food Chem. **43**: 1740-1744.

Singh, A.K., Knox, R.E., Clarke, F.R., Clarke, J.M., Fedak, G., Singh, A., and DePauw, R.M. 2008. Fusarium head blight QTL mapping in durum wheat and *Triticum carthlicum* sources of resistance *In* Proceedings of the 11th International Wheat Genetics Symposium, Brisbane, Australia. pp. 845-847.

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

Singh, R.P., and van Ginkel, M. 1997. Breeding strategies for introgressing diverse scab resistance into adapted wheat. *In* Fusarium Head Scab: Global Status and Future Prospects. *Edited by* H. J. Dubin, L. Gilchrist, J. Reeves, and A. McNab. CIMMYT, Mexico D.F., Mexico. pp. 86-92.

Smith, W.G. 1884. Diseases of Field and Garden Crops. MacMillan & Co., London, UK.

Snijders, C.H.A. 1987. Interactions between winter wheat genotypes and isolates of *Fusarium culmorum*. Mededelingen van de Faculteit Landbouwwetenschappen, Rijksuniversiteit Gent **52**: 807-814.

Snijders, C.H.A. 1990a. Diallel analysis of resistance to head blight caused by *Fusarium culmorum* in winter wheat. Euphytica **50**: 1-9.

Snijders, C.H.A. 1990b. Fusarium head blight and mycotoxin contamination of wheat, a review. Neth. J. Plant Pathol. **96**: 187-198.

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

Snijders, C.H.A. 1990d. The inheritance of resistance to head blight caused by *Fusarium culmorum* in winter wheat. Euphytica **50**: 11-18.

Snijders, C.H.A. 1990e. Systemic fungal growth of *Fusarium culmorum* in stems of winter wheat. J. Phytopathology **129**: 133-140.

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

Snijders, C.H.A., and Van Eeuwijk, F.A. 1991. Genotype x strain interactions for resistance to *Fusarium* head blight caused by *Fusarium culmorum* in winter wheat. Theor. Appl. Genet. **81**: 239-244.

Somers, D.J., Fedak, G., Clarke, J., and Cao, W. 2006. Mapping of FHB resistance QTLs in tetraploid wheat. Genome **49**: 1586-1593.

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

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

Song, Q.J., Fickus, E.W., and Cregan, P.B. 2002. Characterization of trinucleotide SSR motifs in wheat. Theoretical & Applied Genetics **104**: 286-293.

Song, Q.J., Shi, J.R., Singh, S., Fickus, E.W., Costa, J.M., Lewis, J., Gill, B.S., Ward, R., and Cregan, P.B. 2005. Development and mapping of microsatellite (SSR) markers in wheat. Theoretical & Applied Genetics **110**: 550-560.

Sourdille, P., Cadalen, T., Guyomarc'h, H., Snape, J.W., Perretant, M.R., Charmet, G., Boeuf, C., Bernard, S., and Bernard, M. 2003. An update of the Courtot x Chinese Spring

intervarietal molecular marker linkage map for the QTL detection of agronomic traits in wheat. Theoretical & Applied Genetics **106**: 530-538.

Srinivasachary, Gosman, N., Steed, A., Simmonds, J., Leverington-Waite, M., Wang, Y., Snape, J., and Nicholson, P. 2008. Susceptibility to *Fusarium* head blight is associated with the *Rht-D1b* semi-dwarfing allele in wheat. Theor. Appl. Genet. **116**: 1145-1153.

Stack, R.W. 1988. Response of durum wheat to head blight. Biological and Cultural Tests for Control of Plant Diseases **3**: 39.

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

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

Steed, A., Chandler, E., Thomsett, M., Gosman, N., Faure, S., and Nicolson, P. 2005. Identification of type I resistance to Fusarium head blight controlled by a major gene located on chromosome 4A of *Triticum macha*. Theor. Appl. Genet. **111**: 521-529.

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

Strange, R.N., and Smith, H. 1971. A fungal growth stimulant in anthers which predisposes wheat to attack by *Fusarium graminearum*. Physiol. Plant Pathol. **1**: 141-150.

Strange, R.N., and Smith, H. 1978. Effects of choline betaine and wheat germ extract on growth of cereal pathogens. Trans. Br. Mycol. Soc. **70**: 193-200.

Sturz, A.V., and Johnston, H.W. 1985. Characterization of *Fusarium* colonization of spring barley and wheat produced on stubble or fallow soil. Can. J. Plant Pathol. **7**: 270-276.

Suga, H., Karugia, G.W., Ward, T., Gale, L.R., Tomimura, K., Nakajima, T., Miyasaka, A., Koizumi, S., Kageyama, K., and Hyakumachi, M. 2008. Molecular characterization of the Fusarium graminearum species complex in Japan. Phytopathology **98**: 159-166.

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

Swofford, D.L. 2003. PAUP*, phylogenetic analysis using parsimony (*and other methods). Sinauer Associates, Sunderland, MA, USA.

Tanaka, T., Haegawa, A., Yamamoto, S., Lee, U.-S., Sugiura, Y., and Ueno, Y. 1988. Worldwide Contamination of Cereals by the *Fusarium* Mycotoxins Nivalenol Deoxynivalenol and Zearalenone 1. Survey of 19 Countries. J. Agric. Food Chem. **36**: 979-983.

Taylor, J.W., Jacobson, D.J., Kroken, S., Kasuga, T., Geiser, D.M., Hibbett, D.S., and Fisher, M.C. 2000. Phylogenetic species recognition and species concepts in fungi. Fungal Genet. Biol. **31**: 21-32.

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

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

Todd, R.L., Deibert, E.J., Stack, R.W., and Enz, J.W. 2001. Plant residue management and Fusarium head blight. *In* Proceedings of 2001 National Fusarium Head Blight Forum. *Edited by* S. M. Canty, J. Lewis, L. Siler, and R. W. Ward, Erlanger, KY, USA. p. 161.

Tóth, B., Kaszonyi, G., Bartok, T., Varga, J., and Mesterhazy, A. 2008. Common resistance of wheat to members of the *Fusarium graminearum* species complex and *F. culmorum*. Plant Breed. **127**: 1-8.

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

Trail, F., Xu, H., Loranger, R., and Gadoury, D. 2002. Physiological and environmental aspects of ascospore discharge in *Gibberella zeae* (anamorph *Fusarium graminearum*). Mycologia **94**: 181-189.

Trigiano, R.N., Windham, M.T., and Windham, A.S. 2008. Plant Pathology Concepts and Laboratory Exercises. 2nd ed. CRC Press, Boca Raton, FL, USA.

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

Tuite, J., Shaner, G., and Everson, R.J. 1990. Wheat scab in soft red winter wheat in Indiana USA in 1986 and its relation to some quality measurements. Plant Dis. **74**: 959-962.

Ueno, Y., Nakajima, M., Sakai, K., Ishii, K., Sato, N., and Shimada, N. 1973. Comparative Toxicology of Trichothecene Myco Toxins Inhibition of Protein Synthesis in Animal Cells. J. Biochem. **74**: 285-296.

Vales, M.I., Schoen, C.C., Capettini, F., Chen, X.M., Corey, A.E., Mather, D.E., Mundt, C.C., Richardson, K.L., Sandoval-Islas, J.S., Utz, H.F., and Hayes, P.M. 2005a. Effect of population size on the estimation of QTL: a test using resistance to barley stripe rust. Theoretical & Applied Genetics **111**: 1260-1270.

Vales, M.I., Schoen, C.C., Capettini, F., Chen, X.M., Corey, A.E., Mather, D.E., Mundt, C.C., Richardson, K.L., Sandoval-Islas, J.S., Utz, H.F., and Hayes, P.M. 2005b. Effect of population size on the estimation of QTL: a test using resistance to barley stripe rust. Theor. Appl. Genet. **111**: 1260-1270.

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

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

Waalwijk, C., Kastelein, P., de Vries, I., Kerenyi, Z., Van der Lee, T., Hesselink, T., Kohl, J., and Kema, G. 2003. Major changes in *Fusarium* spp. in wheat in the Netherlands. Eur. J. Plant Pathol. **109**: 743-754.

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

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

Wan, Y.-F., Yen, C., and Yang, J.-L. 1997a. The diversity of head-scab resistance in Triticeae and their relation to ecological conditions. Euphytica **97**: 277-281.

Wan, Y.-F., Yen, C., Yang, J.-L., and Liu, F.-Q. 1997b. Evaluation of *Roegneria* for resistance to head scab caused by *Fusarium graminearum* Schwabe. Genet. Res. Crop Evol. **44**: 211-215.

Wang, X.E., Chen, P.D., Liu, D.J., Zhang, P., Zhou, B., Friebe, B., and Gill, B.S. 2001. Molecular cytogenetic characterization of Roegneria ciliaris chromosome additions in common wheat. Theor. Appl. Genet. **102**: 651-657.

Wang, Y.-N., Chen, P.D., and Liu, D.J. 1986. Transfer of useful germplasm from *Elymus giganteus* L. to common wheat. I. Production of (*T. aestivum* L. cv. Chinese Spring x *E. giganteus*) F1. J. Nanjing Agric. Univ. **9**: 10-14 (in Chinese with English abstract).

Wang, Y.-N., Chen, P.D., Wang, Z.T., and Liu, D.J. 1991. Transfer of useful germplasm from *Elymus giganteus* L. to common wheat. II. Cytogenetics and scab resistance of backcross derivatives. J. Nanjing Agric. Univ. **14**: 1-5 (in Chinese with English abstract).

Wang, Y.-Z., and Miller, J.D. 1987. Screening techniques and sources of resistance to Fusarium head blight. *In* Wheat Production Constraints in Tropical Environments, Proceedings of International Conference. CIMMYT, Mexico D.F., Mexico. pp. 239-250.

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

Wang, Y.-Z., Yong, X.-N., and Xiao, Q.-P. 1982. The improvement of identification technique of scab resistance of wheat and the development of resistant sources. Sci. Agric. Sin. **5**: 67-77.

Wang, Z.-Y., Liu, Z.-Z., Zhao, W.-J., Huang, D.-Z., and Huang, X.-M. 1989. Advance of scab resistance testing and improvement in wheat varieties. Jiangsu Agric. Sci. **1** 64-68 (Suppl.).

Wanjiru, W.M., Zhensheng, K., and Buchenauer, H. 2002. Importance of cell wall degrading enzymes produced by *Fusarium graminearum* during infection of wheat heads. Eur. J. Plant Pathol. **108**: 803-810.

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

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

Warner, P., Karakousis, A., Schiemann, A., Eglinton, J.K., Langridge, P., and Barr, A.R. 2002. An investigation of a rapid DNA extraction method for routine MAS in the S.A. Barley Improvement Program. *In* Proceedings of the 10th Australian Barley Technical Symposium. Australian Barley Technical Symposium, Canberra, Australia. pp. www1-www5.

Warren, H.L., and Kommedahl, T. 1973. Fertilization and wheat refuse effects on *Fusarium* species associated with wheat roots in Minnesota. Phytopathology **63**: 103-108.

Weising, K., Nybom, H., and Wolff, K. 2005. DNA Fingerprinting in Plants: Principles, Methods, and Applications. 2nd ed. ed. CRC Press, Boca Raton, FL, USA.

Weng, Y.Q., and Liu, D.J. 1989. Morphology, scab resistance and cytogenetics of intergeneric hybrids of *Triticum aestivum* L. with *Roegneria* C.Koch species. Sci. Agric. Sin. **22**: 1-7 (in Chinese with English abstract).

Weng, Y.Q., and Liu, D.J. 1991. Morphological and cytological investigation of interspecific hybrids between *Roegneria ciliaris, R. japonensis*, and *R. kamoji*. J. Nanjing Agric. Univ. **14**: 1-5 (in Chinese with English abstract).

Wiese, M.V. 1987. Compendium of Wheat Diseases. 2nd ed. ed. APS Press, St. Paul, MN, USA.

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

Williams, J.G.K., Kubelik, A.R., Livak, K.J., Rafalski, J.A., and Tingey, S.V. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. **18**: 6531-6536.

Windels, C.E., Windels, M.B., and Kommedahl, T. 1976. Association of Fusarium species with picnic beetles on corn ears. Phytopathology **66**: 328-331.

Wise, I.L., Lamb, R.J., and Smith, M.A.H. 2001. Domestication of wheats (Gramineae) and their susceptibility to herbivory by Sitodiplosis mosellana (Diptera: Cecidomyiidae). Can. Entomol. **133**: 255-267.

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

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

Wu, Z.-S. 1986. Development of a gene pool with improved resistance to scab in wheat by using the dominant male sterile gene *Ta1*. *In* Proceedings of International Wheat Conference, Rabat, Morocco.

Wu, Z.S., Shen, Q.Q., Lu, W.Z., and Yang, Z.L. 1984. Development of a wheat gene pool with improved resistance to scab. Acta Agron. Sin. **10**: 73-80.

Xu, D.H., Juan, H.F., Nohda, M., and Ban, T. 2001. QTLs mapping of type I and type II resistance to FHB in wheat. *In* Proceedings of 2001 National Fusarium Head Blight Forum. *Edited by* R. Ward, S. M. Canty, J Lewis, and L. Siler, Erlanger, KY, USA. pp. 40-42.

Xue, A.G., Armstrong, K.C., Voldeng, H.D., Fedak, G., and Babcock, C. 2004. Comparative aggressiveness of isolates of *Fusarium* spp. causing head blight on wheat in Canada. Can. J. Plant Pathol. **26**: 81-88.

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

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

Yang, Z.P. 1994. Breeding for resistance to Fusarium head blight of wheat in the mid- to lower Yangtze River valley of China. CIMMYT, Mexico D.F., Mexico.

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

Ye, H.Z. 1980. On the biology of the perfect stage of *Fusarium graminearum* Schw. Acta Phytophylacica Sin. **7**: 35-42 (in Chinese with English abstract).

Ye, M.B., Xu, L.L., Xu, Y.G., and Zhu, B. 1990. Relationship between resistance of wheat to scab and activities of phenylalanine ammonia lyase and amount of chlorogenic acid. J. Nanjing Agric. Univ. **13**: 103-107.

Yli-Mattila, T., Gagkaeva, T., Ward, T.J., Aoki, T., Kistler, H.C., and O'Donnell, K. 2009. A novel Asian clade within the *Fusarium graminearum* species complex includes a newly discovered cereal head blight pathogen from the Russian Far East. Mycologia **101**: 841-852. doi:doi:10.3852/08-217.

Yoshizawa, T., and Jin, Y.-Z. 1995. Natural occurrence of acetylated derivatives of deoxynivalenol and nivalenol in wheat and barley in Japan. Food Additives and Contaminants **12**: 689-694.

Yu, J.-B., Bai, G.-H., Cai, S.-B., and Ban, T. 2006. Marker-assisted characterization of Asian wheat lines for resistance to Fusarium head blight. Theor. Appl. Genet. **113**: 308-320.

Yu, J.B., Bai, G.H., Zhou, W.C., Dong, Y.H., and Kolb, F.L. 2008. Quantitative trait loci for Fusarium head blight resistance in a recombinant inbred population of Wangshuibai/Wheaton. Phytopathology **98**: 87-94.

Yu, Y.-J. 1990. Genetic analysis for scab resistance in four wheat cultivars, PHJZM, MHDTB, CYHM, and YGFZ. *In* Advances in Researches on Inheritance to Diseases in

Major Crops. *Edited by* L. -H. Zhu. Jiangsu Science-Tech. Publishing House, Nanjing, China (in Chinese). pp. 197-205.

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

Yun, S.-H., Arie, T., Kaneko, I., Yoder, O.C., and Turgeon, B.G. 2000. Molecular organization of mating type loci in heterothallic, homothallic, and asexual *Gibberella/Fusarium* species. Fungal Genet. Biol. **31**: 7-20.

Zale, J.M., and Scoles, G.J. 1999. Registration of Crocus hexaploid wheat germplasm. Crop Sci. **39**: 1539-1540.

Zeller, K.A., Bowden, R.L., and Leslie, J.F. 2003. Diversity of epidemic populations of *Gibberella zeae* from small quadrats in Kansas and North Dakota. Phytopathology **93**: 874-880.

Zeller, K.A., Bowden, R.L., and Leslie, J.F. 2004. Population differentiation and recombination in wheat scab populations of *Gibberella zeae* from the United States. Mol. Ecol. **13**: 563-571.

Zhang, G., and Mergoum, M. 2007. Molecular mapping of kernel shattering and its association with Fusarium head blight resistance in a Sumai3 derived population. Theor. Appl. Genet. **115**: 757-766.

Zhang, J.-B., Li, H.-P., Dang, F.-J., Qu, B., Xu, Y.-B., Zhao, C.-S., and Liao, Y.-C. 2007. Determination of the trichothecene mycotoxin chemotypes and associated geographical

distribution and phylogenetic species of the *Fusarium graminearum* clade from China. Mycol. Res. **111**: 967-975.

Zhang, L.-Q., Wang, Y.-Z., and Zhang, L.Q. 1990. Advances of studies on genetics of resistance to scab and its pathogens (review). *In* Advances in Researches on Inheritance to Diseases in Major Crops. *Edited by* L. -H. Zhu. Jiangsu Science-Tech. Publishing House, Nanjing, China (in Chinese). pp. 165-170.

Zhang, L.Q., and Pan, X.P. 1982. A study on resistance to colonization of *Gibberella zeae* in wheat varieties. J. South China Agric. Coll. **3**: 21-29.

Zhang, X., Zhou, M., Ren, L., Bai, G., Ma, H., Scholten, O.E., Guo, P., and Lu, W. 2004. Molecular characterization of *Fusarium* head blight resistance from wheat variety Wangshuibai. Euphytica **139**: 59-64.

Zhou, C.-F. 1985. Production constraints and research priorities in the southern winter wheat region of China. *In* Wheat for More Tropical Environments. CIMMYT, Mexico D.F., Mexico. pp. 72-77.

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

Zhou, W., Kolb, F.L., Yu, J., Bai, G., Boze, L.K., and Domier, L.L. 2004. Molecular characterization of *Fusarium* head blight resistance in Wangshuibai with simple sequence repeat and amplified fragment length polymorphism markers. Genome **47**: 1137-1143.

Zhuang, Q.-S., and Li, Z.-S. 1993. Present status of wheat breeding and related genetic study in China. Wheat Information Service **76**: 1-15.

Appendix

Appendix

List of microsatellite primers used for mapping quantitative trait loci (QTL), forward and reverse primer sequences, annealing temperature, chromosome location, and source of the primers.

Serial number	Primer name	Forward primer sequence	Reverse primer sequence	Annealing temp. (°C)	Chromosome	Source
1	barc3	TTCCCTGTGTCTTTCTAATTTTTTTT	GCGAACTCCCGAACATTTTTAT	51	6A	USDA-ARS
2	barc4	GCGTGTTTGTGTCTGCGTTCTA	CACCACACATGCCACCTTCTTT	51	5B	USDA-ARS
3	barc5	GCGCCTGGACCGGTTTTCTATTTT	GCGTTGGGAATTCCTGAACATTTT	51	7D/2A/6D	USDA-ARS
4	barc7	GCGAAGTACCACAAATTTGAAGGA	CGCCATCTTACCCTATTTGATAACTA	51	2B	USDA-ARS
5	barc8	GCGGGAATCATGCATAGGAAAACAGAA	GCGGGGGCGAAACATACACATAAAAACA	51	1B	USDA-ARS
6	barc10	GCGTGCCACTGTAACCTTTAGAAGA	GCGAGTTGGAATTATTTGAATTAAACAAG	51	7B	USDA-ARS
7	barc13	GCAGGAACAACCACGCCATCTTAC	GCGTCGCAATTTGAAGAAAATCATC	51	2B	USDA-ARS
8	barc17	GCGCAACATATTCAGCTCAACA	TCCACATCTCGTCCCTCATAGTTTG	51	1A	USDA-ARS
9	barc18	CGCTTCCCATAACGCCGATAGTAA	CGCCCGCATCATGAGCAATTCTATCC	51	2B	USDA-ARS
10	barc20	GCGATCCACACTTTGCCTCTTTTACA	GCGATGTCGGTTTTCAGCCTTTT	51	4B	USDA-ARS
11	barc21	GCGTCTTCCGGTTTTGTTTACTTTTC	GCGTTAGGGCTATGGCGGTGTG	61	5B	USDA-ARS
12	barc23	GCGTGAAATAGTGCAAGCCAGAGAT	GCGCTAACACCTCGGCAAGACAA	51	6A/7A	USDA-ARS
13	barc24	CGCCTCTTATGGACCAGCCTAT	GCGGTGAGCCATCGGGTTACAAAG	51		USDA-ARS
14	barc25	GCGGTGCATCAAGGACGACAT	GCGTAGTTCATCCATCCGTAAT	51		USDA-ARS
15	barc28	CTCCCCGGCTAGTGACCACA	GCGGCATCTTTCATTAACGAGCTAGT	61	1A	USDA-ARS
16	barc32	GCGTGAATCCGGAAACCCAATCTGTG	TGGAGAACCTTCGCATTGTGTCATTA	51	7B	USDA-ARS
17	barc35	GCGGTGTGCATGCTTGTCGTGTAGGAGT	GCGTAGTGTAGTATGTGGCCCGATTATT	51	2B	USDA-ARS
18	barc37	CAGCGCTCCCCGACTCAGATCCTT	GCGCCATGTTTCTTTTATTACTCACTTT	51	6A	USDA-ARS
19	barc40	GCCGCCTACCACAGAGTTGCAGCT	GCGGCATTGACAAGACCATAGC	51	5A	USDA-ARS
20	barc42	GCGACTCCTACTGTTGATAGTTC	GCGTTCTTTTATTACTCATTTTGCAT	51	3D	USDA-ARS
21	barc45	CCCAGATGCAATGAAACCACAAT	GCGTAGAACTGAAGCGTAAAATTA	51	3A/2B	USDA-ARS
22	barc48	GCGAGCTGCAGAGGTCCATC	GCGTTAGTCTTCTTGGTCAATCAC	51	6B	USDA-ARS
23	barc49	GTCCCACCAAATTAACAGCTCCTA	AGGCGCAGTGCTCGAAGAATATTAT	51	5D	USDA-ARS
24	barc52	GCGCCATCCATCAACCGTCATCGTCATA	GCGAGGAAGGCGGCCACCAGAATGA	51	3D	USDA-ARS
25	barc53	GCGTCGTTCCTTTGCTTGTACCAGTA	GCGCGTCCTTCCAATGCAGAGTAGA	61	7D	USDA-ARS

List of microsatellite primers used for ... (Continued).

Serial number	Primer name	Forward primer sequence	Reverse primer sequence	Annealing temp. (°C)	Chromosome	Source
26	barc54	GCGAACAGGAGGACAGAGGGCACGAGAG	GCGCTTTCCCACGTTCCATGTTTCT	61	6D	USDA-ARS
27	barc55	GCGGTCAACACACTCCACTCCTCTCTC	CGCTGCTCCCATTGCTCGCCGTTA	51		USDA-ARS
28	barc56	GCGGGAATTTACGGGAAGTCAAGAA	GCGAGTGGTTCAAATTTATGTCTGT	51		USDA-ARS
29	barc59	GCGTTGGCTAATCATCGTTCCTTC	AGCACCCTACCCAGCGTCAGTCAAT	51	2D/5B	USDA-ARS
30	barc60	CATGCTCACAAAACCCACAAGACT	CTCGAAAGGCGGCACCACTA	51	1B/4B	USDA-ARS
31	barc62	TTGCCTGAGACATACATACACCTAA	GCCAGAACAGAATGAGTGCT	51	1D	USDA-ARS
32	barc66	CGCGATCGATCTCCCGGTTTGCT	GGGAAGAGGACCAAGGCCACTA	51	1D	USDA-ARS
33	barc67	GCGGCATTTACATTTCAGATAGA	TGTGCCTGATTGTAGTAACGTATGTA	51	3A	USDA-ARS
34	barc68	CGATGCCAACACACTGAGGT	AGCCGCATGAAGAGATAGGTAGAGAT	51	4B/3D/3B	USDA-ARS
35	barc69	AGGCGGCGGTCGTGGAACA	GCGTACCGAGAAGTGATCAAGAACAT	51	5A	USDA-ARS
36	barc70	GCGAAAAACGATGCGACTCAAAG	GCGCCATATAATTCAGACCCACAAAA	51	7D	USDA-ARS
37	barc71	GCGCTTGTTCCTCACCTGCTCATA	GCGTATATTCTCTCGTCTTCTTGTTGGTT	51	3D	USDA-ARS
38	barc72	CGTCCTCCCCCTCTCAATCTACTCTC	CGTCCCTCCATCGTCTCATCA	51	7B	USDA-ARS
39	barc73	GCGTGTCGTGCTTGTTCTCGGTTCTCAG	CGCTATTTGCCGCCACCTCCATCA	61	3B	USDA-ARS
40	barc75	AGGGTTACAGTTTGCTCTTTTAC	CCCGACGACCTATCTATACTTCTCTA	51	3B	USDA-ARS
41	barc76	ATTCGTTGCTGCCACTTGCTG	GCGCGACACGGAGTAAGGACACC	51	7D/6B/2A	USDA-ARS
42	barc77	GCGTATTCTCCCTCGTTTCCAAGTCTG	GTGGGAATTTCTTGGGAGTCTGTA	51	3B	USDA-ARS
43	barc78	CTCCCCGGTCAAGTTTAATCTCT	GCGACATGGGAATTTCAGAAGTGCCTAA	51	4A	USDA-ARS
44	barc80	GCGAATTAGCATCTGCATCTGTTTGAG	CGGTCAACCAACTACTGCACAAC	51	1B	USDA-ARS
45	barc81	GCGCTAGTGACCAAGTTGTTATATGA	GCGGTTCGGAAAGTGCTATTCTACAGTAA	51	1B	USDA-ARS
46	barc83	AAGCAAGGAACGAGCAAGAGCAGTAG	TGGATTTACGACGACGATGAAGATGA	61		USDA-ARS
47	barc84	CGCATAACCGTTGGGAAGACATCTG	GGTGCAACTAGAACGTACTTCCAGTC	51	3B	USDA-ARS
48	barc85	GCGAACGCTGCCCGGAGGAATCA	GCGTCGCAGATGAGATGGTGGAGCAAT	61	7B	USDA-ARS
49	barc87	GCTCACCGGGCATTGGGATCA	GCGATGACGAGATAAAGGTGGAGAAC	51	7D/3B	USDA-ARS
50	barc89	GGGCGCGGCACCAGCACTACC	CTCCGAGGCCACCGAAGACAAGATG	51	5B	USDA-ARS
51	barc90	GCGCTTGGGTTGCTTCGAGGAGGACA	CGCAATCCTCTTCCCCGTGGCATAG	51	2D	USDA-ARS
52	barc91	TTCCCATAACGCCGATAGTA	GCGTTTAATATTAGCTTCAAGATCAT	51	4D	USDA-ARS
53	barc92	GCGGTTGTGATGTGCTGAAAGATGAATGT	GCGTGGGCTGTTTCTTCCTTTTGTTTTC	51	3B	USDA-ARS
54	barc94	CGAAGAGACCATTGTATTGAGAA	GCGCATCATAGAGGGGTTGTTCATC	51		USDA-ARS
55	barc95	GGGGTGTGGTTGTTTGTAAGG	TGCGAATTCTATATACGATCTTGAGC	51	7B	USDA-ARS

List of microsatellite primers used for ... (Continued).

Serial number	Primer name	Forward primer sequence	Reverse primer sequence	Annealing temp. (°C)	Chromosome	Source
56	barc96	AAGCCTTGTTGTTCCGTATTATT	GCGGTTTATATTTTGTGGTTGAGCATTTT	51		USDA-ARS
57	barc98	CCGTCCTATTCGCAAACCAGATT	GCGGATATGTTCTCTAACTCAAGCAATG	51	2B	USDA-ARS
58	barc99	CGCATTCTTTCGCATTCTCTGTCATA	CGCATACTGTGTCGTGTTCCTGGTTTAGA	51	1D	USDA-ARS
59	barc101	GCTCCTCTCACGATCACGCAAAG	GCGAGTCGATCACACTATGAGCCAATG	51	2B	USDA-ARS
60	barc108	GCGGGTCGTTTCCTGGAAATTCATCTAA	GCGAAATGATTGGCGTTACACCTGTTG	51	7A	USDA-ARS
61	barc109	GGCAAAAGAGAAGGCTCGGAAGAACC	CGCATCGACGTAACATCACCACAATCATTT	51	4B/2D/5B	USDA-ARS
62	barc111	GCGGTCACCAGTAGTTCAACA	GCGTATCCCATTGCTCTTCTTCACTAAC	51	7D	USDA-ARS
63	barc117	TCATGCGTGCTAAGTGCTAA	GAGGGCAGGAAAAAGTGACT	51	5A	USDA-ARS
64	barc119	CACCCGATGATGAAAAT	GATGGCACAAGAAATGAT	51	1A/1D	USDA-ARS
65	barc121	ACTGATCAGCAATGTCAACTGAA	CCGGTGTCTTTCCTAACGCTATG	51	7A/7D	USDA-ARS
66	barc123	GGCCGAATTGAAAAAGCC	CCTGCCGTGTGCCGACTA	61		USDA-ARS
67	barc124	TGCACCCCTTCCAAATCT	TGCGAGTCGTGTGGTTGT	51	2D/2A	USDA-ARS
68	barc125	GCGTCGAGGGTAAAACAACATAT	GTAGCGTCAGTGCTCACACAATGA	51	3D	USDA-ARS
69	barc126	CCATTGAAACCGGATTTGAGTCG	CGTTCCATCCGAAATCAGCAC	51	7D	USDA-ARS
70	barc127	TGCATGCACTGTCCTTTGTATT	AAGATGCGGGCTGTTTTCTA	51	7A	USDA-ARS
71	barc128	GCGGGTAGCATTTATGTTGA	CAAACCAGGCAAGAGTCTGA	51	1B/2B/3D	USDA-ARS
72	barc130	CGGCTAGTAGTTGGAGTGTTGG	ACCGCCTCTAGTTATTGCTCTC	51	5D	USDA-ARS
73	barc134	CCGTGCTGCAAATGAACAC	AGTTGCCGGTTCCCATTGTCA	51	6B	USDA-ARS
74	barc137	GGCCCATTTCCCACTTTCCA	CCAGCCCCTCTACACATTTT	51	1B	USDA-ARS
75	barc138	CTCGATTCGCCGTCAG	GTGGGGGAAGAAGAAACC	51	4A	USDA-ARS
76	barc140	CGCCAACACCTACCATT	TTCTCCGCACTCACAAAC	51	5D/5B	USDA-ARS
77	barc141	GGCCCATGGATAATTTTTGAAATG	CAATTCGGCCAAAGAAGAAGTCA	51	5A/6B	USDA-ARS
78	barc142	CCGGTGAGAGGACTAAAA	GGCCTGTCAATTATGAGC	51	5B	USDA-ARS
79	barc143	TTGTGCCAAATCAAGAACAT	GGTTGGGCTAGGATGAAAAT	51	5D	USDA-ARS
80	barc144	GCGTTTTAGGTGGACGACATAGATAGA	GCGCCACGGGCATTTCTCATAC	51	5D	USDA-ARS
81	barc145	GCAGCCTCGAATCACA	GGGGTGTTGAAGATGA	51	1A/2D	USDA-ARS
82	barc146	AAGGCGATGCTGCAGCTAAT	GGCAATATGGAAACTGGAGAGAAAT	51	6A	USDA-ARS
83	barc147	GCGCCATTTATTCATGTTCCTCAT	CCGCTTCACATGCAATCCGTTGAT	51	3B	USDA-ARS
84	barc148	GCGCAACCACAATGTATGCT	GGGGTGTTTTCCTATTTCTT	51	1A	USDA-ARS
85	barc149	ATTCACTTGCCCCTTTTAAACTCT	GAGCCGTAGGAAGGACATCTAGTG	51	1D	USDA-ARS

List of microsatellite primers used for ... (Continued).

Serial number	Primer name	Forward primer sequence	Reverse primer sequence	Annealing temp. (°C)	Chromosome	Source
86	barc151	TGAGGAAAATGTCTCTATAGCATCC	CGCATAAACACCTTCGCTCTTCCACTC	51	5A/7A	USDA-ARS
87	barc154	GTAATTCCGGTTCCACTTGACATT	GGATGGGCAGCTTCAAGGTATGTT	51	7D/7A	USDA-ARS
88	barc158	TGTGTGGGAAGAAACTGAGTCATC	AGGAATACCAAAAGAAGCAAACCAAC	51	1A	USDA-ARS
89	barc159	CGCAATTTATTATCGGTTTTAGGAA	CGCCCGATAGTTTTTCTAATTTCTGA	51	2B/2D	USDA-ARS
90	barc163	GCGTGTTTTAAGGTATTTTCCATTTTCT	GCGCATCCTGTTCCTCCATTCATA	51	4B	USDA-ARS
91	barc164	TGCAAACTAATCACCAGCGTAA	CGCTTTCTAAAACTGTTCGGGATTTCTAA	51	3B	USDA-ARS
92	barc165	GCGTAGAGCGGCTGTTAGTGTCAAATTA	GCGTTATCTCAAGTTTTGTAGCAGA	51	5A	USDA-ARS
93	barc167	AAAGGCCCATCAACATGCAAGTACC	CGCAGTATTCTTAGTCCCTCAT	51	2B	USDA-ARS
94	barc168	GCGATGCATATGAGATAAGGAACAAATG	GCGGCTCTAAGGCGGTTTCAAAT	51	2D	USDA-ARS
95	barc169	CCGCGAACCATACAAAGGAAAC	GCTATAGAGGCGCCTTGGAGTACC	51	1D	USDA-ARS
96	barc170	CGCTTGACTTTGAATGGCTGAACA	CGCCCACTTTTTACCTAATCCTTTTGAA	51	4A	USDA-ARS
97	barc172	GCGAAATGTGATGGGGTTTATCTA	GCGATTTGATTTAACTTTAGCAGTGAG	51	7D	USDA-ARS
98	barc173	GGGGATCCTTCAACAATAACA	GCGAGATGGCATTTTTAAATAAAGAGAC	51	6D	USDA-ARS
99	barc174	TGGCATTTTTCTAGCACCAATACAT	GCGAACTGGACCAGCCTTCTATCTGTTC	51	2B/7A	USDA-ARS
100	barc175	GCGTAACAGAAGCGGAGAAAGC	GCGAATCATTTAGTGTTAGGTGGCAGTG	51	6D	USDA-ARS
101	barc176	GCGAAAGCCATCAAACACTATCCAACT	GGTAACTAAGCACGTCACAAGCATAAA	51	7B	USDA-ARS
102	barc178	GCGTATTAGCAAAACAGAAGTGAG	GCGACTAGTACGAACACCACAAAA	51	6B	USDA-ARS
103	barc180	GCGATGCTTGTTTGTTACTTCTC	GCGATGGAACTTCTTTTTGCTCTA	51	5A	USDA-ARS
104	barc181	CGCTGGAGGGGGTAAGTCATCAC	CGCAAATCAAGAACACGGGAGAAAGAA	51	1B	USDA-ARS
105	barc182	CCATGGCCAACAGCTCAAGGTCTC	CGCAAAACCGCATCAGGGAAGCACCAAT	51	7B	USDA-ARS
106	barc183	CCCGGGACCACCAGTAAGT	GGATGGGGAATTGGAGATACAGAG	51	6D/2B	USDA-ARS
107	barc184	TTCGGTGATATCTTTTCCCCTTGA	CCGAGTTGACTGTGTGGGCTTGCTG	51	7D	USDA-ARS
108	barc186	GGAGTGTCGAGATGATGTGGAAAC	CGCAGACGTCAGCAGCTCGAGAGG	51		USDA-ARS
109	barc187	GTGGTATTTCAGGTGGAGTTGTTTTA	CGGAGGAGCAGTAAGGAAGG	51		USDA-ARS
110	barc188	CGTGAGATCATGTTATCAGGACAAG	GCGTTGAAAGGTGTTAGTGGGATGG	51	1B	USDA-ARS
111	barc195	CCCACATGTCATTGGCTGTTTAA	GCCCGGCCCAGAACGATTTAAATG	51	7A/6A	USDA-ARS
112	barc196	GGTGGGTTTTATCGAATAGATTTGCT	GCGTTTCGTCAAGATTAATGCAGGTTT	51	6D	USDA-ARS
113	barc197	CGCATGGTCAGTTTTCTTTTAATCCT	GCGCTCTCCTTCATTTATGGTTTGTTG	51	5A	USDA-ARS
114	barc198	CGCTGAAAAGAAGTGCCGCATTATGA	CGCTGCCTTTTCTGGATTGCTTGTCA	51	6B	USDA-ARS
115	barc200	GCGATATGATTTGGAGCTGATTG	GCGATGACGTTAGATGCGGAATTGT	51		USDA-ARS

List of microsatellite primers used for ... (Continued).

Serial number	Primer name	Forward primer sequence	Reverse primer sequence	Annealing temp. (°C)	Chromosome	Source
116	barc204	CGCAGAAGAAAAACCTCGCAGAAAAACC	CGCAGTGTATCCAAATGGGCAAGC	51	6D	USDA-ARS
117	barc206	GCTTTGCCAGGTGAGCACTCT	TGGCCGGGTATTTGAGTTGGAGTTT	51	4A/6A/3B	USDA-ARS
118	barc212	GGCAACTGGAGTGATATAAATACCG	CAGGAAGGGAGGAGAACAGAGG	51		USDA-ARS
119	barc228	CCCTCCTCTCTTTAGCCATCC	GCACGTACTATTCGCCTTCACTTA	51	2D	USDA-ARS
120	barc229	GGCCGCTGGGGATTGCTATGAT	TCGGGATAAGGCAGACCACAT	51	1D	USDA-ARS
121	barc232	CGCATCCAACCATCCCCACCCAACA	CGCAGTAGATCCACCACCCCGCCAGA	61	5A/5B	USDA-ARS
122	barc240	AGAGGACGCTGAGAACTTTAGAGAA	GCGATCTTTGTAATGCATGGTGAAC	51	5B	USDA-ARS
123	barc267	GCGTGCTTTTTATTTTTGTGGACATCTT	GCGAATAATTGGTGGGTGAAACA	51	7B	USDA-ARS
124	cfa2019	GACGAGCTAACTGCAGACCC	CTCAATCCTGATGCGGAGAT	61	7A	INRA
125	cfa2028	TGGGTATGAAAGGCTGAAGG	ATCGCGACTATTCAACGCTT	61	7A	INRA
126	cfa2040	TCAAATGATTTCAGGTAACCACTA	TTCCTGATCCCACCAAACAT	51	7A/7D	INRA
127	cfa2049	TAATTTGATTGGGTCGGAGC	CGTGTCGATGGTCTCCTTG	61	7A	INRA
128	cfa2070	TCTGAACCCTTGATTTTCCG	TTACTGGCAAGCCAGAACTGT	61	5B	INRA
129	cfa2076	CGAAAAACCATGATCGACAG	ACCTGTCCAGCTAGCCTCCA	61	3D	INRA
130	cfa2104	CCTGGCAGAGAAAGTGAAGG	AGTCGCCGTTGTATAGTGCC	61	5A/5D	INRA
131	cfa2106	GCTGCTAAGTGCTCATGGTG	TGAAACAGGGGAATCAGAGG	61	7B	INRA
132	cfa2110	TCACTACCCGCATGAACAAA	TTCTGCACAAACCGTTCTGA	61	7A	INRA
133	cfa2121	TAAATGGCCATCAAGCAATG	GCTTGTGAACTAATGCCTCCC	61	4A/2A	INRA
134	cfa2129	GTTGCACGACCTACAAAGCA	ATCGCTCACTCACTATCGGG	61	1A	INRA
135	cfa2134	TTTACGGGGACAGTATTCGG	AAGACACTCGATGCGGAGAG	61	3A	INRA
136	cfa2141	GAATGGAAGGCGGACATAGA	GCCTCCACAACAGCCATAAT	61	5A/5D	INRA
137	cfa2147	TCATCCCCTACATAACCCGA	ATCGTGCACCAAGCAATACA	61	1B/1D/1B	INRA
138	cfa2155	TTTGTTACAACCCAGGGGG	TTGTGTGGCGAAAGAAACAG	61	5A	INRA
139	cfa2163	TTGATCCTTGATGGGAGGAG	CATCATTGTGTTTACGTTCTTTCA	61	5A	INRA
140	cfa2170	TGGCAAGTAACATGAACGGA	ATGTCATTCATGTTGCCCCT	61	3A/3B	INRA
141	cfa2185	TTCTTCAGTTGTTTTGGGGG	TTTGGTCGACAAGCAAATCA	61	5D	INRA
142	cfa2190	CAGTCTGCAATCCACTTTGC	AAAAGGAAACTAAAGCGATGGA	61	5A	INRA
143	cfa2193	ACATGTGATGTGCGGTCATT	TCCTCAGAACCCCATTCTTG	61	3A	INRA
144	cfa2219	TCTGCCGAGTCACTTCATTG	GACAAGGCCAGTCCAAAAGA	61	1A	INRA
145	cfa2226	GGAGAAAAGCAAACAGCGAC	CAGTAGCATCTTCCATGGCG	61	3B/1A	INRA

List of microsatellite primers used for ... (Continued).

Serial number	Primer name	Forward primer sequence	Reverse primer sequence	Annealing temp. (°C)	Chromosome	Source
146	cfa2234	AATCTGACCGAACAAAATCACA	TCGGAGAGTATTAGAACAGTGCC	61	3A	INRA
147	cfa2250	AGCCATAGATGGCCCTACCT	CACTCAATGGCAGGTCCTTT	61	5A	INRA
148	cfa2256	GGTAATATTCAGGTTACCGCACA	GGTAAAGTTATAAATTGTTGTGGGC	61	4A	INRA
149	cfa2257	GATACAATAGGTGCCTCCGC	CCATTATGTAAATGCTTCTGTTTGA	61	7A	INRA
150	cfa2262	ACAATGTGGAGATGGCACAA	TACCAGCTGCACTTCCATTG	61	2D/3A	INRA
151	cfa2278	GCCTCTGCAAGTCTTTACCG	AAGTCGGCCATCTTCTTCCT	61	2B	INRA
152	cfd1	ACCAAAGAACTTGCCTGGTG	AAGCCTGACCTAGCCCAAAT	61	6A	INRA
153	cfd2	GGTTGCAGTTTCCACCTTGT	CATCTATTGCCAAAATCGCA	61	2A/2D/3A/3D/	INRA
154	cfd3	GCACCAACACACGGAGAAG	TTGAGAGGAGGGCTTGGTTA	61	5D	INRA
155	cfd4	TGCTCCGTCTCCGAGTAGAT	GGGAAGGAGAGATGGGAAAC	61	3D/3B	INRA
156	cfd5	TGCCCTGTCCACAGTGAAG	TTGCCAGTTCCAAGGAGAAT	61	6D	INRA
157	cfd6	ACTCTCCCCCTCGTTGCTAT	ATTTAAGGGAGACATCGGGC	61	7A	INRA
158	cfd7	AGCTACCAGCCTAGCAGCAG	TCAGACACGTCTCCTGACAAA	61	5D/5B	INRA
159	cfd8	ACCACCGTCATGTCACTGAG	GTGAAGACGACAAGACGCAA	61	5D	INRA
160	cfd9	TTGCACGCACCTAAACTCTG	CAAGTGTGAGCGTCGG	61	3D	INRA
161	cfd10	CGTTCTATGACGTGTCATGCT	TCCATTTTCAAAAACACCCTG	61	5D	INRA
162	cfd12	GTTACCCAAACCTGCCCTTT	CTACGAGTCGGGATCAGCAT	61	5D	INRA
163	cfd13	CCACTAACCAAGCTGCCATT	TTTTTGGCATTGATCTGCTG	61	6B/6D	INRA
164	cfd14	CCACCGGCCAGAGTAGTATT	TCCTGGTCTAACAACGAGAAGA	61	7D	INRA
165	cfd15	CTCCCGTATTGAGCAGGAAG	GGCAGGTGTGGTGATGATCT	61	1A/1D	INRA
166	cfd16	GGATCCAAGGGAATCCAAAT	TCCTTCGGTTCCCATATCAC	61	4A	INRA
167	cfd17	AGCACAGAAGGGGTTAGGGT	AGCTGCGGTGTGAGCTAAAT	61	2D	INRA
168	cfd18	CATCCAACAGCACCAAGAGA	GCTACTACTATTTCATTGCGACCA	61	5D	INRA
169	cfd19	TACGCAGGTTTGCTGCTTCT	GGAGTTCACAAGCATGGGTT	61	1D/5D/6D	INRA
170	cfd20	TGATGGGAAGGTAATGGGAG	ATCCAGTTCTCGTCCAAAGC	61	1B	INRA
171	cfd21	CCTCCATGTAGGCGGAAATA	TGTGTCCCATTCACTAACCG	61	7D/1D	INRA
172	cfd22	GGTTGCAAACCGTCTTGTTT	AGTCGAGTTGCGACCAAAGT	61	4B	INRA
173	cfd23	TAGCAGTAGCAGCAGCAGGA	GCAAGGAAGAGTGTTCAGCC	61	4D	INRA
174	cfd25	CATCGCTCATGCTAAGGTCA	CGTGTCTGTTAGCTGGGTGG	61	2B/7D/5D	INRA
175	cfd26	TCAAGATCGTGCCAAATCAA	ACTCCAAGCTGAGCACGTTT	61	5D	INRA

List of microsatellite primers used for ... (Continued).

Serial number	Primer name	Forward primer sequence	Reverse primer sequence	Annealing temp. (°C)	Chromosome	Source
176	cfd28	TGCATCTTATTACTGGAGGCATT	CGCATGCCCTTATACCAACT	61	1D	INRA
177	cfd29	GGTTGTCAGGCAGGATATTTG	TATTGATAGATCAGGGCGCA	61	5D	INRA
178	cfd30	AATCGCACAACAATGGTTCA	GCCTCTCCTCTCTGCTCCTT	61	6A	INRA
179	cfd31	GCACCAACCTTGATAGGGAA	GTGCCTGATGATTTTACCCG	61	4A/7D	INRA
180	cfd34	GGAAGAACCGCAACAGACAT	GCATCTTCTCCTCCCTCCTC	61	3D	INRA
181	cfd35	GGGATGACACATAACGGACA	ATCAGCGGCGCTATAGTACG	61	3D	INRA
182	cfd36	GCAAAGTGTAGCCGAGGAAG	TTAGAGTTTTGCAGCGCCTT	61	2D/2A	INRA
183	cfd37	GCTTCTTTTGCTGCTTTTGC	CCCCCACATACAGAGGCTAA	61	6D	INRA
184	cfd39	CCACAGCTACATCATCTTTCCTT	CAAAGTTTGAACAGCAGCCA	61	4B/5A/4D	INRA
185	cfd40	GCGACAAGTAATTCAGAACGG	CGCTTCGGTAAAGTTTTTGC	61	5D	INRA
186	cfd41	TAAAGTCTCAGGCGACCCAC	AGTGATAGACGGATGGCACC	61	7D	INRA
187	cfd42	AGGTTCTAGGGGGCATGTCT	GCTCTCAATGACTGCACTGG	61	6D	INRA
188	cfd43	AACAAAAGTCGGTGCAGTCC	CCAAAAACATGGTTAAAGGGG	61	2D	INRA
189	cfd46	TGGTGGTATAGTCGTTGGAGC	CCACACACACACACCATCAA	61	7D	INRA
190	cfd48	ATGGTTGATGGTGGGTGTTT	ATGTATCGATGAAGGGCCAA	61	1B	INRA
191	cfd49	TGAGTTCTTCTGGTGAGGCA	GAATCGGTTCACAAGGGAAA	61	6D	INRA
192	cfd51	GGAGGCTTCTCTATGGGAGG	TGCATCTTATCCTGTGCAGC	61	2D	INRA
193	cfd55	CCAGTAGCCGGCCCTACTAT	GCACGAGATACGGACAATCA	61	3D	INRA
194	cfd56	TTGCATAATTACTTGCCCTCC	CTGGTCCAACTTCCATCCAT	61	2D	INRA
195	cfd57	ATCGCCGTTAACATAGGCAG	TCACTGCTGTATTTGCTCCG	61	5D	INRA
196	cfd59	TCACCTGGAAAATGGTCACA	AAGAAGGCTAGGGTTCAGGC	61	1D/1B/6B/1D	INRA
197	cfd60	TGACCGGCATTCAGTATCAA	TGGTCACTTTGATGAGCAGG	61	6D	INRA
198	cfd61	ATTCAAATGCAACGCAAACA	GTTAGCCAAGGACCCCTTTC	61	1D	INRA
199	cfd62	CAAGAGCTGACCAATGTGGA	ACGGCGGTGAGATGAG	61	2D/7A	INRA
200	cfd63	TCCTGAGGATGTTGAGGACC	GAGAGAGGCGAAACATGGAC	61	1D	INRA
201	cfd65	AGACGATGAGAAGGAAGCCA	CCTCCCTTGTTTTTGGGATT	61	1D/1B	INRA
201	cfd66	AGGTCTTGGTGGTTTTGGTG	TTTTCACATGCCCACAGTTG	61	7D	INRA
203	cfd67	GCGGACAAATTGAGCCTTAG	TGTGCGTGTGTGTGTGTTTT	61	5D	INRA
204	cfd69	AAATACCTTGAATTGTGAGCTGC	TCTGTTCATCCCCAAAGTCC	61	7D	INRA
205	cfd70	GTCGGCATAGTCGCACATAC	ACTATGCCAAGGGGAGTGTG	61	3D	INRA

List of microsatellite primers used for ... (Continued).

Serial number	Primer name	Forward primer sequence	Reverse primer sequence	Annealing temp. (°C)	Chromosome	Source
206	cfd71	CAATAAGTAGGCCGGGACAA	TGTGCCAGTTGAGTTTGCTC	61	4D/4A	INRA
207	cfd72	CTCCTTGGAATCTCACCGAA	TCCTTGGGAATATGCCTCCT	61	1D	INRA
208	cfd73	GATAGATCAATGTGGGCCGT	AACTGTTCTGCCATCTGAGC	61	2B/2D	INRA
209	cfd75	GCATAAACTTGGACCCTGGA	GCTAAGCCACGCTACCACTC	61	6D	INRA
210	cfd76	GCAATTTCACACGCGACTTA	CGCTCGACAACATGACACTT	61	6D	INRA
211	cfd78	ATGAAATCCTTGCCCTCAGA	TGAGATCATCGCCAATCAGA	61	5D	INRA
212	cfd79	TCTGGTTCTTGGGAGGAAGA	CATCCAACAATTTGCCCAT	61	3D/3B	INRA
213	cfd80	ATAGGGGTTTTGAATCACTCC	TTGGATTTGCAGAGCCTTCT	61	6D	INRA
214	cfd81	TATCCCCAATCCCCTCTTTC	GTCAATTGTGGCTTGTCCCT	61	7B/5D/4D	INRA
215	cfd84	GTTGCCTCGGTGTCGTTTAT	TCCTCGAGGTCCAAAACATC	61	4D	INRA
216	cfd86	TTAATGAGCGTCAGTACTCCC	GCAACCATGTTTAAGCCGAT	61	5D/5B	INRA
217	cfd88	TAGGCATAGTTTTGGGCCTG	GGTAGAAGGAAGCTTCGGGA	61	4A	INRA
218	cfd92	CTTGTTGATCTCCTTCCCCA	TTCTCTCATGACGGCAACAC	61	1D	INRA
219	cfd102	TTGTGGAAGGGTTTGATGAAG	TGCAGGACCAAACATAGCTG	61	5D	INRA
220	cfd106	ACGGGTGGTTTTGCTCAGT	ACTCCACCAGCGGAGAAATA	61	4D	INRA
221	cfd116	TTTGCCCATTACAACAAGCA	CAAGCAGCACCTCATGACAG	61	2D	INRA
222	cfd127	TAAACACCAGGGAGGTCCAC	ACCTACGATCGACGAAATGG	61	3D	INRA
223	cfd132	CAAATGCTAATCCCCGCC	TGTAAACAAGGTCGCAGGTG	61	6D	INRA
224	cfd135	GGATCTCGGGGATGTCCT	TAAGCACCTTCTTCATGGGG	61	6D	INRA
225	cfd141	CGTAAAGATCCGAGAGGGTG	TCCGAGGTGCTACCTACCAG	61	3D	INRA
226	cfd152	TGGAAGTCTGGAACCACTCC	GCAACCAGACCACACTCTCA	61	3D	INRA
227	cfd156	AGCAGTGTAATAAAAGGGCG	GTATTCGCACCAGAATCCGT	61	5B	INRA
228	cfd160	CCACTACTGCGGCTAGGTCT	CTTTTCCGTGTCTCCCTAGC	61	2D	INRA
229	cfd161	GTAAGGCATCTTCGCGTCTC	CCATGATAGATTTGGACGGG	61	2D	INRA
230	cfd168	CTTCGCAAATCGAGGATGAT	TTCACGCCCAGTATTAAGGC	61	2D	INRA
231	cfd175	TGTCGGGGACACTCTCTCTT	ACCAATGGGATGCTTCTTTG	61	2D	INRA
232	cfd183	ACTTGCACTTGCTATACTTACGAA	GTGTGTCGGTGTGTGGAAAG	61	5D	INRA
233	cfd188	AATGGCTTCACTGTTTGCCT	AAATGGTCCCAGCATTCAAG	61	6D	INRA
234	cfd189	GCTAAAGCCACATAGGACGG	GCACAAGATTTTGCAAGGCT	61	5D	INRA
235	cfd190	CAATCAGAAGCGCCATTGTT	CCCTGATGTTTTCTTTTTCTCC	61	6A	INRA

List of microsatellite primers used for ... (Continued).

Serial number	Primer name	Forward primer sequence	Reverse primer sequence	Annealing temp. (°C)	Chromosome	Source
236	cfd193	GCTGCCGCTACTGTCTGTC	GGCACACTCACACACCACAC	61	4D	INRA
237	cfd201	ACAAGACCACACCTCCAAGG	CGGTTTGGGTTTTGTGATCT	61	3D	INRA
238	cfd211	AGAAGACTGCACGCAAGGAT	TGCACTAAAGCATCTTCGTGTT	61	3D	INRA
239	cfd219	GGCCCATCTGTCATTGACTT	CAGCTTGTGTTGCTCGCTTA	61	5B/3D	INRA
240	cfd223	AAGAGCTACAATGACCAGCAGA	GCAGTGTATGTCAGGAGAAGCA	61	3D	INRA
241	cfd233	GAATTTTTGGTGGCCTGTGT	ATCACTGCACCGACTTTTGG	61	2D	INRA
242	cfd239	CTCTCGTTCTCTCCAGGCTC	GAGAGGAGAGCTTGCCATTG	61	2D	INRA
243	cfd242	CCAGTTTGCAGCAGTCACAT	CAGACCTTAACGGGGTTGAA	61	7A	INRA
244	cfd257	TCTCAACTTGCAACTGCCAC	CCCTCCATGGATTCTTGCTA	61	4A	INRA
245	cfd266	GAAAACAAAACCCATTTGCG	AAGCTTCAGTGCCTTTGGAA	61	5D	INRA
246	cfd282	TCTCATCCCTGTTCCTCTGC	GTCGACGTCTGCACATTGTT	61	1D	INRA
247	cfd283	CCCGTGGTCTTGGGTTC	AGTTTTGCCATCGGCTGTAT	61	4B/5D	INRA
248	cfd287	TCAAGAAGATGCGTTCATGC	GGGAGCTTTCCCTAGTGCTT	61	6D	INRA
249	gdm33	GGCTCAATTCAACCGTTCTT	TACGTTCTGGTGGCTGCTC	61	1A, 1D	Roder
250	gdm36	ATGCAAAGGAATGGATTCAA	CAAATCCGCATCCAGAAAAT	51	6D	Roder
251	gdm63	GCCCCCTATTCCATAGGAAT	CCTTTTGATGGTGCATAGGA	61	5D	Roder
252	gdm67	AAGCAAGGCACGTAAAGAGC	CTCGAAGCGAACACAAAACA	61	7D	Roder
253	gdm72	TGGTTTTCTCGAGCATTCAA	TGCAACGATGAAGACCAGAA	61	3D	Roder
254	gdm88	TCCCACCTTTTTGCTGTAGA	AAGGACAAATCCCTGCATGA	61	4A	Roder
255	gdm99	AGGTTGTCCACTGCCTGTTC	ATGTCGTCCTCGTCTCATCC	61	5D	Roder
256	gdm101	GTCTCCATGACAAGGAGGGA	TGAAACCTCAAAGGGAAAGA	61	5B, 2A / 1B	Roder
257	gdm109	GGTCCGCCTGACAGACC	AAAGCTGCTCATCGTGGTG	51	5A	Roder
258	gdm111	CACTCACCCCAAACCAAAGT	GATGCAATCGGGTCGTTAGT	61	1D	Roder
259	gdm113	ACCCATCTGATATTTTGGGG	AAAATGCCCTTCCCAACC	51	2B	Roder
260	gdm116	GCTGCAATGCAAGGTCTCTT	GATGTGGCTTTCTAAGGCAA	55	5D	Roder
261	gdm126	TCCATCATATCCGTAGCACA	CGTGGTTGATTTCAGGAGGT	61	1D	Roder
262	gdm132	ACCGCTCGGAGAAAATCC	AGGGGGGCAGAGGTAGG	61	6D	Roder
263	gdm133	ACGATTCATAACACAGCGCA	TGAGAACAATTTCACGGCTG	61	4D, 5B, 5D	Roder
264	gdm136	CTCATCCGGTGAGTGCATC	CCCGCATGTCTACATGAGAA	61	5D, 1A	Roder
265	gdm138	CATGAGCCGATTCAGCG	CGCTTAAATTGAAGTACCGC	61	5D	Roder

List of microsatellite primers used for ... (Continued).

Serial number	Primer name	Forward primer sequence	Reverse primer sequence	Annealing temp. (°C)	Chromosome	Source
266	gdm145	TGAAGGACAAATCCCTGCAT	TCCCACCTTTTTGCTGTAGA	61	4A	Roder
267	gdm146	ATCCTGACGGCCACCAC	CAAAGCCTGCGATACATCAA	61	5B	Roder
268	gdm153	TATAGGCAAATTAATTAAGACG	ATCTTTATGTGAGTACACTGC	61	5D	Roder
269	gwm2	CTGCAAGCCTGTGATCAACT	CATTCTCAAATGATCGAACA	61	3A,3D	Roder
270	gwm3	AATATCGCATCACTATCCCA	GCAGCGGCACTGGTACATTT	61	3D	Roder
271	gwm4	GCTGATGCATATAATGCTGT	CACTGTCTGTATCACTCTGCT	61	4A	Roder
272	gwm5	GCCAGCTACCTCGATACAACTC	AGAAAGGGCCAGGCTAGTAGT	61	3A	Roder
273	gwm6	CGTATCACCTCCTAGCTAAACTAG	AGCCTTATCATGACCCTACCTT	61	4B	Roder
274	gwm10	CGCACCATCTGTATCATTCTG	TGGTCGTACCAAAGTATACGG	61	2A	Roder
275	gwm11	GGATAGTCAGACAATTCTTGTG	GTGAATTGTGTCTTGTATGCTTCC	61	1B	Roder
276	gwm16	GCTTGGACTAGCTAGAGTATCATAC	CAATCTTCAATTCTGTCGCACGG	61	2B,5D,7B	Roder
277	gwm18	TGGCGCCATGATTGCATTATCTTC	GGTTGCTGAAGAACCTTATTTAGG	61	1B	Roder
278	gwm30	ATCTTAGCATAGAAGGGAGTGGG	TTCTGCACCCTGGGTGAT	61	3A	Roder
279	gwm32	TATGCCGAATTTGTGGACAA	TGCTTGGTCTTGAGCATCAC	61	3A	Roder
280	gwm33	GGAGTCACACTTGTTTGTGCA	CACTGCACACCTAACTACCTGC	61	1A, 1B, 1D	Roder
281	gwm37	ACTTCATTGTTGATCTTGCATG	CGACGAATTCCCAGCTAAAC	61	7D	Roder
282	gwm43	CACCGACGGTTTCCCTAGAGT	GGTGAGTGCAAATGTCATGTG	61	7B	Roder
283	gwm44	GTTGAGCTTTTCAGTTCGGC	ACTGGCATCCACTGAGCTG	61	7D	Roder
284	gwm46	GCACGTGAATGGATTGGAC	TGACCCAATAGTGGTGGTCA	61	7B	Roder
285	gwm47	TTGCTACCATGCATGACCAT	TTCACCTCGATTGAGGTCCT	61	2B	Roder
286	gwm52	CTATGAGGCGGAGGTTGAAG	TGCGGTGCTCTTCCATTT	61	3D	Roder
287	gwm55	GCATCTGGTACACTAGCTGCC	TCATGGATGCATCACATCCT	61	6D	Roder
288	gwm60	TGTCCTACACGGACCACGT	GCATTGACAGATGCACACG	61	7A	Roder
289	gwm63	TCGACCTGATCGCCCCTA	CGCCCTGGGTGATGAATAGT	61	7A	Roder
290	gwm66	CCAAAGACTGCCATCTTTCA	CATGACTAGCTAGGGTGTGACA	61	4B, 5B	Roder
291	gwm67	ACCACACAAACAAGGTAAGCG	CAACCCTCTTAATTTTGTTGGG	61	5B	Roder
292	gwm68	AGGCCAGAATCTGGGAATG	CTCCCTAGATGGGAGAAGGG	51	5B	Roder
293	gwm70	AGTGGCTGGGAGAGTGTCAT	GCCCATTACCGAGGACAC	61	6B	Roder
294	gwm71	GGCAGAGCAGCGAGACTC	CAAGTGGAGCATTAGGTACACG	61	3D	Roder
295	gwm72	TGGTCCCTCTCCCTTTCTCT	ACAGAATTGAAGATTGTCGGTC	61	3B	Roder

List of microsatellite primers used for ... (Continued).

Serial number	Primer name	Forward primer sequence	Reverse primer sequence	Annealing temp. (°C)	Chromosome	Source
296	gwm77	ACAAAGGTAAGCAGCACCTG	ACCCTCTTGCCCGTGTTG	61	3B	Roder
297	gwm88	CACTACAACTATGCGCTCGC	TCCATTGGCTTCTCTCTCAA	61	6B	Roder
298	gwm95	GATCAAACACACACCCCTCC	AATGCAAAGTGAAAAACCCG	61	2A	Roder
299	gwm99	AAGATGGACGTATGCATCACA	GCCATATTTGATGACGCATA	61	1A	Roder
300	gwm102	TCTCCCATCCAACGCCTC	TGTTGGTGGCTTGACTATTG	61	2D	Roder
301	gwm106	CTGTTCTTGCGTGGCATTAA	AATAAGGACACAATTGGGATGG	61	1D	Roder
302	gwm107	ATTAATACCTGAGGGAGGTGC	GGTCTCAGGAGCAAGAACAC	61	4B	Roder
303	gwm108	CGACAATGGGGTCTTAGCAT	TGCACACTTAAATTACATCCGC	61	3B	Roder
304	gwm111	TCTGTAGGCTCTCTCCGACTG	ACCTGCTCAGATCCCACTCG	61	7D	Roder
305	gwm112	CTAAACACGACAGCGGTGG	GATATGTGAGCAGCGGTCAG	61	3B	Roder
306	gwm113	ATTCGAGGTTAGGAGGAAGAGG	GAGGGTCGGCCTATAAGACC	61	4B	Roder
307	gwm114	ACAAACAGAAAATCAAAACCCG	ATCCATCGCCATTGGAGTG	61	3B	Roder
308	gwm120	GATCCACCTTCCTCTCTCTC	GATTATACTGGTGCCGAAAC	51	2B	Roder
309	gwm121	TCCTCTACAAACAAACACAC	CTCGCAACTAGAGGTGTATG	61	5D, 7D	Roder
310	gwm122	GGGTGGGAGAAAGGAGATG	AAACCATCCTCCATCCTGG	61	2A	Roder
311	gwm124	GCCATGGCTATCACCCAG	ACTGTTCGGTGCAATTTGAG	61	1B	Roder
312	gwm126	CACACGCTCCACCATGAC	GTTGAGTTGATGCGGGAGG	61	5A	Roder
313	gwm129	TCAGTGGGCAAGCTACACAG	AAAACTTAGTAGCCGCGT	61	2B, 5A	Roder
314	gwm130	AGCTCTGCTTCACGAGGAAG	CTCCTCTTTATATCGCGTCCC	61	7A	Roder
315	gwm131	AATCCCCACCGATTCTTCTC	AGTTCGTGGGTCTCTGATGG	61	1B, 3B	Roder
316	gwm132	TACCAAATCGAAACACATCAGG	CATATCAAGGTCTCCTTCCCC	61	6B	Roder
317	gwm133	ATCTAAACAAGACGGCGGTG	ATCTGTGACAACCGGTGAGA	61	6B	Roder
318	gwm135	TGTCAACATCGTTTTGAAAAGG	ACACTGTCAACCTGGCAATG	61	1A	Roder
319	gwm136	GACAGCACCTTGCCCTTTG	CATCGGCAACATGCTCATC	61	1A	Roder
320	gwm140	ATGGAGATATTTGGCCTACAAC	CTTGACTTCAAGGCGTGACA	61	1B	Roder
321	gwm146	CCAAAAAAACTGCCTGCATG	CTCTGGCATTGCTCCTTGG	61	7B	Roder
322	gwm147	AGAACGAAAGAAGCGCGCTGAG	ATGTGTTTCTTATCCTGCGGGC	61		Roder
323	gwm148	GTGAGGCAGCAAGAGAGAAA	CAAAGCTTGACTCAGACCAAA	51	2B	Roder
324	gwm149	CATTGTTTTCTGCCTCTAGCC	CTAGCATCGAACCTGAACAAG	61	4B	Roder
325	gwm153	GATCTCGTCACCCGGAATTC	TGGTAGAGAAGGACGGAGAG	61	1B	Roder

List of microsatellite primers used for ... (Continued).

Serial number	Primer name	Forward primer sequence	Reverse primer sequence	Annealing temp. (°C)	Chromosome	Source
326	gwm154	TCACAGAGAGAGAGGGAGGG	ATGTGTACATGTTGCCTGCA	51	5A	Roder
327	gwm155	CAATCATTTCCCCCTCCC	AATCATTGGAAATCCATATGCC	61	3A	Roder
328	gwm156	CCAACCGTGCTATTAGTCATTC	CAATGCAGGCCCTCCTAAC	51	5A	Roder
329	gwm157	GTCGTCGCGGTAAGCTTG	GAGTGAACACACGAGGCTTG	61	2D	Roder
330	gwm159	GGGCCAACACTGGAACAC	GCAGAAGCTTGTTGGTAGGC	61	5B	Roder
331	gwm160	TTCAATTCAGTCTTGGCTTGG	CTGCAGGAAAAAAAGTACACCC	61	4A	Roder
332	gwm161	GATCGAGTGATGGCAGATGG	TGTGAATTACTTGGACGTGG	61	3D	Roder
333	gwm162	AGTGGATCGACAAGGCTCTG	AGAAGAAGCAAAGCCTTCCC	61	3A	Roder
334	gwm164	ACATTTCTCCCCCATCGTC	TTGTAAACAAATCGCATGCG	51	1A	Roder
335	gwm165	TGCAGTGGTCAGATGTTTCC	CTTTTCTTTCAGATTGCGCC	61	4A, 4B, 4D	Roder
336	gwm169	ACCACTGCAGAGAACACATACG	GTGCTCTGCTCTAAGTGTGGG	61	6A	Roder
337	gwm174	GGGTTCCTATCTGGTAAATCCC	GACACACATGTTCCTGCCAC	61	5D	Roder
338	gwm179	AAGTTGAGTTGATGCGGGAG	CCATGACCAGCATCCACTC	61	5A	Roder
339	gwm181	TCATTGGTAATGAGGAGAGA	GAACCATTCATGTGCATGTC	51	3B	Roder
340	gwm182	TGATGTAGTGAGCCCATAGGC	TTGCACACAGCCAAATAAGG	61	5D	Roder
341	gwm183	GTCTTCCCATCTCGCAAGAG	CTCGACTCCCATGTGGATG	61	3D	Roder
342	gwm186	GCAGAGCCTGGTTCAAAAAG	CGCCTCTAGCGAGAGCTATG	61	5A	Roder
343	gwm190	GTGCTTGCTGAGCTATGAGTC	GTGCCACGTGGTACCTTTG	61	5D	Roder
344	gwm191	AGACTGTTGTTTGCGGGC	TAGCACGACAGTTGTATGCATG	61	2B, 5B, 6B	Roder
345	gwm192	GGTTTTCTTTCAGATTGCGC	CGTTGTCTAATCTTGCCTTGC	61	4A, 4B, 4D	Roder
346	gwm193	CTTTGTGCACCTCTCTCTCC	AATTGTGTTGATGATTTGGGG	61	6B	Roder
347	gwm194	GATCTGCTCTACTCTCCTCC	CGACGCAGAACTTAAACAAG	61	4D	Roder
348	gwm205	CGACCCGGTTCACTTCAG	AGTCGCCGTTGTATAGTGCC	61	5A, 5D	Roder
349	gwm210	TGCATCAAGAATAGTGTGGAAG	TGAGAGGAAGGCTCACACCT	61	2B, 2D	Roder
350	gwm212	AAGCAACATTTGCTGCAATG	TGCAGTTAACTTGTTGAAAGGA	61	5D	Roder
351	gwm213	TGCCTGGCTCGTTCTATCTC	CTAGCTTAGCACTGTCGCCC	61	5B	Roder
352	gwm219	GATGAGCGACACCTAGCCTC	GGGGTCCGAGTCCACAAC	61	6B	Roder
353	gwm232	ATCTCAACGGCAAGCCG	CTGATGCAAGCAATCCACC	61	1D	Roder
354	gwm233	TCAAAACATAAATGTTCATTGGA	TCAACCGTGTGTAATTTTGTCC	61	7A	Roder
355	gwm234	GAGTCCTGATGTGAAGCTGTTG	CTCATTGGGGTGTGTACGTG	61	5B	Roder

List of microsatellite primers used for ... (Continued).

Serial number	Primer name	Forward primer sequence	Reverse primer sequence	Annealing temp. (°C)	Chromosome	Source
356	gwm247	GCAATCTTTTTTCTGACCACG	ATGTGCATGTCGGACGC	61	3B	Roder
357	gwm249	CAAATGGATCGAGAAAGGGA	CTGCCATTTTTCTGGATCTACC	61	2A, 2D	Roder
358	gwm251	CAACTGGTTGCTACACAAGCA	GGGATGTCTGTTCCATCTTAG	61	4B	Roder
359	gwm257	AGAGTGCATGGTGGGACG	CCAAGACGATGCTGAAGTCA	61	2B	Roder
360	gwm259	AGGGAAAAGACATCTTTTTTTTC	CGACCGACTTCGGGTTC	61	1B	Roder
361	gwm260	GCCCCCTTGCACAATC	CGCAGCTACAGGAGGCC	61	7A	Roder
362	gwm261	CTCCCTGTACGCCTAAGGC	CTCGCGCTACTAGCCATTG	61	2D	Roder
363	gwm264	GAGAAACATGCCGAACAACA	GCATGCATGAGAATAGGAACTG	61	1B, 3B	Roder
364	gwm268	AGGGGATATGTTGTCACTCCA	TTATGTGATTGCGTACGTACCC	61	1B	Roder
365	gwm269	TGCATATAAACAGTCACACACCC	TTTGAGCTCCAAAGTGAGTTAGC	61	5D	Roder
366	gwm271	CAAGATCGTGGAGCCAGC	AGCTGCTAGCTTTTGGGACA	61	5D	Roder
367	gwm272	TGCTCTTTGGCGAATATATGG	GTTCAAAACAAATTAAAAGGCCC	61	5D	Roder
368	gwm273	ATTGGACGGACAGATGCTTT	AGCAGTGAGGAAGGGGATC	61	1B	Roder
369	gwm274	AACTTGCAAAACTGTTCTGA	TATTTGAAGCGGTTTGATTT	51	1B, 7B	Roder
370	gwm275	AATTTTCTTCCTCACTTATTCT	AACAAAAAATTAGGGCC	51	2A	Roder
371	gwm276	ATTTGCCTGAAGAAAATATT	AATTTCACTGCATACACAAG	51	7A	Roder
372	gwm282	TTGGCCGTGTAAGGCAG	TCTCATTCACACACAACACTAGC	61	7A	Roder
373	gwm284	AATGAAAAAACACTTGCGTGG	GCACATTTTTCACTTTCGGG	61	3B	Roder
374	gwm285	ATGACCCTTCTGCCAAACAC	ATCGACCGGGATCTAGCC	61	3B	Roder
375	gwm291	CATCCCTACGCCACTCTGC	AATGGTATCTATTCCGACCCG	61	5A	Roder
376	gwm292	TCACCGTGGTCACCGAC	CCACCGAGCCGATAATGTAC	61	5D	Roder
377	gwm293	TACTGGTTCACATTGGTGCG	TCGCCATCACTCGTTCAAG	61	5A	Roder
378	gwm294	GGATTGGAGTTAAGAGAGAACCG	GCAGAGTGATCAATGCCAGA	61	2A	Roder
379	gwm295	GTGAAGCAGACCCACAACAC	GACGGCTGCGACGTAGAG	61	7D	Roder
380	gwm296	AATTCAACCTACCAATCTCTG	GCCTAATAAACTGAAAACGAG	61	2A, 2D	Roder
381	gwm297	ATCGTCACGTATTTTGCAATG	TGCGTAAGTCTAGCATTTTCTG	61	7B	Roder
382	gwm299	ACTACTTAGGCCTCCCGCC	TGACCCACTTGCAATTCATC	61	3B	Roder
383	gwm301	GAGGAGTAAGACACATGCCC	GTGGCTGGAGATTCAGGTTC	61	2D	Roder
384	gwm302	GCAAGAAGCAACAGCAGTAAC	CAGATGCTCTTCTCTGCTGG	61	7B	Roder
385	gwm304	AGGAAACAGAAATATCGCGG	AGGACTGTGGGGAATGAATG	61	5A	Roder

List of microsatellite primers used for ... (Continued).

Serial number	Primer name	Forward primer sequence	Reverse primer sequence	Annealing temp. (°C)	Chromosome	Source
386	gwm311	TCACGTGGAAGACGCTCC	CTACGTGCACCACCATTTTG	61	2A, 2B	Roder
387	gwm312	ATCGCATGATGCACGTAGAG	ACATGCATGCCTACCTAATGG	51	2A	Roder
388	gwm314	AGGAGCTCCTCTGTGCCAC	TTCGGGACTCTCTTCCCTG	61	3D	Roder
389	gwm319	GGTTGCTGTACAAGTGTTCACG	CGGGTGCTGTGTGTAATGAC	61	2B	Roder
390	gwm320	CGAGATACTATGGAAGGTGAGG	ATCTTTGCAAGGATTGCCC	61	2D	Roder
391	gwm325	TTTCTTCTGTCGTTCTCTTCCC	TTTTTACGCGTCAACGACG	61	6D	Roder
392	gwm328	GCAATCCACGAGAAGAGAGG	CACAAACTCTTGACATGTGCG	61	2A	Roder
393	gwm332	AGCCAGCAAGTCACCAAAAC	AGTGCTGGAAAGAGTAGTGAAGC	61	7A	Roder
394	gwm333	GCCCGGTCATGTAAAACG	TTTCAGTTTGCGTTAAGCTTTG	61	7B	Roder
395	gwm334	AATTTCAAAAAGGAGAGAGA	AACATGTGTTTTTAGCTATC	51	6A	Roder
396	gwm335	CGTACTCCACTCCACACGG	CGGTCCAAGTGCTACCTTTC	61	5B	Roder
397	gwm337	CCTCTTCCTCCCTCACTTAGC	TGCTAACTGGCCTTTGCC	61	1D	Roder
398	gwm339	AATTTTCTTCCTCACTTATT	AAACGAACAACCACTCAATC	51	2A	Roder
399	gwm340	GCAATCTTTTTTCTGACCACG	ACGAGGCAAGAACACACATG	61	3B	Roder
400	gwm341	TTCAGTGGTAGCGGTCGAG	CCGACATCTCATGGATCCAC	61	3D	Roder
401	gwm344	CAAGGAAATAGGCGGTAACT	ATTTGAGTCTGAAGTTTGCA	61	7B	Roder
402	gwm349	GGCTTCCAGAAAACAACAGG	ATCGGTGCGTACCATCCTAC	61	2D	Roder
403	gwm350	ACCTCATCCACATGTTCTACG	GCATGGATAGGACGCCC	61	7A, 7D	Roder
404	gwm356	AGCGTTCTTGGGAATTAGAGA	CCAATCAGCCTGCAACAAC	61	2A	Roder
405	gwm357	TATGGTCAAAGTTGGACCTCG	AGGCTGCAGCTCTTCTTCAG	61	1A	Roder
406	gwm358	AAACAGCGGATTTCATCGAG	TCCGCTGTTGTTCTGATCTC	61	5D	Roder
407	gwm359	CTAATTGCAACAGGTCATGGG	TACTTGTGTTCTGGGACAATGG	61	2A	Roder
408	gwm361	GTAACTTGTTGCCAAAGGGG	ACAAAGTGGCAAAAGGAGACA	61	6B	Roder
409	gwm368	CCATTTCACCTAATGCCTGC	AATAAAACCATGAGCTCACTTGC	61	4B	Roder
410	gwm369	CTGCAGGCCATGATGATG	ACCGTGGGTGTTGTGAGC	61	3A	Roder
411	gwm371	GACCAAGATATTCAAACTGGCC	AGCTCAGCTTGCTTGGTACC	61	5B	Roder
412	gwm372	AATAGAGCCCTGGGACTGGG	GAAGGACGACATTCCACCTG	61	2A	Roder
413	gwm374	ATAGTGTGTTGCATGCTGTGTG	TCTAATTAGCGTTGGCTGCC	61	2B	Roder
414	gwm376	GGGCTAGAAAACAGGAAGGC	TCTCCCGGAGGGTAGGAG	61	3B	Roder
415	gwm382	GTCAGATAACGCCGTCCAAT	CTACGTGCACCACCATTTTG	61	2A, 2B, 2D	Roder

List of microsatellite primers used for ... (Continued).

Serial number	Primer name	Forward primer sequence	Reverse primer sequence	Annealing temp. (°C)	Chromosome	Source
416	gwm383	ACGCCAGTTGATCCGTAAAC	GACATCAATAACCGTGGATGG	61	3D	Roder
417	gwm388	CTACAATTCGAAGGAGAGGGG	CACCGCGTCAACTACTTAAGC	61	2B	Roder
418	gwm389	ATCATGTCGATCTCCTTGACG	TGCCATGCACATTAGCAGAT	61	3B	Roder
419	gwm397	TGTCATGGATTATTTGGTCGG	CTGCACTCTCGGTATACCAGC	61	4A	Roder
420	gwm400	GTGCTGCCACCACTTGC	TGTAGGCACTGCTTGGGAG	61	7B	Roder
421	gwm403	CGACATTGGCTTCGGTG	ATAAAACAGTGCGGTCCAGG	61	1B	Roder
422	gwm408	TCGATTTATTTGGGCCACTG	GTATAATTCGTTCACAGCACGC	61	5B	Roder
423	gwm410	GCTTGAGACCGGCACAGT	CGAGACCTTGAGGGTCTAGA	61	2B, 5A	Roder
424	gwm413	TGCTTGTCTAGATTGCTTGGG	GATCGTCTCGTCCTTGGCA	61	1B	Roder
425	gwm415	GATCTCCCATGTCCGCC	CGACAGTCGTCACTTGCCTA	61	5A	Roder
426	gwm425	GAGCCCACAAGCTGGCA	TCGTTCTCCCAAGGCTTG	61	2A	Roder
427	gwm427	AAACTTAGAACTGTAATTTCAGA	AGTGTGTTCATTTGACAGTT	51	6A	Roder
428	gwm428	CGAGGCAGCGAGGATTT	TTCTCCACTAGCCCCGC	61	7D	Roder
429	gwm429	TTGTACATTAAGTTCCCATTA	TTTAAGGACCTACATGACAC	51	2B	Roder
430	gwm437	GATCAAGACTTTTGTATCTCTC	GATGTCCAACAGTTAGCTTA	61	7D	Roder
431	gwm443	GGGTCTTCATCCGGAACTCT	CCATGATTTATAAATTCCACC	51	5B	Roder
432	gwm445	TTTGTTGGGGGTTAGGATTAG	CCTTAACACTTGCTGGTAGTGA	61	2A	Roder
433	gwm448	AAACCATATTGGGAGGAAAGG	CACATGGCATCACATTTGTG	61	2A	Roder
434	gwm455	ATTCGGTTCGCTAGCTACCA	ACGGAGAGCAACCTGCC	61	2D	Roder
435	gwm456	TCTGAACATTACACAACCCTGA	TGCTCTCTCTGAACCTGAAGC	61	3D	Roder
436	gwm458	AATGGCAATTGGAAGACATAGC	TTCGCAATGTTGATTTGGC	61	1D	Roder
437	gwm459	ATGGAGTGGTCACACTTTGAA	AGCTTCTCTGACCAACTTCTCG	51	6A	Roder
438	gwm469	CAACTCAGTGCTCACACAACG	CGATAACCACTCATCCACACC	61	6D	Roder
439	gwm471	CGGCCCTATCATGGCTG	GCTTGCAAGTTCCATTTTGC	61	7A	Roder
440	gwm473	TCATACGGGTATGGTTGGAC	CACCCCCTTGTTGGTCAC	61	2A	Roder
441	gwm480	TGCTGCTACTTGTACAGAGGAC	CCGAATTGTCCGCCATAG	61	3A	Roder
442	gwm484	ACATCGCTCTTCACAAACCC	AGTTCCGGTCATGGCTAGG	61	2D	Roder
443	gwm493	TTCCCATAACTAAAACCGCG	GGAACATCATTTCTGGACTTTG	61	3B	Roder
444	gwm494	ATTGAACAGGAAGACATCAGGG	TTCCTGGAGCTGTCTGGC	61	6A	Roder
445	gwm495	GAGAGCCTCGCGAAATATAGG	TGCTTCTGGTGTTCCTTCG	61	4B	Roder

List of microsatellite primers used for ... (Continued).

Serial number	Primer name	Forward primer sequence	Reverse primer sequence	Annealing temp. (°C)	Chromosome	Source
446	gwm497	GTAGTGAAGACAAGGGCATT	CCGAAAGTTGGGTGATATAC	61	1A,2A,3D	Roder
447	gwm498	GGTGGTATGGACTATGGACACT	TTTGCATGGAGGCACATACT	61	1B	Roder
448	gwm499	ACTTGTATGCTCCATTGATTGG	GGGGAGTGGAAACTGCATAA	61	5B	Roder
449	gwm501	GGCTATCTCTGGCGCTAAAA	TCCACAAACAAGTAGCGCC	61	2B	Roder
450	gwm508	GTTATAGTAGCATATAATGGCC	GTGCTGCCATGATATTT	51	6B	Roder
451	gwm512	AGCCACCATCAGCAAAAATT	GAACATGAGCAGTTTGGCAC	61	2A	Roder
452	gwm513	ATCCGTAGCACCTACTGGTCA	GGTCTGTTCATGCCACATTG	61	4B	Roder
453	gwm515	AACACAATGGCAAATGCAGA	CCTTCCTAGTAAGTGTGCCTCA	61	2A, 2D	Roder
454	gwm518	AATCACAACAAGGCGTGACA	CAGGGTGGTGCATGCAT	61	6B	Roder
455	gwm526	CAATAGTTCTGTGAGAGCTGCG	CCAACCCAAATACACATTCTCA	61	2B	Roder
456	gwm533	AAGGCGAATCAAACGGAATA	GTTGCTTTAGGGGAAAAGCC	61	3B	Roder
457	gwm537	ACATAATGCTTCCTGTGCACC	GCCACTTTTGTGTCGTTCCT	61	7B	Roder
458	gwm538	GCATTTCGGGTGAACCC	GTTGCATGTATACGTTAAGCGG	61	4B	Roder
459	gwm539	CTGCTCTAAGATTCATGCAACC	GAGGCTTGTGCCCTCTGTAG	61	2D	Roder
460	gwm540	TCTCGCTGTGAAATCCTATTTC	AGGCATGGATAGAGGGGC	51	5B	Roder
461	gwm544	TAGAATTCTTTATGGGGTCTGC	AGGATTCCAATCCTTCAAAATT	61	5B	Roder
462	gwm547	GTTGTCCCTATGAGAAGGAACG	TTCTGCTGCTGTTTTCATTTAC	51	3B	Roder
463	gwm550	CCCACAAGAACCTTTGAAGA	CATTGTGTGTGCAAGGCAC	51	1B	Roder
464	gwm554	TGCCCACAACGGAACTTG	GCAACCACCAAGCACAAAGT	61	5B	Roder
465	gwm558	GGGATTGCATATGAGACAACG	TGCCATGGTTGTAGTAGCCA	61	2A	Roder
466	gwm565	GCGTCAGATATGCCTACCTAGG	AGTGAGTTAGCCCTGAGCCA	61	5D	Roder
467	gwm566	TCTGTCTACCCATGGGATTTG	CTGGCTTCGAGGTAAGCAAC	61	3B	Roder
468	gwm569	GGAAACTTATTGATTGAAAT	TCAATTTTGACAGAAGAATT	51	7B	Roder
469	gwm570	TCGCCTTTTACAGTCGGC	ATGGGTAGCTGAGAGCCAAA	61	6A	Roder
470	gwm573	AAGAGATAACATGCAAGAAA	TTCAAATATGTGGGAACTAC	51	7A, 7B	Roder
471	gwm577	ATGGCATAATTTGGTGAAATTG	TGTTTCAAGCCCAACTTCTATT	61	7B	Roder
472	gwm582	AAGCACTACGAAAATATGAC	TCTTAAGGGGTGTTATCATA	51	1B	Roder
473	gwm583	TTCACACCCAACCAATAGCA	TCTAGGCAGACACATGCCTG	61	5D	Roder
474	gwm595	GCATAGCATCGCATATGCAT	GCCACGCTTGGACAAGATAT	61	5A	Roder
475	gwm601	ATCGAGGACGACATGAAGGT	TTAAGTTGCTGCCAATGTTCC	61	4A	Roder

List of microsatellite primers used for ... (Continued).

Serial number	Primer name	Forward primer sequence	Reverse primer sequence	Annealing temp. (°C)	Chromosome	Source
476	gwm604	TATATAGTTCAATATGACCCG	ATCTTTTGAACCAAATGTG	51	5B	Roder
477	gwm608	ACATTGTGTGTGCGGCC	GATCCCTCTCCGCTAGAAGC	61	2D, 4D	Roder
478	gwm609	GCGACATGACCATTTTGTTG	GATATTAAATCTCTCTATGTGTG	61	4D	Roder
479	gwm610	CTGCCTTCTCCATGGTTTGT	AATGGCCAAAGGTTATGAAGG	61	4A	Roder
480	gwm611	CATGGAAACACCTACCGAAA	CGTGCAAATCATGTGGTAGG	61	7B	Roder
481	gwm613	CCGACCCGACCTACTTCTCT	TTGCCGTCGTAGACTGG	61	6B	Roder
482	gwm614	GATCACATGCATGCGTCATG	TTTTACCGTTCCGGCCTT	61	2A	Roder
483	gwm617	GATCTTGGCGCTGAGAGAGA	CTCCGATGGATTACTCGCAC	61	5A, 6A	Roder
484	gwm624	TTGATATTAAATCTCTCTATGTG	AATTTTATTTGAGCTATGCG	61	4D	Roder
485	gwm626	GATCTAAAATGTTATTTTCTCTC	TGACTATCAGCTAAACGTGT	55	6B	Roder
486	gwm630	GTGCCTGTGCCATCGTC	CGAAAGTAACAGCGCAGTGA	51	2B	Roder
487	gwm635	TTCCTCACTGTAAGGGCGTT	CAGCCTTAGCCTTGGCG	61	7A,7D	Roder
488	gwm636	CGGTAGTTTTTAGCAAAGAG	CCTTACAGTTCTTGGCAGAA	61	2A	Roder
489	gwm637	AAAGAGGTCTGCCGCTAACA	TATACGGTTTTGTGAGGGGG	55	4A	Roder
490	gwm639	CTCTCTCCATTCGGTTTTCC	CATGCCCCCCTTTTCTG	61	5A, 5B, 5D	Roder
491	gwm642	ACGGCGAGAAGGTGCTC	CATGAAAGGCAAGTTCGTCA	61	1D	Roder
492	gwm644	GTGGGTCAAGGCCAAGG	AGGAGTAGCGTGAGGGGC	61	6B,7B	Roder
493	gwm645	TGACCGGAAAAGGGCAGA	GCCCCTGCAGGAGTTTAAGT	61	3D	Roder
494	gwm654	TGCTGATGTTGTAAGAAGGC	TGCGTCAGATATGCCTACCT	61	5D	Roder
495	gwm664	CAGTCAGTGCCGTTTAGCAA	AGCTTTGCTCTATTGGCGAG	61	3D	Roder
496	gwm666	GCACCCACATCTTCGACC	TGCTGCTGGTCTCTGTGC	61	1A, 5A, 7A	Roder
497	gwm674	TCGAGCGATTTTTCCTGC	TGACCGAGTTGACCAAAACA	61	3A	Roder
498	gwm705	TCTCCCTCATTAGAGTTGTCCA	ATGCAAGTTTAGAGCAACACCA	61		Roder
499	wmc1	ACTGGGTGTTTGCTCGTTGA	CAATGCTTAAGCGCTCTGTG	61		Agrogene
500	wmc9	AACTAGTCAAATAGTCGTGTCCG	GTCAAGTCATCTGACTTAACCCG	51		Agrogene
501	wmc10	GATCCGTTCTGAGGTGAGTT	GGCAGCACCCTCTATTGTCT	61		Agrogene
502	wmc11	TTGTGATCCTGGTTGTGTTGTGA	CACCCAGCCGTTATATATGTTGA	61	3A	Agrogene
503	wmc14	ACCCGTCACCGGTTTATGGATG	TCCACTTCAAGATGGAGGGCAG	61		Agrogene
504	wmc15	AGTCCGATTCGGACTCCTCAAG	GGACTAACCGAGGGTAGTTCAG	51		Agrogene
505	wmc16	ACCGCCTGCATTCTCATCTACA	GTGGCGCCATGGTAGAGATTTG	61		Agrogene

List of microsatellite primers used for ... (Continued).

Serial number	Primer name	Forward primer sequence	Reverse primer sequence	Annealing temp. (°C)	Chromosome	Source
506	wmc17	ACCTGCAAGAAATTAGGAACTC	CTAGTGTTTCAAATATGTCGGA	61	7A	Agrogene
507	wmc18	CTGGGGCTTGGATCACGTCATT	AGCCATGGACATGGTGTCCTTC	61	2D	Agrogene
508	wmc24	GTGAGCAATTTTGATTATACTG	TACCCTGATGCTGTAATATGTG	51	1A	Agrogene
509	wmc25	TCTGGCCAGGATCAATATTACT	TAAGATACATAGATCCAACACC	51	2B,2D	Agrogene
510	wmc27	AATAGAAACAGGTCACCATCCG	TAGAGCTGGAGTAGGGCCAAAG	61	5B?	Agrogene
511	wmc28	ATCACGCATGTCTGCTATGTAT	ATTAGACCATGAAGACGTGTAT	61		Agrogene
512	wmc31	GTTCACACGGTGATGACTCCCA	CTGTTGCTTGCTCTGCACCCTT	61		Agrogene
513	wmc36	TTCTCTTTTCCTTTCGCACTCC	CATCAGTTGTGGGGTTTCTTCA	61		Agrogene
514	wmc41	TCCCTCTTCCAAGCGCGGATAG	GGAGGAAGATCTCCCGGAGCAG	51		Agrogene
515	wmc42	GCCCTTGGTCCTGGGGTGAGCC	GCCTCATCCAGAGAGCCTGCGG	51		Agrogene
516	wmc43	TAGCTCAACCACCACCCTACTG	ACTTCAACATCCAAACTGACCG	61	3D,3B	Agrogene
517	wmc44	GGTCTTCTGGGCTTTGATCCTG	TGTTGCTAGGGACCCGTAGTGG	61	1B	Agrogene
518	wmc47	GAAACAGGGTTAACCATGCCAA	ATGGTGCTGCCAACAACATACA	61		Agrogene
519	wmc48	GAGGGTTCTGAAATGTTTTGCC	ACGTGCTAGGGAGGTATCTTGC	61	4B,4D	Agrogene
520	wmc49	CTCATGAGTATATCACCGCACA	GACGCGAAACGAATATTCAAGT	51		Agrogene
521	wmc51	TTATCTTGGTGTCTCATGTCAG	TCGCAAGATCATCAGAACAGTA	61	1B	Agrogene
522	wmc52	TCCAATCAATCAGGGAGGAGTA	GAACGCATCAAGGCATGAAGTA	61	1B	Agrogene
523	wmc59	TCATTCGTTGCAGATACACCAC	TCAATGCCCTTGTTTCTGACCT	61		Agrogene
524	wmc63	GTGCTCTGGAAACCTTCTACGA	CAGTAGTTTAGCCTTGGTGTGA	61		Agrogene
525	wmc65	TGGATGGGAAGGAGAATAAGTG	ATCCAACCGGAACTACCGTCAG	61		Agrogene
526	wmc70	GGGGAGCACCCTCTATTGTCTA	TAATGCTCCCAGGAGAGAGTCG	61		Agrogene
527	wmc73	TTGTGCACCGCACTTACGTCTC	ACACCCGGTCTCCGATCCTTAG	61		Agrogene
528	wmc74	AACGGCATTGAGCTCACCTTGG	TGCGTGAAGGCAGCTCAATCGG	61		Agrogene
529	wmc75	GTCCGCCGCACACATCTTACTA	GTTTGATCCTGCGACTCCCTTG	61		Agrogene
530	wmc76	CTTCAGAGCCTCTTTCTCTACA	CTGCTTCACTTGCTGATCTTTG	61	7B	Agrogene
531	wmc78	AGTAAATCCTCCCTTCGGCTTC	AGCTTCTTTGCTAGTCCGTTGC	61		Agrogene
532	wmc79	CATCAATGCATATGGCTGAAAT	AAAAGTTGTCATGAGCGAAGAA	61		Agrogene
533	wmc83	TGGAGGAAACACAATGGATGCC	GAGTATCGCCGACGAAAGGGAA	61	7A,2B,7A	Agrogene
534	wmc89	ATGTCCACGTGCTAGGGAGGTA	TTGCCTCCCAAGACGAAATAAC	61		Agrogene
535	wmc93	ACAACTTGCTGCAAAGTTGACG	CCAACTGAGCTGAGCAACGAAT	61	1A	Agrogene

List of microsatellite primers used for ... (Continued).

Serial number	Primer name	Forward primer sequence	Reverse primer sequence	Annealing temp. (°C)	Chromosome	Source
536	wmc94	TTCTAAAATGTTTGAAACGCTC	GCATTTCGATATGTTGAAGTAA	51	7D	Agrogene
537	wmc95	GTTTTTGTGATCCCGGGTTT	CATGCGTCAGTTCAAGTTTT	61		Agrogene
538	wmc96	TAGCAGCCATGCTTAGCATCAA	GTTTCAGTCTTTCACGAACACG	61		Agrogene
539	wmc97	GTCCATATATGCAAGGAGTC	GTACTCTATCGCAAAACACA	61	2A or 2B	Agrogene
540	wmc99	ATTACAATTGCTTCAGTGAGTG	TCATGATCATTGTTATAACGGT	51		Agrogene
541	wmc104	TCTCCCTCATTAGAGTTGTCCA	ATGCAAGTTTAGAGCAACACCA	61		Agrogene
542	wmc105	AATGTCATGCGTGTAGTAGCCA	AAGCGCACTTAACAGAAGAGGG	51		Agrogene
543	wmc109	AATTCGGGAAGAGTCTCAGGGG	TTCGAAGGGCTCAAGGGATACG	61		Agrogene
544	wmc110	GCAGATGAGTTGAGTTGGATTG	GTACTTGGAAACTGTGTTTGGG	61		Agrogene
545	wmc111	ATTGATGTGTACGATGTGCCTG	CATGTCAATGTCATGATGAAGC	61	2D	Agrogene
546	wmc112	TGAGTTGTGGGGTCTTGTTTGG	TGAAGGAGGGCACATATCGTTG	61	2D	Agrogene
547	wmc118	AGAATTAGCCCTTGAGTTGGTC	CTCCCATCGCTAAAGATGGTAT	61		Agrogene
548	wmc121	GGCTGTGGTCTCCCGATCATTC	ACTGGACTTGAGGAGGCTGGCA	61	7D	Agrogene
549	wmc125	ATACCACCATGCATGTGGAAGT	ACCGCTTGTCATTTCCTTCTGT	61		Agrogene
550	wmc128	CGGACAGCTACTGCTCTCCTTA	CTGTTGCTTGCTCTGCACCCTT	61		Agrogene
551	wmc134	CCAAGCTGTCTGACTGCCATAG	AGTATAGACCTCTGGCTCACGG	61		Agrogene
552	wmc139	TGTAACTGAGGGCCATGAAT	CATCGACTCACAACTAGGGT	61		Agrogene
553	wmc144	GGACACCAATCCAACATGAACA	AAGGATAGTTGGGTGGTGCTGA	61		Agrogene
554	wmc145	GGCGGTGGGTTCAAGTCGTCTG	GGACGAGTCGCTGTCCTCCTGG	61		Agrogene
555	wmc147	AGAACGAAAGAAGCGCGCTGAG	ATGTGTTTCTTATCCTGCGGGC	61		Agrogene
556	wmc149	ACAGACTTGGTTGGTGCCGAGC	ATGGGCGGGGGTGTAGAGTTTG	61	5B	Agrogene
557	wmc150	CATTGATTGAACAGTTGAAGAA	CTCAAAGCAACAGAAAAGTAAA	51		Agrogene
558	wmc152	CTATTGGCAATCTACCAAACTG	TCTCTTCTTGCCACATATTCGT	61		Agrogene
559	wmc153	ATGAGGACTCGAAGCTTGGC	CTGAGCTTTTGCGCGTTGAC	61		Agrogene
560	wmc154	ATGCTCGTCAGTGTCATGTTTG	AAACGGAACCTACCTCACTCTT	61	2B	Agrogene
561	wmc156	GCCTCTAGGGAGAAAACTAACA	TCAAGATCATATCCTCCCCAAC	61	1B	Agrogene
562	wmc158	AACTGGCATCATGTTTTGTAGG	AATGTAGTCAAAAGAGGTGGTG	61		Agrogene
563	wmc160	CATGGCTCCAAGATACAAAAAG	AGGCCTGGATTCATGATAGATA	51		Agrogene
564	wmc161	ACCTTCTTTGGGATGGAAGTAA	GTACTGAACCACTTGTAACGCA	61	5D	Agrogene
565	wmc166	ATAAAGCTGTCTCTTTAGTTCG	GTTTTAACACATATGCATACCT	51		Agrogene

List of microsatellite primers used for ... (Continued).

Serial number	Primer name	Forward primer sequence	Reverse primer sequence	Annealing temp. (°C)	Chromosome	Source
566	wmc167	AGTGGTAATGAGGTGAAAGAAG	TCGGTCGTATATGCATGTAAAG	61	2D	Agrogene
567	wmc168	AACACAAAAGATCCAACGACAC	CAGTATAGAAGGATTTTGAGAG	61		Agrogene
568	wmc169	TACCCGAATCTGGAAAATCAAT	TGGAAGCTTGCTAACTTTGGAG	61		Agrogene
569	wmc173	TGCAGTTGCGGATCCTTGA	TAACCAAGCAGCACGTATT	61		Agrogene
570	wmc175	GCTCAGTCAAACCGCTACTTCT	CACTACTCCAATCTATCGCCGT	61	2B	Agrogene
571	wmc177	AGGGCTCTCTTTAATTCTTGCT	GGTCTATCGTAATCCACCTGTA	61	2A	Agrogene
572	wmc179	CATGGTGGCCATGAGTGGAGGT	CATGATCTTGCGTGTGCGTAGG	61		Agrogene
573	wmc181	TCCTTGACCCCTTGCACTAACT	ATGGTTGGGAGCACTAGCTTGG	61		Agrogene
574	wmc182	GTATCTCACGAGCATAACACAA	GAAAGTGTATGGATCATTAGGC	61	6B	Agrogene
575	wmc183	CAGAAACGGCTCAACTTAACAA	TCTGATCTCGTGATCAGAATAG	51		Agrogene
576	wmc201	CATGCTCTTTCACTTGGGTTCG	GCGCTTGCAGGAATTCAACACT	61	6A	Agrogene
577	wmc206	TTGTGCTCGTGAATTGCATACC	GCCAAAATGGCAGCTTCTCTTA	51		Agrogene
578	wmc213	ATTTTCTCAAACACACCCCG	TAGCAGATGTTGACAATGGA	51		Agrogene
579	wmc215	CATGCATGGTTGCAAGCAAAAG	CATCCCGGTGCAACATCTGAAA	61	5D	Agrogene
580	wmc216	ACGTATCCAGACACTGTGGTAA	TAATGGTGGATCCATGATAGCC	61	1D	Agrogene
581	wmc218	TCTCCTGTCGGCTGAAAGTGTT	CCATGGAGGTTCACCTAGCAAA	61		Agrogene
582	wmc219	TGCTAGTTTGTCATCCGGGCGA	CAATCCCGTTCTACAAGTTCCA	51		Agrogene
583	wmc221	ACGATAATGCAGCGGGGAAT	GCTGGGATCAAGGGATCAAT	61		Agrogene
584	wmc222	AAAGGTGCGTTCATAGAAAATTAGA	AGAGGTGTTTGAGACTAATTTGGTA	61	1D	Agrogene
585	wmc231	CATGGCGAGGAGCTCGGTGGTC	GTGGAGCACAGGCGGAGCAAGG	61		Agrogene
586	wmc232	GAGATTTGTTCATTTCATCTTCGCA	TATATTAAAGGTTAGAGGTAGTCAG	61	4A	Agrogene
587	wmc233	GACGTCAAGAATCTTCGTCGGA	ATCTGCTGAGCAGATCGTGGTT	61		Agrogene
588	wmc235	ACTGTTCCTATCCGTGCACTGG	GAGGCAAAGTTCTGGAGGTCTG	61	5B	Agrogene
589	wmc238	TCTTCCTGCTTACCCAAACACA	TACTGGGGGATCGTGGATGACA	61		Agrogene
590	wmc243	CGTCATTTCCTCAAACACACCT	ACCGGCAGATGTTGACAATAGT	61		Agrogene
591	wmc245	GCTCAGATCATCCACCAACTTC	AGATGCTCTGGGAGAGTCCTTA	61	2D	Agrogene
592	wmc254	AGTAATCTGGTCCTCTCTTCTTCT	AGGTAATCTCCGAGTGCACTTCAT	51		Agrogene
593	wmc256	CCAAATCTTCGAACAAGAACCC	ACCGATCGATGGTGTATACTGA	61	6A	Agrogene
594	wmc257	GGCTACACATGCATACCTCT	CGTAGTGGGTGAATTTCGGA	51		Agrogene
595	wmc258	GCGATGTCAGATATCCGAAAGG	ACCAGGACACCAGAACAGCAAT	61	4A	Agrogene

List of microsatellite primers used for ... (Continued).

Serial number	Primer name	Forward primer sequence	Reverse primer sequence	Annealing temp. (°C)	Chromosome	Source
596	wmc261	GATGTGCATGTGAATCTCAAAAGTA	AAAGAGGGTCACAGAATAACCTAAA	61	2A	Agrogene
597	wmc262	GCTTTAACAAAGATCCAAGTGGCAT	GTAAACATCCAAACAAAGTCGAACG	61	4A	Agrogene
598	wmc264	CTCCATCTATTGAGCGAAGGTT	CAAGATGAAGCTCATGCAAGTG	61	3A	Agrogene
599	wmc265	GTGGATAACATCATGGTCAAC	TACTTCGCACTAGATGAGCCT	61	2B	Agrogene
600	wmc269	GCACCTTCTAACCTTCCCCAGC	CCCTAATCCAGGACTCCCTCAG	61		Agrogene
601	wmc272	TCAGGCCATGTATTATGCAGTA	ACGACCAGGATAGCCAATTCAA	61	2B	Agrogene
602	wmc273	AGTTATGTATTCTCTCGAGCCTG	GGTAACCACTAGAGTATGTCCTT	61		Agrogene
603	wmc274	AAGCAAGCAGCAAAACTATCAA	GAATGAATGAATGAATCGAGGC	61		Agrogene
604	wmc276	GACATGTGCACCAGAATAGC	AGAAGAACTATTCGACTCCT	61	7B	Agrogene
605	wmc278	AAACGATAGTAAAATTACCTCGGAT	TCAAAAAATAGCAACTTGAAGACAT	61		Agrogene
606	wmc283	CGTTGGCTGGGTTATATCATCT	GACCCGCGTGTAAGTGATAGGA	61		Agrogene
607	wmc285	TGTGGTTGTATTTGCGGTATGG	TTGTGGTGCTGAGTTAGCTTGT	61		Agrogene
608	wmc289	CATATGCATGCTATGCTGGCTA	AGCCTTTCAAATCCATCCACTG	61		Agrogene
609	wmc291	TACCACGGGAAAGGAAACATCT	CACGTTGAAACACGGTGACTAT	61		Agrogene
610	wmc296	GAATCTCATCTTCCCTTGCCAC	ATGGAGGGGTATAAAGACAGCG	61		Agrogene
611	wmc307	GTTTGAAGACCAAGCTCCTCCT	ACCATAACCTCTCAAGAACCCA	61		Agrogene
612	wmc310	TGTGAGGCTGGGAGGAAAAGAG	GCTAGGTTGTGTCCCACAATGC	51		Agrogene
613	wmc311	GGGCCTGCATTTCTCCTTTCTT	CTGAACTTGCTAGACGTTCCGA	61		Agrogene
614	wmc312	TGTGCCCGCTGGTGCGAAG	CCGACGCAGGTGAGCGAAG	61		Agrogene
615	wmc313	GCAGTCTAATTATCTGCTGGCG	GGGTCCTTGTCTACTCATGTCT	61		Agrogene
616	wmc317	TGCTAGCAATGCTCCGGGTAAC	TCACGAAACCTTTTCCTCCTCC	61		Agrogene
617	wmc318	CGTAAAATTACGGTGCATTGAT	GTGGACTTTTGTGGTTTTTGAG	61		Agrogene
618	wmc323	ACATGATTGTGGAGGATGAGGG	TCAAGAGGCAGACATGTGTTCG	61		Agrogene
619	wmc326	GGAGCATCGCAGGACAGA	GGACGAGGACGCCTGAAT	61		Agrogene
620	wmc331	CCTGTTGCATACTTGACCTTTTT	GGAGTTCAATCTTTCATCACCAT	61		Agrogene
621	wmc332	CATTTACAAAGCGCATGAAGCC	GAAAACTTTGGGAACAAGAGCA	61		Agrogene
622	wmc335	TGCGGAGTAGTTCTTCCCCC	ACATCTTGGTGAGATGCCCT	61		Agrogene
623	wmc336	GTCTTACCCCGCGATCTGC	GCGGCCTGAGCTTCTTGAG	61		Agrogene
624	wmc339	CCGCTCGCCTTCTTCCAG	TCCGGAACATGCCGATAC	61		Agrogene
625	wmc344	ATTTCAGTCTAATTAGCGTTGG	AACAAAGAACATAATTAACCCC	61		Agrogene

List of microsatellite primers used for ... (Continued).

Serial number	Primer name	Forward primer sequence	Reverse primer sequence	Annealing temp. (°C)	Chromosome	Source
626	wmc349	ACACACACTCGATCGCAC	GCAGTTGATCATCAAAACACA	61		Agrogene
627	wmc356	GCCGTTGCCCAATGTAGAAG	CCAGAGAAACTCGCCGTGTC	61		Agrogene
628	wmc357	TAGTGGGTGACCGGTCAAGA	TGGACGGATTTGGTCATTTC	61		Agrogene
629	wmc361	AATGAAGATGCAAATCGACGGC	ATTCTCGCACTGAAAACAGGGG	61		Agrogene
630	wmc363	TCTGTAACGCATAATAGAATAGCCC	ATGATTGCGTTATCTTCATATTTGG	61		Agrogene
631	wmc364	ATCACAATGCTGGCCCTAAAAC	CAGTGCCAAAATGTCGAAAGTC	61		Agrogene
632	wmc366	TACCTCTCTACGATGAAGCC	TGGAGTCTTAGTGTGGTGTT	61		Agrogene
633	wmc367	CTGACGTTGATGGGCCACTATT	GTGGTGGAAGAGGAAGGAGAGG	61		Agrogene
634	wmc376	TCTCAACCACCGACTTGTAA	ACATGTAATTGGGGACACTG	61		Agrogene
635	wmc382	CATGAATGGAGGCACTGAAACA	CCTTCCGGTCGACGCAAC	61		Agrogene
636	wmc386	ATCACTGAAACGAAATGAGCGG	TGGTTGGCGGTTTTTCTCTACA	61		Agrogene
637	wmc388	TGTGCGGAATGATTCAATCTGT	GGCCATTAGACTGCAATGGTTT	61		Agrogene
638	wmc396	TGCACTGTTTTACCTTCACGGA	CAAAGCAAGAACCAGAGCCACT	61		Agrogene
639	wmc397	AGTCGTGCACCTCCATTTTG	CATTGGACATCGGAGACCTG	61		Agrogene
640	wmc398	GGAGATTGACCGAGTGGAT	CGTGAGAGCGGTTCTTTG	61		Agrogene
641	wmc399	CTTCAGAGATGTTTGATTACCT	GGTATTGCTAACTGAATGATGT	61		Agrogene
642	wmc405	GTGCGGAAAGAGACGAGGTT	TATGTCCACGTTGGCAGAGG	61		Agrogene
643	wmc406	TATGAGGGTCGGATCAATACAA	CGAGTTTACTGCAAACAAATGG	61		Agrogene
644	wmc407	GGTAATTCTAGGCTGACATATGCTC	CATATTTCCAAATCCCCAACTC	51		Agrogene
645	wmc413	CACTGGAAACATCTCTTCAACT	ACAGGAAAGGATGATGTTCTCT	61		Agrogene
646	wmc415	AATTCGATACCTCTCACTCACG	TCAACTGCTACAACCTAGACCC	61		Agrogene
647	wmc416	AGCCCTTTCTACCGTGTTTCTT	TATGGTCGATGGACTGTCCCTA	61		Agrogene
648	wmc417	GTTCTTTTAGTTGCGACTGAGG	CGATGTATGCCGTATGAATGTT	61		Agrogene
649	wmc418	AGAGCAGCAAGTTGTGTAGCCA	TGAAGCTATTGCCAGCACGAG	61		Agrogene
650	wmc419	GTTTCGGATAAAACCGGAGTGC	ACTACTTGTGGGTTATCACCAGCC	61		Agrogene
651	wmc420	ATCGTCAACAAAATCTGAAGTG	TTACTTTTGCTGAGAAAACCCT	51		Agrogene
652	wmc422	GGACTACTGAACTGGAGAGTGTG	GCATTAGAATTTGGAGTTTGGAG	61		Agrogene
653	wmc426	GACGATCGTTTCTCCTACTTTA	ACTACACAAATGACTGCTGCTA	61		Agrogene
654	wmc428	TTAATCCTAGCCGTCCCTTTTT	CGACCTTCGTTGGTTATTTGTG	61		Agrogene
655	wmc429	CGTAAAGATTTTCATTTGGCG	AACGGCAGCTTGAAAACATAG	61		Agrogene

List of microsatellite primers used for ... (Continued).

Serial number	Primer name	Forward primer sequence	Reverse primer sequence	Annealing temp. (°C)	Chromosome	Source
656	wmc430	CAGTTGCAAGTTGGCCATAG	TAGGGACCCCTTGACAAAAA	61		Agrogene
657	wmc432	ATGACACCAGATCTAGCAC	AATATTGGCATGATTACACA	51		Agrogene
658	wmc434	GGAGCCTGATTAGGCTGGAC	AGCCAAACAGCCAACAGAGT	61		Agrogene
659	wmc435	GCACTATACTTATTGGATTGTCA	CATGGTATCCCTAGTAAGTTTTT	61		Agrogene
660	wmc438	GACCGTTGGGCTGTATAGCATT	CTCTGACAGTGGTGGAGCTTGA	61		Agrogene
661	wmc441	TCCAGTAGAGCACCTTTCATT	ATCACGAAGATAAACAAACGG	61		Agrogene
662	wmc443	CCTCCTCTGTTTTCCCTCTGTT	CACACTCTGTGCTTCTGTTTGC	61		Agrogene
663	wmc445	AGAATAGGTTCTTGGGCCAGTC	GAGATGATCTCCTCCATCAGCA	51		Agrogene
664	wmc446	CCAGCTAGTACTCTATATCTACATC	TATTTGAACAAGAGTTATGTGG	61		Agrogene
665	wmc450	GCAGGACAGGAGGTGAAGAAG	AGGCGTTGCTGATGACACTAC	61		Agrogene
666	wmc453	ACTTGTGTCCATAACCGACCTT	ATCTTTTGAGGTTACAACCCGA	61		Agrogene
667	wmc455	GCGTCATTTCCTCAAACACATC	AGAAGGAGAAGTGCCTCACCAA	61		Agrogene
668	wmc457	CTTCCATGAATCAAAGCAGCAC	CATCCATGGCAGAAACAATAGC	61		Agrogene
669	wmc463	GATTGTATAGTCGGTTACCCCT	ATTAGTGCCCTCCATAATTGTG	61		Agrogene
670	wmc468	AGCTGGGTTAATAACAGAGGAT	CACATAACTGTCCACTCCTTTC	61		Agrogene
671	wmc469	AGGTGGCTGCCAACG	CAATTTTATCAGATGCCCGA	51		Agrogene
672	wmc470	ACTTGCAACTGGGGACTCTC	TCCCCAATTGCATATTGACC	61		Agrogene
673	wmc471	GGCAATAATAGTGCAAGGAATG	GCCGATAATGGGCAATATAAGT	61		Agrogene
674	wmc473	TCTGTTGCGCGAAACAGAATAG	CCCATTGGACAACACTTTCACC	61		Agrogene
675	wmc474	ATGCTATTAAACTAGCATGTGTCG	AGTGGAAACATCATTCCTGGTA	61		Agrogene
676	wmc475	AACACATTTTCTGTCTTTCGCC	TGTAGTTATGCCCAACCTTTCC	61		Agrogene
677	wmc476	TACCAACCACACCTGCGAGT	CTAGATGAACCTTCGTGCGG	61		Agrogene
678	wmc477	CGTCGAAAACCGTACACTCTCC	GCGAAACAGAATAGCCCTGATG	61		Agrogene
679	wmc479	GACCTAAGCCCAGTGTCATCAG	AGACTCTTGGCTTTGGATACGG	61		Agrogene
680	wmc486	CCGGTAGTGGGATGCATTTT	ATGCATGCTGAATCCGGTAA	61		Agrogene
681	wmc487	CAAATTTGGCCACCATTTTACA	CGGTTCAATCCTTGGATTTACA	61		Agrogene
682	wmc488	AAAGCACAACCAGTTATGCCAC	GAACCATAGTCACATATCACGAGG	61		Agrogene
683	wmc489	CGAAGGATTTGTGATGTGAGTA	GGACAACATCATAGAGAAGGAA	61		Agrogene
684	wmc491	GGTAAAACTTCGTGTCCCTTGC	TAGTTGCGAGTCGGTAGTCTGC	61		Agrogene
685	wmc492	AGGATCAGAATAGTGCTACCC	ATCCCGTGATCAGAATAGTGT	61		Agrogene

List of microsatellite primers used for ... (Continued).

Serial number	Primer name	Forward primer sequence	Reverse primer sequence	Annealing temp. (°C)	Chromosome	Source
686	wmc494	GGATCGAGTCTCAAGTCTACAA	AGAAGGAACAAGCAACATCATA	61		Agrogene
687	wmc497	CCCGTGGTTTTCTTTCCTTCT	AACGACAGGGATGAAAAGCAA	51		Agrogene
688	wmc498	CGATGAAGAGAGCCATCAAAA	TGACATTCCGGTAGGTCAGTT	61		Agrogene
689	wmc500	ATAGCATGTTGGAACAGAGCAC	CTTAGATGCAACTCTATGCGGT	61		Agrogene
690	wmc503	GCAATAGTTCCCGCAAGAAAAG	ATCAACTACCTCCAGATCCCGT	61		Agrogene
691	wmc505	AGGGGAGGAAAACCTTGTAATC	ACGACCTACGTGGTAGTTCTTG	61		Agrogene
692	wmc506	CACTTCCTCAACATGCCAGA	CTTTCAATGTGGAAGGCGAC	61		Agrogene
693	wmc508	AGCCCTTGAGTTGGTCTCATTT	GAGCAGAGCTCCACTCACATTT	61		Agrogene
694	wmc511	CGCACTCGCATGATTTTCCT	ATGCCCGGAAACGAGACTGT	61		Agrogene
695	wmc513	TGAATTGAATCTGGTTGCGG	TGGCAATTCACAGGCACATA	61		Agrogene
696	wmc516	GGGCCACGAATAAACAG	GACTCGCAACTAGGGGT	51		Agrogene
697	wmc517	ATCCTGACGTTACACGCACC	ACCTGGAACACCACGACAAA	61		Agrogene
698	wmc522	AAAAATCTCACGAGTCGGGC	CCCGAGCAGGAGCTACAAAT	61		Agrogene
699	wmc524	TAGTCCACCGGACGGAAAGTAT	GTACCACCGATTGATGCTTGAG	61		Agrogene
700	wmc525	GTTTGACGTGTTTGCTGCTTAC	CTACGGATAATGATTGCTGGCT	51		Agrogene
701	wmc526	TCCCATTGGTTCACAAACTCG	GATGGTATCGCATTCATCGGT	61		Agrogene
702	wmc527	ACCCAAGATTGGTTGCAGAA	GCTACAGAAAACCGGAGCCTAT	61		Agrogene
703	wmc529	ATTGCATGCAAATTAGTAGTAG	GTGTTGACAAATTTTGAGTTAG	61		Agrogene
704	wmc532	GATACATCAAGATCGTGCCAAA	GGGAGAAATCATTAACGAAGGG	61		Agrogene
705	wmc533	AATTGGATCGGCAGTTGGAG	AGCAAGCAGAGCATTGCGTT	61		Agrogene
706	wmc537	TCTTCTGTACATTGAACAACGA	ATGCAGAACCGTGATAGGAT	61		Agrogene
707	wmc539	GCAAGTAGGACCTTACAGTTCT	GTTATAACCTTTGTCCCTTCAC	61		Agrogene
708	wmc540	CGGGGTCCTAACTACGGTGA	CCTGTAATGGAGGACGGCTG	61		Agrogene
709	wmc544	CCATTTGAGGTTTGGTCGCTAC	TATATGTGATTTGTCGTGCCCC	61		Agrogene
710	wmc546	CGGCTAAAATCGTACACTACACA	CTCACTTGCACGATTTCCCTAT	61	7B/ 7B/ 4B	Agrogene
711	wmc549	TTGTCACACACGCACTCCC	GTCCTTCCCTCGTTCATCCT	61		Agrogene
712	wmc552	ACTAAGGAGTGTGAGGGCTGTG	CTCTCGCGCTATAAAAGAAGGA	61	3D	Agrogene
713	wmc553	CGGAGCATGCAGCTAGTAA	CGCCTGCAGAATTCAACAC	61	6A	Agrogene
714	wmc557	GGTGCTTGTTCATACGGGCT	AGGTCCTCGATCCGCTCAT	61		Agrogene
715	wmc559	ACACCACGAATGATGTGCCA	ACGACGCCATGTATGCAGAA	61	3A	Agrogene

List of microsatellite primers used for ... (Continued).

Serial number	Primer name	Forward primer sequence	Reverse primer sequence	Annealing temp. (°C)	Chromosome	Source
716	wmc574	TCCCCTACTGGAACCACGAC	ATCCATCGACCGACAAGAGC	61		Agrogene
717	wmc577	CTGTCCGACTCCCCAGATG	CCCTGTCAGAGGCTGGTTG	61	5A	Agrogene
718	wmc580	AAGGCGCACAACACAATGAC	GGTCTTTTGTGCAGTGAACTGAAG	61	6A	Agrogene
719	wmc581	CATGTTGCCATCAAACTCGC	GCTATTGACATGCAACTATGGACCT	61	7B	Agrogene
720	wmc590	CGCACGAAGCTATCTGATACCA	GGAAAACCTAACCCTAGCCACC	61		Agrogene
721	wmc592	GGTGGCATGAACTTTCACCTGT	TGTGTGGTGCCCATTAGGTAGA	61		Agrogene
722	wmc593	GGGGAGAAGCAGCAGGG	CGCGCGGTTGCCGGTGG	61		Agrogene
723	wmc594	CCCCTCACTGCCG	ATATCCATATAGTACTCGCAC	51		Agrogene
724	wmc596	TCAGCAACAAACATGCTCGG	CCCGTGTAGGCGGTAGCTCTT	61		Agrogene
725	wmc597	AACACACCTTGCTTCTCTGGGA	GACTAGGGTTTCGGTTGTTGGC	61		Agrogene
726	wmc598	TCGAGGAGTCAACATGGGCTG	ACGGTCGCTAGGGAGGGGAG	61		Agrogene
727	wmc601	ACAGAGGCATATGCAAAGGAGG	CTTGTCTCTTTATCGAGGGTGG	61		Agrogene
728	wmc602	TACTCCGCTTTGATATCCGTCC	GTTTGTTGTTGCCATCACATTC	61		Agrogene
729	wmc603	ACAAACGGTGACAATGCAAGGA	CGCCTCTCTCGTAAGCCTCAAC	61		Agrogene
730	wmc606	CCGATGAACAGACTCGACAAGG	GGCTTCGGCCAGTAGTACAGGA	61		Agrogene
731	wmc607	ATATATGCCCATGAAGCTCAAG	GATCGAGCTAAAGCTGATACCA	61		Agrogene
732	wmc608	ACTGGAACGCGAAACAAATGG	CAGGAGCCCCTCCTAGATTGG	61		Agrogene
733	wmc609	CATCCAGCCCATGTAGACGC	AACGGTGCCCATCATCTCCC	61		Agrogene
734	wmc611	GGTTCGCTTTCAAGGTCCACTC	CGGGACACTAGTGCTCGATTCT	61		Agrogene
735	wmc612	GAGGTCAGTACCCGGAGA	CCACCCCAATTCAAAAAG	61		Agrogene
736	wmc613	ACAACTGTGAAACGAGACGGTG	GTGAGTGTGAAAACCAAGACGC	61		Agrogene
737	wmc615	TGCCCACAACTTATCTCAG	GGTAAGTGGCCCAGGTAGT	51		Agrogene
738	wmc616	TAAAGCTAGGAGATCAGAGGCG	TAATCCCATCTTGAGAAGCGTC	61		Agrogene
739	wmc617	CCACTAGGAAGAAGGGGAAACT	ATCTGGATTACTGGCCAACTGT	61		Agrogene
740	wmc619	TTCCCTTTCCCCTCTTTCCG	TACAATCGCCACGAGCACCT	61		Agrogene
741	wmc621	GACGTAGGGCGGCGGATA	TGCGCCGTGTTTAATTGCTC	51		Agrogene
742	wmc622	CAGGAAGAAGAGCTCCGAGAAA	CTTGCTAACCCGCGCC	61		Agrogene
743	wmc623	ACGATTGGCCACAGAGGAG	CAGTGACCAATAGTGGAGGTCA	61		Agrogene
744	wmc625	CACAGACCTCAACCTCTTCTT	AGTACTGTTCACAGCAGACGA	61		Agrogene
745	wmc626	AGCCCATAAACATCCAACACGG	AGGTGGGCTTGGTTACGCTCTC	61		Agrogene

List of microsatellite primers used for ... (Continued).

Serial number	Primer name	Forward primer sequence	Reverse primer sequence	Annealing temp. (°C)	Chromosome	Source
746	wmc627	GATCCGAGAAGGGCAATGGTAG	AGCAACAGCAGCGTACCATAAA	61		Agrogene
747	wmc629	TTTGTGTGTTGGATGCGTGC	AATAAAACGCGACCTCCCCC	61		Agrogene
748	wmc630	ATAATGCACGGTAGGACTGAGG	CATACTGAGACAATTTGGGGGT	61		Agrogene
749	wmc631	TTGCTCGCCCACCTTCTACC	GGAAACCATGCGCTTCACAC	61		Agrogene
750	wmc632	GTTTGATTGGTCGTTCCTGGTC	AACAGCGAATGGAGGGCTTTAG	61		Agrogene
751	wmc633	ACACCAGCGGGGATATTTGTTAC	GTGCACAAGACATGAGGTGGATT	61		Agrogene
752	wmc634	AGCGAGGAGGATGCATCTTATT	GACATACACATGATGGACACGG	61		Agrogene
753	wmc636	AATTACAGAAGGCCATACAGTC	ATTAAGAGAAAAGGGAAGGATG	51		Agrogene
754	wmc640	AATTTATCTCGATCATGTGAGC	TGAGTAGTTCCCTTAGGACCTT	61		Agrogene
755	wmc644	GACCCTGGTATTCGCACCTCTG	CGTGACGGCCATTACATAGGAG	61		Agrogene
756	wmc646	GGAGTAAATGGAGACGGGGAC	GCCAGTGTGATGCATGTGAC	61		Agrogene
757	wmc650	AAAGCAAGAGCAGACTGGC	GCACATCAGTAACGCATCTC	61		Agrogene
758	wmc651	CGACGACGTCCGGGTG	CATTTCCTCTCCCATATCTCTCATC	61		Agrogene
759	wmc652	ATACGGCAAAGGAGAAGCGG	GGTAGCGCTAATGCAGGGTG	61		Agrogene
760	wmc653	AGTGTTTTAGGGGTGGAAGGGA	CGGAACCCTAAACCCTAGTCG	61		Agrogene
761	wmc654	CTGTGATGAACTGAAATAACCA	TATTCTACTTTTCTCTTCCCCC	61		Agrogene
762	wmc656	AAGTAGGCGAGCGTTGT	TTTCCCTGGCGAGATG	61		Agrogene
763	wmc657	CGGGCTGCGGGGGTAT	CGGTTGGGTCATTTGTCTCA	61		Agrogene
764	wmc658	CTCATCGTCCTCCTCCACTTTG	GCCATCCGTTGACTTGAGGTTA	61		Agrogene
765	wmc661	CCACCATGGTGCTAATAGTGTC	AGCTCGTAACGTAATGCAACTG	61		Agrogene
766	wmc662	AGTGGAGCCATGGTACTGATTT	TGTGTACTATTCCCGTCGGTCT	61		Agrogene
767	wmc664	GGGCCAACAAATCCAAT	TCTACTTCCTTCATCCACTCC	61		Agrogene
768	wmc667	GAGGAGAGGAAAAGGCAGGCTA	AACTCTTGCGTGTCTCAAACCG	61		Agrogene
769	wmc671	GTACGTCAAAGAAAGAGAATTACCTC	CTCAGAGATATATCTTCGTTGTCAGT	61		Agrogene
770	wmc672	GGAGGAGCAAGCTAGGCAA	TTTATAGAGGGAGGGGAGGCAG	61		Agrogene
771	wmc673	AGGAAACAAGAGTGTGTGTGGG	AGGAATAAGGACTCGCAAAACG	61		Agrogene
772	wmc674	TTTGAAAACTCCTCGGGTCGTC	CACGAGCTCGAGGTGTTTGTAG	61		Agrogene
773	wmc675	TTGCTAGTTAGCGAACACCATC	GGGCTGTCATGTGAAGTAAAGA	61		Agrogene
774	wmc679	TAGGGGACAGGAGGGAGGG	CGGATCCAGACCAGGAAGGT	61		Agrogene
775	wmc680	TGAGTGTTCAGGCCGCACTATG	ATCCTTGTTCAGGAATCCCCGT	61		Agrogene

List of microsatellite primers used for ... (Continued).

Serial number	Primer name	Forward primer sequence	Reverse primer sequence	Annealing temp. (°C)	Chromosome	Source
776	wmc682	GAGCGTGCGAAAAAACTGAAT	TTCTATCGCACGCATCCAAA	61		Agrogene
777	wmc684	CGAATCCAACGAGGCCATAGA	GCAATCAGGAGGCATCCACC	61		Agrogene
778	wmc687	AGGACGCCTGAATCCGAG	GGGAGCGTAGGAGGACTAACA	61		Agrogene
779	wmc692	TTATCTTGATCCGAGCGA	ATGTGATTAGTCCTAAGGTCTCTCT	51		Agrogene
780	wmc693	CAGCGCCGCTCCCAAGA	GCACACTGATTGCAGCCCCAT	61		Agrogene
781	wmc694	ATTTGCCCTTGTGAGCCGTT	GACCTGGGTGGGACCCATTA	61		Agrogene
782	wmc695	GAGGGCACCTCGTAAGTTGG	GGCAGGAGCCCCTACAAGAT	61		Agrogene
783	wmc696	ACCCGAGAGAGATTAGGGCTTG	CACTCGCAGCCTCTCTTCTACC	61		Agrogene
784	wmc698	GTGAAGGGAGAGCTAGCAA	ACAGTTGGCCCAGCTAGTA	51		Agrogene
785	wmc702	GAATCACATCGAATGGATCTCA	GAGGCCTTTTTCGATATTCTGC	61		Agrogene
786	wmc705	GGTTGGGCTCCTGTCTGTGAA	TCTTGCACCTTCCCATGCTCT	61		Agrogene
787	wmc707	GCTAGCTGACACTTTTCCTTTG	TCAGTTTCCCACTCACTTCTTT	61		Agrogene
788	wmc710	GTAAGAAGGCAGCACGTATGAA	TAAGCATTCCCAATCACTCTCA	51 or 61		Agrogene
789	wmc713	ACATAGCATCCCATACTGAGAGAGG	ATGCGGGGAATAGAGACACAC	61	5A	Agrogene
790	wmc716	CATTTATGTGCACGCCGAAG	CCATAAGCATCGTCACCCTG	61		Agrogene
791	wmc718	GGTCGGTGTTGATGCACTTG	TCGGGGTGTCTTAGTCCTGG	61	4A	Agrogene
792	wmc719	TTGTGGGAATCTACATCAGAAGG	AACAGCCACGCTCTATCTTCAGT	61	1B	Agrogene
793	wmc720	CACCATGGTTGGCAAGAGA	CTGGTGATACTGCCGTGACA	61		Agrogene
794	wmc722	GCTTTTCGATGGGATGGTGC	TTTGTCCACTGCCTTCTGCC	61		Agrogene
795	wmc723	CTCGCTCGATCCCCTTTC	CGAGGTGGAGTCCCGTCTAT	61		Agrogene
796	wmc726	GCAAAGAACCGTGCCCTGAC	CGGGGTGGCCCGAGA	61		Agrogene
797	wmc727	CATAATCAGGACAGCCGCAC	TAGTGGCCTGATGTATCTAGTTGG	61	5A	Agrogene
798	wmc728	GCAGGCTCTGCATCTTCTTG	CGCAGAGCTGAGCTGAAATC	61		Agrogene
799	wmc732	ACTGCCCGTAGAACACCGTC	ACGGGGTTCTCCTTCCTCAA	55		Agrogene
800	wmc734	GGTGACCAGCGGTGAGC	CCGTCTCGGCCTCTCTAGATTT	61		Agrogene
801	wmc737	CGACTAGGACTAGACGACTCTAACGG	GTCGATCACCAGAGGCATTG	61	6B	Agrogene
802	wmc740	CTTGGTTGCAGACGGGG	GCTGGGTGCAATGCAGATAG	61	5B	Agrogene
803	wmc741	CAACAACGCTAGAGGCCAAC	GGGCTCCATGCTCTTCC	61		Agrogene
804	wmc744	AAAACAACAGGTTTCTCATCGC	GGTTAATCCTAAGGCATCTCTCC	61		Agrogene
805	wmc745	AAACAGAGGAGGGGGAGAGC	TAGACGATGCCAGCACGATG	61		Agrogene

List of microsatellite primers used for ... (Continued).

Serial number	Primer name	Forward primer sequence	Reverse primer sequence	Annealing temp. (°C)	Chromosome	Source
806	wmc748	CCAGCCCAGATGCTTCAATG	ACGTGGGTGCAATTCTCAGG	61	6A/ 6D/ 6B	Agrogene
807	wmc749	GGGTACAGGAGGATCTGACAGG	TCTCGTCTCCGTCTAGGTTCG	61	6D	Agrogene
808	wmc751	ATTGCCGGGTTGAGTTTGAT	ACATCTTCAGCATTATAGGGGGT	51		Agrogene
809	wmc752	CCGATTGTAGATCAAAAGCC	TCTAGAGAGTCTTTTTCCCGAGC	61		Agrogene
810	wmc753	AAGGTGAAGATGATGCTCGC	TGACTGATCATGGATTGCCC	61		Agrogene
811	wmc754	ATCCACATGAACCTCAACTTATGG	GGCATTGTTGTTGTACTGCAGTC	61	3B	Agrogene
812	wmc756	TTCCGTGGCCTCTCGTTC	CATTGCCATCAGTCACCCTC	51		Agrogene
813	wmc757	AAGTCTCACGCCCTCTCCAA	CCCTCCCCGTGGACCT	61		Agrogene
814	wmc758	TAGGGGAGGCGACGGAG	GTTGCTGGAGAGTGGATTGC	61		Agrogene
815	wmc759	CCTTACCTCCGTCTCCCTT	GGAGTGTGCGGCCAAA	61	5B	Agrogene
816	wmc760	ATCATACGGCTTCCCCTTCC	CAGGCGGTGTATTGTGTTCG	61		Agrogene
817	wmc762	CCTTGAAGGCGCGACG	GTCTGTACCTCCCTGCACCG	61		Agrogene
818	wmc764	CCTCGAACCTGAAGCTCTGA	TTCGCAAGGACTCCGTAACA	61	2B	Agrogene
819	wmc765	GGGATCAGACTGGGACTGGAG	GGGTTGGCTTGGCAGAGAA	61	5D	Agrogene
820	wmc766	AGATGGAGGGGATATGTTGTCAC	TCGTCCCTGCTCATGCTG	61		Agrogene
821	wmc770	TGTCAGACTTCCTTTGATCCCC	AAGACCATGTGACGTCCAGC	61	2B	Agrogene
822	wmc773	GAGGCTTGCATGTGCTTGA	GCCAACTGCAACCGGTACTCT	61	5B	Agrogene
823	wmc776	CCATGACGTGACAACGCAG	ATTGCAGGCGCGTTGGTA	61		Agrogene
824	wmc777	GCCATCAAGCGGATCAACT	GTAGCGCCCTGTTTCACCTC	61	3B	Agrogene
825	wmc783	AGGTTGGAGATGCAGGTGGG	TCTTCCTTCTCCTGCCGCTA	61	5B	Agrogene
826	wmc786	GGGTCACCAACCCGCTC	CGTGGGTGCAATTCTCAGG	61	6A/ 6D/ 6B	Agrogene
827	wmc787	GCTTGCTAGCAGCATCAGAGG	CGATGCTTCTCTCTGCAGGTC	61		Agrogene
828	wmc788	GGTTATTCCTTGCATTCCCG	CTCTTAGCTCTAGCTCGTGCTCATC	61		Agrogene
829	wmc790	AATTAAGATAGACCGTCCATATCATCCA	CGACAACGTACGCGCC	61	7A	Agrogene
830	wmc792	GGATGCAGTAGCAGTCAGGGA	CTCCATCGCTAGGCAGGG	61		Agrogene
831	wmc794	GTAAACTGGAAAGAAAACGAACCTG	CTATCCACACGTGGAAAAGAAATC	61		Agrogene
832	wmc795	GGCTCGATTCCGTTACCTCA	GGCGATTCGCCACACCT	61		Agrogene
833	wmc797	CGAAACCCTAGATGAAGC	ACACAACCACAGGTGAGTTGTTCT	51		Agrogene
834	wmc798	GTGTGGTAGTGTAGCTGCCAAAAG	GTTAGCATGGCACATAGAAGCAG	61		Agrogene
835	wmc799	CGTACGTACGCCTGTACCCTTG	AATCTTGGGCGTCTAATCTTTTGC	61	5D	Agrogene

List of microsatellite primers used for ... (Continued).

Serial number	Primer name	Forward primer sequence	Reverse primer sequence	Annealing temp. (°C)	Chromosome	Source
836	wmc805	GATGCTGCTGCACCAAACTC	GCCTTTTCCATGCCACACT	61	5A/ 5D	Agrogene
837	wmc807	ATCCAACAAGGCCTCACCAT	GCAGGTTTGATCTGGATTTCATC	61		Agrogene
838	wmc808	TGAACCATCATCGGAGCTTG	TTTTAGCCGAAGTCAAACATTGC	61		Agrogene
839	wmc809	CAGGTCGTAGTTGGTACCCTGAA	TGAACACGGCTGGATGTGA	61		Agrogene
840	wmc810	GGCACCGATGCTTCCA	GCCCCAACCACCTCCC	61		Agrogene
841	wmc813	TGTTGGATGCGTGCGAC	CCTCTCCCGGACTCCTGC	61		Agrogene
842	wmc815	GACAGAATTGAAGATTGTCGGC	GCACGAAAAACTTGTTGGTCC	61	3B	Agrogene
843	wmc817	TGACGGGGATGATGATAACG	CGGTGAGATGAGAAAGGAAAAC	61	2D/ 2B	Agrogene
844	wmc818	TGAAGGGTGCGTGTGGTC	GCGTCGATTTTAATTTGATGATGG	61	5D/ 2D/ 4D	Agrogene
845	wmc819	GATTCGGTCGGTTGGCTAAG	GTTTGTGGTGGGTGGATTGC	61	2A	Agrogene
846	wmc822	CACCCGTCGACCTAGACACC	CGACTGCCCTCTGCTATCCT	61	6D	Agrogene
847	wmc824	CCGATGAACTTAAAAGTACCACCTG	CATGGATTGACACGATTGGC	61	7D	Agrogene
848	wmc825	GCTAGCTGCTGGTTCCACTTG	TGTCCACTCCACTCCAGCATTAC	51		Agrogene
849	wmc826	GAGGTAGATGACCACGCCG	CACGATCCCCCAAGCAC	61	1A/ 4B/ 7A	Agrogene
850	wmc827	ACGGTGACCTCAGTGCTCAC	ATGCTTGCCTCAGCAAAACC	61	1A/ 4B/ 7A	Agrogene
851	wmc830	ACCTTTTCCTGCATCGGCT	CTCCGCTCGTGTCCAACTATC	61	1B	Agrogene