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Understanding the basis of resistance to Fusarium head blight in UK winter wheat (REFAM)

by

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ABSTRACT

Fusarium head blight (FHB) of wheat is caused predominantly by *Fusarium graminearum* and *F. culmorum* although other *Fusarium* species and *Microdochium majus* and *M. nivale* are also important in some regions. The disease can often contaminate the grain with mycotoxins such as deoxynivalenol (DON) and nivalenol (NIV). Of 53 UK National List varieties tested for response to FHB, only three (Soissons, Spark and Vector) had significant stable resistance over trials. UK barley varieties differed significantly in FHB resistance.

Three FHB resistant varieties were studied to identify the location of quantitative trait loci (QTL) associated with FHB resistance. Analysis of Spark and Soissons was combined with study of near-isogenic semi-dwarf lines for *Rht1* and *Rht2*. Our results demonstrated that *Rht2* is associated with a significant increase in susceptibility to initial infection (Type I resistance) while being largely unaffected in resistance to spread within the spike (Type II resistance). In contrast, *Rht1* conferred no negative effect on FHB resistance, even conferring a very minor positive effect in one trial. Under high disease pressure both *Rht1* and *Rht2* significantly decreased Type 1 resistance. However, while *Rht2* had no effect on Type 2 resistance *Rht1* significantly increased Type 2 resistance. Enhanced susceptibility associated with *Rht2* is probably due to linkage to deleterious genes rather than to pleiotropy and the positive effect of *Rht1* on FHB resistance is due either to pleiotropy conferring Type 2 resistance or very tight linkage to resistance genes. In the third variety (RL4137), we identified FHB resistance QTL on chromosomes 1B and 2B.

Correlation for resistance to *F. culmorum* (DON-producer) and *M. majus* (non toxin-producer) was moderate across 29 European varieties following spray inoculation. Following point inoculation *M. majus* was not able to spread. Type 2 resistance appears to be important to restrict spread of DON-producing isolates of some species but may be largely irrelevant for other pathogens. Spread of a NIV-producing isolate of *F. graminearum* was much slower than that of a DON producing isolate. These isolates were used to identify and characterise new sources of FHB resistance among 300 lines from CIMMYT. 60 lines were shown to have moderate/high levels of FHB resistance. A few lines possessed a high level of Type I resistance only whereas a greater number possessed both Type I and Type II resistance. These lines merit further study as potential sources of novel FHB resistance. Furthermore, we propose that spray inoculation with an appropriate aggressive non DON-producing FHB pathogens may be used to identify the Type I FHB resistance component in wheat.

PROJECT SUMMARY

Introduction

Fusarium head blight (FHB) of wheat (also known as Fusarium ear blight), is caused by several fungal species that produce similar symptoms. *Fusarium graminearum* is the major pathogen worldwide, while *F. culmorum* tends to predominate in maritime regions. *Fusarium avenaceum* and *F. poae* are also frequently associated with FHB, particularly in Northern Europe. In addition to the true *Fusarium* species, two *Microdochium* species, *M. majus* and *M. nivale,* also cause FHB and are particularly prevalent where cooler, wetter conditions prevail such as in the UK.

FHB is of particular concern because many of the *Fusarium* species produce mycotoxins in infected grain and pose a risk to human and animal consumers. The most common mycotoxin in blighted grain is the trichothecene deoxynivalenol (DON), produced by *Fusarium graminearum* and *F. culmorum.* A second, closely related trichothecene, produced by certain isolates of these species is nivalenol (NIV).

The development and deployment of FHB-resistant cultivars is generally accepted as the most cost-effective and environmentally benign way to minimise disease and potential risk to consumers. However, resistance to FHB is quantitatively inherited and the influence of environment on disease makes reliable disease screening (phenotyping) difficult. To date no variety has been found to be immune to FHB. Advances in phenotyping, combined with statistical methods to detect regions on wheat chromosomes harbouring genes for resistance to FHB, have led to the identification of numerous so-called quantitative trait loci (QTL) for FHB resistance. Several important sources of FHB resistance have been identified among Chinese spring wheat varieties that have been deployed in breeding programs worldwide. The resistance of some of these varieties, notably Sumai 3, appears to be controlled by a few genes of major effect and hence may be amenable for use in marker-assisted breeding programmes that can greatly facilitate the rapid introgression of resistance into new varieties.

Although a number of European winter wheat varieties show resistance to FHB, in only a few varieties has the basis for the resistance been genetically characterised. In contrast to the resistance in some spring wheat varieties, FHB resistance in winter wheat germplasm appears to be due to numerous QTL of moderate to small effect. DNA markers, such as 'simple sequence repeats' (SSRs) can be used to infer the origin and genetic relationship between FHB resistance QTL. This information is used

by plant breeders to identify appropriate parental combinations in order to 'pyramid' resistance genes when developing new varieties.

Due to the previously low incidence of FHB in the UK, a comprehensive assessment of resistance in elite UK winter wheat germplasm has not yet been undertaken. One aim of the current study was to assess the FHB reaction of winter wheat and spring barley varieties currently on the UK National List (2003). A second aim was to compare the sizes of SSR markers at FHB QTL in Chinese varieties with those in UK and European winter wheat varieties to establish whether different genes might be responsible. Diversification of resistance sources in breeding programs should reduce the risk of the emergence of virulent pathogen strains.

An important aim of this project was to understand the genetic basis of FHB resistance in selected wheat varieties. Initial studies showed Soissons and Spark to be the most resistant UK varieties and QTL analysis was undertaken of Spark x Rialto and Soissons x Orvantis doubled haploid populations. A third population consisted of recombinant inbred lines from RL4137 (FHB resistant) x Timgalen. Field and polytunnel disease trials were established and each line scored at several locations. Genetic maps were produced for all three crosses and QTL analysis carried out for FHB resistance traits. Several authors have reported a negative relationship between plant height and FHB resistance and, for this reason we also undertook QTL analysis for selected morphological traits to determine the genetic basis of FHB resistance and identify any associations between resistance to FHB and characteristics such as plant height.

The mycotoxin DON is required to facilitate the spread of fungus from the point of infection into other parts of the wheat head via the rachis. Comparison of DON and NIV chemotypes of *F. graminearum* suggest that DON production is associated with greater disease causing potential. The reduced aggressiveness of NIV, relative to DON producing isolates (chemotypes), may stem from the much lower phytotoxicity of NIV towards wheat. In contrast to *F. graminearum* and *F. culmorum*, *Microdochium majus* is not known to produce mycotoxins.

Resistance of wheat to FHB appears to be horizontal and non-species specific with no clear evidence for any differential effect on different pathogen species. An additional aim of the current study was to investigate whether FHB resistance effective against toxin-producing species is also effective against non toxigenic species. Two components of host resistance to FHB are widely recognised: resistance to initial infection (Type I resistance) and resistance to spread within the spike (Type II). It is generally accepted that single spikelet (point) inoculation assesses Type II

resistance only while spraying a conidial suspension on spikes and scoring disease incidence on a plot basis assesses Type I resistance. However, accurate assessment of Type I resistance can be hindered by differences among varieties in their degree of Type II resistance leading to altered disease severity.

Several major QTL conditioning Type II resistance have been reported but only a few studies have identified QTL for Type I resistance. This may reflect a paucity of Type I resistance, but it is also probable that the need to infer Type I resistance is hampering the identification of this form of resistance. If species, or isolates, that produce little or no toxin can infect but not spread within the spike they might be used as tools to identify Type I resistance. We undertook studies to establish whether particular species, or isolates, of FHB causing pathogens could be used to facilitate the identification of Type I resistance and established trials to identify and characterise potential sources of FHB resistance among a collection of wheat lines obtained from the International Maize and Wheat Improvement Center (CIMMYT), Mexico.

Materials and methods

Plant materials, map construction and QTL analysis

The Fusarium head blight (FHB) reaction of 53 varieties from the (2003) National List of winter wheat varieties approved for sale in the United Kingdom (UK) was compared with 19 reference cultivars from Continental Europe which had previously been characterised for resistance by collaborative partners. A similar assessment was made for UK spring barley varieties.

Recombinant inbred lines (RILs) of a cross between RL4137 and Timgalen and doubled haploid (DH) populations: Spark/Rialto, Soissons/Orvantis, was used in the study. Near isogenic lines (NILs) differing in their *Rht1* and *Rht2* semi-dwarfing alleles in Mercia and Maris Huntsman background were used to assess the relationship between FHB resistance and height.

Thirty winter wheat varieties were used to assess the efficacy of FHB resistance against toxin-producing and non-producing species. The level and type of FHB resistance was assessed in a collection of 300 lines from a CIMMYT FHB resistance breeding programme.

FHB disease screening was carried out in field, glasshouse and polytunnel experiments conducted either at the John Innes Centre (JIC), Norwich, National Institute for Agricultural Botany (NIAB), Cambridge, Central Science Laboratory (CSL), York or at sites of participating commercial partners (Nickerson Seeds Ltd, Advanta Seeds (UK) Ltd. Elsoms Seeds Ltd.). Plants were inoculated at mid-anthesis with

conidial suspensions of highly virulent DON producing isolates of *Fusarium culmorum*, *F. graminearum* or a NIV producing isolate of *F. graminearum* or *Microdochium majus* (non toxin-producer), either by spray or point inoculation as appropriate. Following spray inoculation, disease was assessed several times and expressed as area under the disease progress curve (AUDPC). For point inoculation experiments disease was generally measured as the number of spikelets showing symptoms. In addition to measuring disease, selected morphological traits such as presence/absence of awns, plant height (PH) and weight of infected spikelets (WIS) were recorded.

The genetic maps for the mapping populations were constructed using SSRs, Amplified Fragment Length Polymorphism (AFLP) and Diversity Array Technology (DArT) markers. Linkage maps were constructed and QTL detection was carried out by Interval Mapping (IM) and using the Multiple QTL Model (MQM). The QTL that explained more than 10 % of the variance (R^2) in at least one environment/experiment were classified as major QTL and those explaining less than 10 % as minor QTL.

DON analysis, DNA extraction and quantitative PCR

The DON content of milled grain was assessed using an enzyme linked immuno-assay (ELISA) according to the manufacturer's instructions. DNA extraction and competitive PCR were performed using specific primers developed within our laboratory and the amount of fungal DNA was expressed as a percentage of the total DNA content of the sample.

SSR haplotyping

Comparison of SSR allele sizes from known, genetically characterised FHB resistant and susceptible cultivars from Asia, Europe and USA were used to infer the origin of FHB resistance QTL in the trial varieties and to identify potentially novel loci.

Statistical analyses

All the statistical analysis was performed using GenStat for Windows 9th edition (copyright Lawes Agricultural Trust, Rothamsted Experimental Station, UK).

Results

Fusarium head blight status of UK winter wheat and spring barley varieties

The Fusarium head blight (FHB) reaction of 53 varieties from the (2003) National List of winter wheat varieties was compared with 19 reference cultivars from Continental Europe that had previously been characterised for resistance. Of the National List varieties tested, only Soissons, Spark and Vector had stable resistance over trial sites. In addition, under moderate disease pressure, a total of 24 National List varieties had levels of the trichothecene mycotoxin deoxynivalenol (DON) above the EU limit of 1.25 parts per million (ppm) in grain. Significant and consistent differences in resistance to FHB were found among the barley varieties and disease levels were found to correlate with DON content.

Comparison of SSR allele size was used to infer the origin of FHB resistance and to identify germplasm with potentially novel loci. A total of 17 SSR loci were selected from published studies of resistance on chromosomes 3BS, 5A and 6B associated with resistance in the Chinese cultivar Sumai-3, chromosomes 1B, and 5A associated with resistance in the Romanian cultivar Fundulea F201R (F201R) and chromosome 5AL associated with resistance in the French cultivar Renan. No variety appeared to possess FHB QTL similar to those of Sumai-3 (3BS, 5A and 6B), F201R (1B) and Renan (5A). However, the highly resistant German reference cultivar Petrus had an identical haplotype to F201R on 1B indicating that this cultivar has an allelic QTL at that location.

FHB resistance quantitative trait loci (QTL) detected in Spark x Rialto

Spark is more resistant to FHB than most other UK winter wheat varieties but the genetic basis for this is not known. Spark carries no known mutation at either *Rht-B1* or *Rht-D1* loci whereas Rialto carries the *Rht2* allele at the *Rht-D1* locus. A mapping population from a cross between Spark and the FHB susceptible variety Rialto was used to identify QTL associated with resistance. QTL analysis across environments revealed nine QTL for FHB resistance and four QTL for plant height. Spark contributed seven QTL (2A, 3A, 4D (2 QTL), 5A, 6A, 7A) while two QTL were derived from Rialto (1B, 3A). Two QTL for PH were contributed by Spark (4D, 6A) and two by Rialto (2A, 3B). One FHB QTL was coincident with the *Rht-1D* (*Rht-2*) locus and accounted for up to 51% of the phenotypic variance. None of the other height QTL was associated with FHB resistance.

FHB resistance QTL detected in Soissons x Orvantis

Soissons is one of the most resistant varieties grown in the UK. Soissons carries *Rht-D1a* (*Rht1*) while Orvantis carries (*Rht2*). QTL analysis of FHB revealed only a single major FHB QTL on chromosome 4D effective in all three field trials. Soissons (*Rht-D1a*) contributed the FHB resistance allele. Although this QTL is in the region of the *Rht-D1* locus, the peak of the QTL was closer to an adjacent marker. The major PH-QTL associated with the *Rht-B1* (*Rht1*) locus also co-localised with a putative minor FHB QTL on 4BS but surprisingly, and in contrast to the effect around the *Rht-D1* locus, FHB resistance was associated with the *Rht-B1b* (*Rht1*) allele (contributed by Soissons) rather than the *Rht-B1a* (tall) allele (Orvantis) as might have been expected if the effect were due to differences in plant height. Putative QTL for FHB resistance, often appearing in more than one trial, were also detected (1BL, 3BL, 4BS, and 7AL). Soissons contributed all the alleles for FHB resistance except that on 1B.

Association between FHB susceptibility and plant height determined by *Rht* alleles

In both the Spark x Rialto and Soissons x Orvantis populations a major FHB QTL was found on chromosome 4D at, or close to, the *Rht-D1* locus with the *Rht2* allele associated with FHB susceptibility. In the Soissons x Orvantis population a minor FHB QTL was detected on 4BS at the *Rht-B1* locus but surprisingly, and in contrast to the effect around the *Rht-D1* locus, FHB resistance was associated with the *Rht-B1b* allele (*Rht1*), contributed by Soissons, rather than the *Rht-B1a* allele (Orvantis) as might have been expected if the effect were due to differences in plant height. These results indicated that the *Rht-1* and *Rht2* differ in their effects on FHB resistance. The influence of the *Rht-B1b* and *Rht-D1b* alleles on FHB resistance was further investigated using both Mercia and Maris Huntsman near isogenic lines. Under high disease pressure both *Rht-B1b* and *Rht-D1b* significantly decreased Type I resistance (resistance to initial infection). However, *Rht-D1b* had no effect on Type II resistance (resistance to spread of the fungus within the spike), while *Rht-B1b* significantly increased Type II resistance.

Mapping of QTL associated with Fusarium head blight in RL4137

RL4137 is a FHB resistant line derived from the Brazilian variety Frontana. The study used 90 recombinant inbred lines (RIL) derived from a cross between RL4137 and the moderately FHB resistant variety Timgalen. QTL analyses identified a total of six FHB resistance QTL (1B, 2B, 3A, 6A, 6B and 7A). In all but one instance, the alleles from

RL4137 had a positive effect on FHB resistance. The FHB QTL on 1B, 2B and 6B were detected in multiple trials, with alleles from RL4137 contributing a positive QTL for resistance on 1B and 2B and the alleles from Timgalen contributing a positive QTL for resistance on 6B. Our study also identified three QTL for plant height (PH) (2B, 4A and 5B), two QTL for weight of infected spikelet (WIS) from infected ears (2B and 6A) and one QTL for awns (2B). The QTL mapped on 2B for PH, WIS and awns co-localized with that for FHB resistance.

Assessing non-specificity of FHB resistance, development of methodologies to detect type I resistance and identification of novel sources of FHB resistance.

Fusarium head blight (FHB) of wheat is caused predominantly by *Fusarium graminearum* and *F. culmorum* although other *Fusarium* species and *Microdochium majus* and *M. nivale* are also important in some regions. The few reports to date suggest that FHB resistance is effective against all pathogen species. However, because biosynthesis of DON has been shown to be critical for spread of *F. graminearum* within the spike we reasoned that isolates that do not produce DON might be unable to spread within the spike. Appropriate non DON-producing isolates might be used to reveal Type I resistance (resistance to initial infection) without the confounding effects of differences in Type II resistance (resistance to spread within the spike). In initial experiments, thirty European winter wheat varieties were spray and point inoculated with a DON-producing isolate of *F. culmorum* or an isolate of *M. majus* in glasshouse tests. Resistance to the two pathogens following spray inoculation was well correlated whereas, following point inoculation, no correlation was observed because *M. majus* was unable to spread beyond the inoculated spikelet. However, spray inoculation with *M. majus* produced only low levels of disease making it unsuitable for use in routine screening. In a second set of experiments we found that a NIV-producing isolate of *F. graminearum* caused high levels of disease following spray inoculation but spread only very slowly within the spike. Comparative spray and point trials using DON and NIV-producing isolates of *F. graminearum* were carried out to characterise a set of wheat lines for their Type I and II FHB resistance components. From 300 lines, three possessed a high level of Type I resistance and several possessed high levels of both Type I and Type II resistance.

Discussion

FHB resistance of UK National List entries was assessed and compared to resistant European cultivars in three contrasting environments. Although the resistance of varieties correlated well across sites, some varieties differed markedly in resistance between sites. This effect is due primarily to the differences in environment at the three sites but we also found evidence that suggests that the period of optimal susceptibility to FHB can differ significantly between cultivars. Assessment of visual disease, DON and FDNA data from three inoculation timings indicated that some varieties had a very narrow (three days or less) period of optimal susceptibility whereas others remained highly susceptible over a six day period. These findings make it imperative that lines are inoculated at the same developmental stage when carrying out trials at different locations or in different years.

Among the National List varieties (2003), only Soissons, Spark and Vector showed evidence for moderate FHB resistance. Even under the moderate disease pressure 24 National List varieties had DON levels which were above the proposed EU action limits of 1.25 parts per million (ppm). These results indicate that a significant effort will be required by the UK plant breeding community to improve overall levels of FHB resistance. For barley, significant genetic variation exists for resistance to FHB among UK varieties. In general, decreased symptoms correlated with reduced DON content of grain for both wheat and barley.

None of the FHB resistant varieties from the UK or mainland Europe had SSR haplotypes indicating that their resistance is derived from Sumai-3. Thus the introduction of potent FHB QTL from this source should complement those of the FHB resistant European varieties to increase overall levels of resistance. While several varieties carried the 1RS-1BL rye translocation that confers type II resistance, only the resistant German variety Petrus carries the entire region associated with this QTL. Selection of lines that carry all the markers relating to this QTL should ensure a minimal level of FHB resistance among varieties carrying the 1RS-1BL translocation. The genetic basis of FHB resistance of Soissons and Spark, the two most resistant UK varieties, was assessed along with that of a Frontana-derived line. QTL analysis of the Spark x Rialto population revealed that the main effect (up to 51% of phenotypic variance) was coincident with the dwarfing locus *Rht-1D* (*Rht2*). No other height QTL was associated with FHB resistance in this cross. Surprisingly, the main effect in the Soissons x Orvantis population also occurred close to the same locus. However, in this population, the QTL peak lay over an adjacent marker suggesting that the susceptibility is due to a tightly linked gene rather than pleiotropy associated with

Rht2. In contrast, *Rht1* (carried by Soissons) conferred no negative effect on FHB resistance, even conferring a very minor positive effect in one trial. Additional experiments with near-isogenic lines supported these findings. Under high disease pressure both *Rht-B1b* and *Rht-D1b* significantly decreased resistance to initial infection. However, while *Rht-D1b* had no effect on resistance to spread within the spike, *Rht-B1b* significantly increased resistance to spread. Combined with the evidence from the population studies above our study suggests that the enhanced susceptibility of *Rht-D1b* allele is due to linkage to deleterious genes rather than to pleiotropy and that the positive effect of *Rht-B1b* allele on FHB resistance is due either to pleiotropy conferring Type II resistance or very tight linkage to resistance genes.

The majority of UK winter wheat varieties are highly susceptible to FHB and almost all these carry the semi-dwarfing *Rht-D1b* (*Rht2*) allele. Neither Soissons nor Spark carry *Rht-D1b*: Soissons possesses *Rht-B1b* (*Rht1*) and Spark has a tall (*rht*) genotype with its reduced height being due to non-*Rht* genes. It appears that the difference in FHB resistance between these two varieties and the others on the UK National List of 2003 may, in large part, be simply a reflection of the presence or absence of *Rht-D1b*. Under conditions of moderate disease pressure, use of the *Rht-B1b* semi-dwarfing allele may provide the desired crop height without compromising resistance to FHB to the same extent as lines carrying *Rht-D1b*.

In the Frontana-derived population we identified a major stable QTL on chromosome 2B and one on 3A that was only effective in field conditions with low disease pressure. Similar QTL have been observed in Frontana, indicating that they retain their efficacy in different genetic backgrounds.

Disease among 30 varieties was correlated following spray inoculation with *F. culmorum* and *M. majus* although *M. majus* was much less aggressive. In contrast, following point inoculation, *M. majus* was unable to spread beyond the infected spikelet whereas the DON-producing *F. culmorum* isolate spread into the rachis and throughout the head. Symptoms produced by *M. majus*, a non toxin-producing species are almost identical to those produced by *Tri5*$^{-}$transformants, being restricted to single spikelets and unable to spread throughout the spike.

Experiments comparing NIV and DON producing isolates of *F. graminearum* showed that, while the two isolates caused similar levels of disease initially, the DON producer spread much more rapidly in the spike than the NIV producer. We concluded that use of appropriate virulent NIV chemotype isolates of *F. graminearum* might be used in spray inoculation trials to determine relative levels of Type I resistance in wheat. To complement this, point inoculation with a virulent DON-producing isolate

can be used to evaluate levels of type II resistance. We used such isolates to identify FHB resistance within a large collection of wheat lines from the International Wheat and Maize Centre (CIMMYT), Mexico. Several lines exhibiting high levels of FHB resistance in field trials were found to also possess high levels of Type II resistance following point inoculation. More significantly, a few lines exhibited high levels of FHB resistance in field trials, but were highly susceptible to point inoculation indicating that their resistance is predominantly of Type I. Only a few sources of this type of resistance have been identified to date due, in large part, to the greater technical challenges associated with the unequivocal identification of Type I resistance. We propose that the use of appropriate non DON-producing FHB species or isolates in spray inoculation trials combined with point inoculation using DON-producing isolates will greatly aid the identification and characterisation of wheat for Type I and Type II resistance to FHB.

FULL PROJECT REPORT

1 Introduction

Fusarium ear blight (FEB), more commonly termed Fusarium head blight (FHB), is a damaging disease of wheat and barley in many cereal-growing areas of the world. The disease has become devastating in the mid-west of USA, resulting in losses of well over $1 billion over the last decade. The disease can be caused by a number of fungi, chiefly *Fusarium* species. Whereas *F. graminearum* is predominant worldwide, it was, until recently, not often found in the UK where the main pathogens were *F. culmorum* and *Microdochium nivale*. However, this species has now become established across the UK and is now frequently isolated from wheat ears (Turner et al, 1999). As well as causing loss of yield and quality this disease is of major concern because of the production of mycotoxins by many of the *Fusarium* species responsible (*M. nivale* does not produce mycotoxins). The chief mycotoxins of concern are trichothecenes and the most prevalent in cereals is deoxynivalenol (DON) although a derivative nivalenol (NIV) is also of relevance to the UK and Europe.

To date, the disease has occurred only sporadically in the UK. However, observed changes in the species detected in the UK indicate an increasing risk. In addition, altered climatic conditions in the future may lead to an increase in the incidence of this disease, irrespective of changes in the pathogen species responsible. In an effort to ensure continued and improved health of consumers the EU recently introduced legislation setting limits to the amount DON mycotoxin permitted in grain and wheat products. It is imperative that the UK is positioned to comply with any such legislation and maintain consumer confidence through ensuring a disease and toxin free crop. It is widely accepted that host resistance is an essential component of any attempt to reduce the level of disease and associated mycotoxins.

The problem addressed in the REFAM project was that progress in breeding varieties resistant to *Fusarium* species and at low risk of mycotoxin accumulation is currently constrained because very little is known about the genetics of resistance.

Efforts to study resistance are hindered because of the quantitative nature of the resistance with several genes being required to provide significant protection. Disease symptoms do not always appear to be related to toxin accumulation. Although this may partly reflect the inadequacy of the assessment techniques (Nicholson et al, unpublished) it is clearly important to assess resistance to both disease and mycotoxin accumulation .In the field, the disease consists of a complex of different species (some producing toxins while others do not) and it remains unclear whether

resistance to toxin producing species also always confers resistance to species such as *M. nivale* that do not produce toxins. It is highly likely that the effect will depend upon the mechanism(s) behind a particular resistance and, hence, depends upon the wheat variety involved.

Wheat heads differ in susceptibility as they mature, being most susceptible at mid-anthesis. In artificially inoculated tests, failure to apply the fungus at the correct time will lead to escape and prevent assessment of true resistance. In the field, other characters, such as plant height and ear morphology may influence humidity and hence infection. This will also interfere with attempts to distinguish true resistance from disease escape in a particular environment. Relatively few researchers have addressed these problems because of the requirement for significant investment in personnel and infrastructure.

The genetic basis for resistance has been investigated for only a few varieties. The best studied of these is the Chinese variety "Sumai 3" in which it is thought that a major part of the resistance is due to a factor on the short arm of chromosome 3B. Resistance from this variety has yet to be incorporated into backgrounds suitable for growth in the UK. There is little published information on the genetic basis of other potential FHB resistance sources although moderate levels of resistance have been identified in UK wheat varieties (Nicholson et al., unpublished) The genetic basis for the resistance of these varieties is not known.

It is important that further resistance sources are identified, and the genetic basis of resistance understood, in order to complement that of Sumai 3 or its derivatives. Reliance upon one resistance factor of a single variety would pose a risk to the long term security of the wheat crop in the UK and elsewhere.
For barley, even less is known than for wheat about the status of FEB resistance, as varieties are not routinely screened for resistance during breeding or official trials. There is an urgent need to examine current UK and European material for resistance to *Fusarium* spp. / *M. nivale* and mycotoxin production, in order to establish what resistance is available for immediate exploitation, before proceeding to search more widely for sources of improved resistance.

The purpose of the REFAM project was to identify and characterise the best available sources of FHB resistance in wheat and barley and develop rigorous and detailed knowledge of the genetics of FHB resistance in wheat. The approach will facilitate long-term progress in the scientific study of resistance to the disease and toxin accumulation and enable the development of efficient marker assisted selection (MAS) procedures within breeding programmes of the companies that are partners in

this application.

Advances in molecular marker technology, combined with statistical methods to analyse quantitative traits, such as FHB resistance, provide an opportunity to significantly improve the understanding of the genetic basis of FHB resistance. In recent work funded by the EU and DEFRA, the JIC has demonstrated that a rigorous approach to disease assessment and analysis of the traits that form components of the overall resistance can be used to investigate the genetic basis of FEB resistance. This approach allows the dissection of resistance and identification of molecular markers linked tightly to the resistance components.

Plant breeders require more information about the genetics of resistance to FHB, particularly in respect to the relationship between disease and mycotoxin accumulation as well as the relative efficacy of resistance towards toxin producing and non-toxin producing FHB species. There is a scarcity of sources of FHB resistance and this project will contribute towards the needs of breeders through the identification and characterisation of new sources of resistance. This information will help them to accumulate genes from different sources (and with differing chromosomal locations), in order to build up effective, durable resistance in their breeding programmes.

The project consisted of three work packages (WP) with associated objectives.

1a Evaluation of UK RL and selected European winter wheat and barley lines with known FHB resistance.

1b Comparison of resistance efficacy against toxin producing and non-toxin producing FHB species by spray and point inoculation.

1c Identification and characterisation of new sources of FHB resistance by screening wheat collections held at the JIC.

2 Analysis of resistance in populations from resistant/susceptible crosses. Three populations were studied Spark x Rialto, Soissons x Orvantis and RL4137 x Timgalen.

3a Microsatellite (SSR), amplified fragment length polymorphism (AFLP) and Diversity Array (DArT) markers were used to develop genetic maps of the selected crosses. Quantitative trait locus (QTL) analysis was undertaken to determine the precise location and relative magnitude of FHB resistance and the identification of molecular markers tightly linked to FHB resistance.

3b Identification of molecular markers tightly linked to FHB/toxin resistance, suitable for inclusion in marker assisted selection procedures within breeding programmes.

2 Materials and methods

2.1 Plant materials

Recombinant inbred lines (RILs) of a Cross between RL4137 (TRL1) and Timgalen (TRL2) and two doubled haploid (DH) populations: Spark/Rialto, Soissons/Orvantis, was used in the study. In additions to the mapping populations, the near isogenic lines (NILs) of *Rht1* and *Rht2* semi-dwarfing alleles in Mercia and Maris Huntsman background (Flintham et al, 1997), 79 winter wheat varieties and 300 lines from CIMMYT FHB resistance breeding programme were used in the study.

2.2 Genotyping

The genetic maps for the mapping populations were constructed using various markers including simple sequence repeats (SSR), amplified fragment length polymorphism (AFLP) (Vos et al, 1995) and Diversity Array Technology (DArT^{TM}) markers (Jaccoud et al, 2001). The DArT markers were added by The Triticarte Pvt. Ltd., Australia. SSRs were visualised on Polyacrylamide silver staining gels or ran on ABI sequencing machine and scored. The *Rht1* and *Rht2* perfect markers were mapped as described in Ellis et al (2002). SSR and DArT markers permitted the assignment of linkage groups (LGs) to chromosomes according to the previously published wheat genetic maps (http://www.triticarte.com.au/pdf/TriticartewhtmapalignV1-2.xls; Semagn et al, 2007; Somers et al, 2004).

2.3 Phenotyping for FHB resistance

The FHB phenotyping was carried out both in field and polytunnel experiments using either using randomized complete blocks design or randomised incomplete block design after classification of the genotypes according to flowering date. The field plot size was double rows of 1 m length with 17 cm row spacing. The field trials were conducted either at the John Innes Centre (JIC), Norwich, Nickerson Seeds Ltd, Suffolk, Advanta Seeds (UK) Ltd., Norfolk, National Institute for Agricultural Botany (NIAB), Cambridge, Central Science Laboratory (CSL), York or Elsoms Seeds Ltd., Lincolnshire. All the polytunnel experiments were conducted at JIC. Polytunnel experiments used 12 plants of each line grown separately in 1 litre pots of John Innes

potting mix, with one seed per pot and grown in polytunnel. The pots containing individual plants were arranged in a randomised complete block design, prior to inoculation. Plants were spray inoculated at mid-anthesis (growth stage 65 (Zadoks et al, 1974) with a conidial suspension (1 x 10^5 spores ml^{-1}) of highly virulent DON producing isolate *Fusarium culmorum* (Fu42 or UK1) or a NIV producing isolate of *F. grameniarum* (F86). The inoculum preparation, plant husbandry, trial set-up and disease assessment were carried out as described in Gosman et al (2005). All the inocula were amended with 0.05 % Tween 20 and the inoculations were carried out in the evening.

The variety Mercia was assessed for resistance to a DON-producing isolate UK1 and a NIV-producing isolate F86 of *F. graminearum* following spray and point inoculation. Two experiments were performed in an unheated polytunnel with different inoculum loads. In the high titre trial, conidial suspensions were 1 x 10^5 ml^{-1} and 1 x 10^6 ml^{-1} for spray and point inoculations, respectively. For the low titre trial, conidial suspensions were 1 x 10^4 ml^{-1} and 1 x 10^5 ml^{-1} for spray and point inoculations, respectively.

Disease was assessed as the percentage (0 to 100 %) of visually infected spikelets. In the polytunnel trials, disease was assessed several times at intervals of 7 days from the date of post inoculation and area under the disease progress curve (AUDPC) was calculated (Buerstmayr et al, 2000) and used in analysis. In addition to disease, the morphological traits such as awns, plant height (PH) and weight of infected spikelets (WIS) were recorded.

2.4 DON analysis, DNA extraction and quantitative PCR

DON was extracted from milled grain samples using 10% methanol (10 ml g^{-1}) and stored at -20 ^{0}C prior to analysis. An appropriate dilution in distilled water was made from each sample and 50 µl aliquots assessed for DON content using the Ridascreen® Fast DON™ (R-Biopharm Rhône Ltd.) enzyme linked immuno-assay (ELISA) according to the manufacturer's instructions. Absorbance was measured at 450 nm using a Wallec 400 plate reader and the sample data converted to DON concentration by reference to a standard curve generated from DON standards provided in the kit. DNA extraction and competitive PCR were performed as described previously (Gosman et al, 2004). Quantitative PCR analysis using *F. culmorum* specific primers was carried out according to the method of Nicholson et al (1998) and the amount of fungal DNA was expressed as a percentage of the total DNA content of the sample as described by Gosman et al (2004).

2.5 SSR haplotyping

Comparison of microsatellite allele size with FHB resistant and susceptible cultivars from Asia, Europe and USA described in published QTL mapping studies were used to infer the origin of FHB resistance QTL in the trial varieties and to identify potentially novel loci. The microsatellite loci analysed were reported in those studies to flank FHB resistance QTL on chromosomes 1B, 3B, 5A and 6B. SSR loci Xgwm389, Xgwm533 and Xgwm493 (3BS) and Xgwm293, Xgwm156 and Xgwm304 (5A) were used to identify QTL *Qfhs.ndsu-3BS* and *Qfhs.ifa-5A* derived from Sumai-3 (Anderson et al, 2001; Buerstmayr et al, 2003) (Table 2.1). The presence of a QTL in a similar location to *Qfhs.ifa-5A* identified in the Romainian cultivar Fundulea F201R (Shen et al, 2003) was monitored using Xgwm304. Two QTL on 5AL of Renan (Gervais et al, 2003) were identified using Xgwm639, Wmc415 and Barc151 for *QFhs.inra-5a2* and Wmc524 and Xgwm410 for *QFhs.inra-5a3*. The presence of the ω-secalin gene and microsatellite markers Xbarc008 and Xgwm018 were used to identify the QTL on 1B derived from Fundulea F201R (Shen et al, 2003). As an additional reference, the Russian cultivar Aurora was included in the analysis as it is likely that F201R inherited the 1RS-1BL translocation from Aura, which has Aurora (carrier of the 1RS-1BL translocation) in its parentage (Shen et al. 2003). On 6BS an interval was identified which encompassed QTL in Sumai-3 (Wmc105) and Ning 894037 (Xgwm088) reported by McCartney et al. (2004) and Shen et al (2003b), respectively. Additional loci (Xgwm133 and Barc198) identified from the consensus wheat map of Somers et al (2004) were used as flanking markers for this QTL. Polymorphic information content, or PIC values were calculated using the formula of Botstein et al (1980).

The sources of primer sequences for microsatellites are identified by the marker prefix, e.g. (Xgwm) published in Röder et al (1998), (Barc) published in Song et al (2002) and (Wmc) published by Somers & Isaac (2004). PCR amplification for SSR analysis was carried out in 6.25µl volumes using Qiagen HotStarTaq Mastermix (Qiagen), primers at 0.2µM; one of which was fluorescently labelled with FAM, VIC, NED, PET or HEX (Applied Biosystems) using approximately 20ng of genomic DNA. PCR was performed using a MJ Research Tetrad Thermocycler (MJ Research). The reaction mixtures were denatured at 95 ^{0}C for 15 minutes, followed by 34 cycles consisting of 94 ^{0}C for 1 minute, primer dependent annealing temperature for 1 minute (ramp speed 0.5 ^{0}C sec^{-1}), and extension at 72 ^{0}C for 1 minute. Amplification was completed with a 10 minute extension at 72 ^{0}C and held at 10 ^{0}C.

Table 2.1 Summary of chromosomal locations of FHB resistance QTL on chromosomes 1B, 3BS, 5A and 6B, flanking markers and references to published studies

Chromosome	Cultivar	Markers(s)	Reference
1B	Fundulea F201R	Barc008, Xgwm018	Shen et al.(2003a)
1B	CM82036	*Glu-B1*	Buerstmayr et al (2002)
3BS	Sumai-3	Xgwm389, Xgwm533, Xgwm493	Anderson et al (2001)
3BS	Ning 7840	Xgwm389, Xgwm533	Guo et al. (2003)
3BS	CM82036	Xgwm533, Barc133, Xgwm493	Buerstmayr et al (2003)
3BS	Ning 894037	Xgwm389, Xgwm533, Xgwm493	Shen et al (2003b)
3BS	Huapei 57-2	Xgwm389,Barc133, Xgwm493	Bourdoncle & Ohm (2003)
5A	CM82036	Xgwm293, Xgwm304, Xgwm156	Buerstmayr et al (2003)
5A	Fundulea F201R	Xgwm304	Shen et al (2003a)
5A	Sumai-3	*B1* awning suppressor	Ban & Suenaga (2000)
5A-1	Renan	Xpsr0170a	Gervais et al (2003)
5A-2	Renan	Xgwm639b	Gervais et al (2003)
5A-3	Renan	*B1* awning suppressor	Gervais et al (2003)
6B	Ning 894037	Xgwm088, Xgwm644	Shen et al (2003b)

Hierarchical cluster analysis was performed using the Euclidian test with single link as the clustering method. The similarity matrix was calculated using allele size data generated from SSR loci associated with the six FHB resistance QTL described previously.

2.6 Statistical analysis

All the statistical analysis was performed using GenStat for Windows 9th edition (copy right Lowes Agricultural Trust, Rothamsted Experimental Station, UK). Analysis of variance (ANOVA) was carried out using the generalised linear model (GLM) of regression analysis. Broad sense heritability (h^2) was estimated from the ANOVA using the formula: $h^2 = \sigma_G^2/[\sigma_G^2 + (\sigma_e^2/r)]$, with σ_G^2, the genetic variance; r, the number of replicates per genotype (Nyquist, 1991).

In the current project, linkage maps were constructed for RL4137 (TRL1) X Timgalen (TRL2) and Soissons X Orvantis mapping populations using JoinMap (version 3.0) (Van Ooijen & Voorrips, 2001) and the map distances were calculated using Kosambi mapping function (Kosambi 1944). The QTL detection was carried out using MapQTL® 4.0 (Van Ooijen, 2004). First, for each trait, Krusskall-Wallis test was performed to detect the association between markers and traits individually. In a second step, interval mapping (IM) was performed to identify the major QTL. Automatic cofactor selection was used to fit the multiple QTL model (MQM) (backward elimination (P>0.02)) and detect significantly associated markers as cofactors. For each trait, a 1000X permutation test was performed to identify the LOD threshold corresponding to a genome-wide false discovery rate of 5 % (P<0.05). The QTL

detected above the LOD threshold that explained more than 10 % of the variance (R^2) in at least one environment/experiment were classified as major QTL and those explaining less than 10 % as minor QTL. Linkage maps were drawn using MapChart (Vorrips, 2002).

3 Evaluation of UK released varieties and selected European winter wheat lines with known FHB resistance.

Fusarium head blight (FHB) of wheat is caused by several fungal species that cause similar symptoms. *Fusarium graminearum* is the major pathogen worldwide, while *F. culmorum* tends to predominate in maritime regions (McMullen et al, 1997; Windels, 2000). *F. avenaceum* and *F. poae* are also frequently associated with FHB, particularly in Northern Europe. In addition to the true *Fusarium* species, two *Microdochium* species *M. majus* and *M. nivale* also cause FHB and are particularly prevalent where cooler, wetter conditions prevail such as the UK (Lemanczyk & Sadowski, 2002).

FHB is of particular concern because many of the *Fusarium* species produce mycotoxins that contaminate infected grain and pose a risk to human and animal consumers (Pestka & Smolinski, 2005). The most common mycotoxin in blighted grain is the trichothecene deoxynivalenol (DON), produced by *F. graminearum* and *F. culmorum.* A second, closely related trichothecene, produced by certain isolates of these species is nivalenol (NIV).

The use FHB-resistant cultivars is the most cost-effective and environmentally pragmatic approach to manage this devastating disease. FHB resistance is quantitatively inherited and is heavily influenced environment during anthsis. To date no variety has been found to be immune to FHB (Snijiders, 1990). Advances in phenotyping, combined with statistical methods to detect quantitative trait loci (QTL) have led to the identification of numerous QTL for FHB resistance in spring wheat varieties which could be introgressed into elite agronomically superior cultivars (Van Sanford et al, 2001). Several sources of FHB resistance genes have been identified among Chinese, and Chinese derived spring wheat notably Sumai 3 has already been widely deployed in breeding programs worldwide.

Despite the existence of a significant amount of FHB resistant European winter wheats, resistance in only a few varieties has been genetically characterised to date (Paillard et al, 2004; Scmolke et al, 2005; Shen et al, 2003b). In contrast to the resistance in some spring wheat varieties, FHB resistance in winter wheat germplasm appears to be due to numerous QTL of moderate to small effect. Microsatellite markers can be used to infer the origin and genetic relationship between FHB resistance QTL which could be used to identify appropriate parental combinations in order to 'pyramid' previously characterised resistance genes and to identify potentially novel sources of resistance (McCartney et al, 2004). Diversification of resistance sources in breeding programs should reduce the risk of the emergence of virulent pathogen strains.

Due to the previously low incidence of FHB in the UK, a comprehensive assessment of resistance in elite UK winter wheat germplasm has not yet been undertaken. One aim of the current study was to assess the FHB reaction of winter wheat varieties currently on the UK National List (NIAB, Cambridge) and to identify the presence of characterised FHB QTL and infer potentially novel sources of resistance through allele size comparison of microsatellite loci associated with QTL in Chinese and Chinese derived germplasm.

3.1 Results

3.1.1 Fusarium head blight resistance

In (2003) 72 winter wheat varieties from the UK National List (NIAB), plus additional entries from France and Germany were phenotyped for resistance to FHB (Table 3.1). Trials were carried out at two field sites in the UK; one in the South East of England at NIAB in Cambridge, and the other in the North of England at CSL in York. A third trial was carried out in a high temperature and humidity facility (polytunnel) at the JIC in Norwich. Relative to the most susceptible variety at each site, average disease levels were highest at JIC (56.35%) followed by NIAB (28.65%) and CSL (15.34%). Coefficients of correlation between sites were low for the relationship between JIC and NIAB (0.36, $P = 0.002$) to high for the relationship between JIC and CSL (0.40, $P = 0.001$) and between NIAB and CSL (0.52, $P < 0.001$). Analysis of variance (ANOVA) indicated that genotypic variance was highly significant ($P < 0.001$) and within year heritability calculated from variance component estimates over all sites was medium sized ($H^2 = 0.57$). Comparison between the average score of equal sized groups of varieties from the UK and control entries from Continental Europe over all three sites indicated that UK varieties were significantly ($t = -3.05$, $P = 0.005$) more susceptible.

Across sites, relative to the cultivar Wizard (which in previous tests had been shown to be FHB susceptible), the UK variety Macro was the most susceptible (highest post inoculation score of 35% infection) and the most resistant was the French cultivar Nirvana (highest post inoculation score of 2.4 % infection). The most resistant varieties, all with significantly ($P \leq 0.05$) less visual disease than Wizard, were (most resistant first) Nirvana, Renan, Tambor, Petrus, Soissons, Vector, Atlantis, Centrum, Ornicar, Spark and Piko (Figure 3.1). All these varieties, with the exception of Vector and Spark, were from Continental Europe. The most susceptible varieties were (most susceptible first) Macro, Darwin, Tanker, Scorpion 25, Goodwood, Xi19, Warlock,

Riband, Einstein and Bentley (Figure 3.1). With the exception of Bentley, all these varieties were from the UK.

Table 3.1 Origin and pedigree information of 79 winter wheat trial entries comprised of 7 FHB resistant reference varieties used in previous QTL studies (REF), 41 varieties undergoing recommended list trials, or were on the UK recommended list in (2003) (RL)[1], 12 UK national list varieties (NL) and 19 control varieties which have previously been tested for FHB resistance by collaborative partners (CON)

Variety / line code	Breeder / seed source	Status	Country of origin
Sumai-3	Agriculture and Agri-food Canada	REF	China
Wuhan 2-37E	John Innes Centre	REF	China
CM82036	CIMMYT	REF	Mexico
Aurora	CIMMYT	REF	Russia
Fundulea F201R	Institute for Cereals & Industrial Crops - Fundulea	REF	Romania
WEK0609	Pioneer HiBred Seeds	REF	USA
Renan	INRA	REF	France
01ST2031	Monsanto	CON	France
A42-02	Advanta	NL	UK
A48-02	Advanta	NL	UK
Access	CPB Twyford	RL	UK
Apache	Monsanto	CON	France
Arran	Nickerson	RL	UK
Atlantis	Nickerson	CON	Germany
Bandit	Nickerson	NL	UK
Batis	Monsanto	CON	Germany
Bentley	Desprez	RL	France
Biscay	CPB Twyford	RL	UK
Carlton	Elsoms	NL	UK
Centrum	Monsanto	CON	France
Charger	Monsanto	NL	UK
Claire	Nickerson	RL	UK
Consort	Monsanto	RL	UK
Contra	Monsanto	CON	Germany
Cordiale (CPBT W83)	CPB Twyford	RL	UK
CPBT W87	CPB Twyford	RL	UK
CWW 00/22	Monsanto	RL	UK
Dart (CWW 00/47)	Monsanto	RL	UK
Darwin	Nickerson	NL	UK
Deben	Nickerson	RL	UK
Dekan	Nickerson	CON	Germany
Dick	Cebeco	RL	UK
Einstein	Nickerson	RL	UK
ELS 00/21	Elsoms	RL	UK
Excellence	Monsanto	NL	UK
German A	Monsanto	CON	Germany
Gladiator (CWW 00/33)	Monsanto	RL	UK
Goodwood	Cebeco	RL	UK
Grief	Monsanto	CON	Germany
Hereward	Monsanto	RL	UK
Istabraq (NSL WW47)	Nickerson	RL	UK
Macro	Monsanto	NL	UK
Malacca	CPB Twyford	RL	UK

[1] Information provided by the (2003) NIAB Recommended List and UK National list

Variety / line code	Breeder / seed source	Status	Country of origin
Napier	Monsanto	RL	UK
Nirvana	Monsanto	CON	France
Nijinsky (NSL WW46)	Nickerson	RL	UK
Option	Monsanto	RL	UK
Ornicar	Monsanto	CON	France
Orvantis	Monsanto	CON	France
Pennant	Elsoms	NL	UK
Petrus	Monsanto	CON	Germany
Piko	Monsanto	CON	Germany
Rialto	Monsanto	NL	UK
Riband	Monsanto	RL	UK
Richmond	Cebeco	RL	UK
Robigus	CPB Twyford	RL	UK
Romanus	Nickerson	CON	Germany
Savannah	Advanta	RL	UK
Scorpion 25	Advanta	RL	UK
Senator (CPBT W90)	CPB Twyford	RL	UK
Skater	Nickerson	RL	Germany
Smuggler (A30-00)	Advanta	RL	UK
Soissons	Desprez	RL	France
Solstice	Advanta	NL	UK
Spark	Nickerson	NL	UK
Steadfast (CWW 00/40)	Monsanto	RL	UK
SW Tataros	Hadmersleben	RL	Germany
Tambor	Monsanto	CON	Germany
Tanker	Elsoms	RL	UK
Tellus	CPB Twyford	RL	UK
Travix	Nickerson	CON	UK
Vector	Advanta	RL	UK
Vergas	Nickerson	CON	Germany
Warlock 24	Advanta	RL	UK
Welford	Elsoms	RL	UK
Winnetou	Nickerson	CON	Germany
Wizard	CPB Twyford	RL	UK
Xi19	Advanta	RL	UK
Zebedee (A45-02)	Advanta	NL	UK

[1] Information provided by the (2003) NIAB Recommended List and UK National list

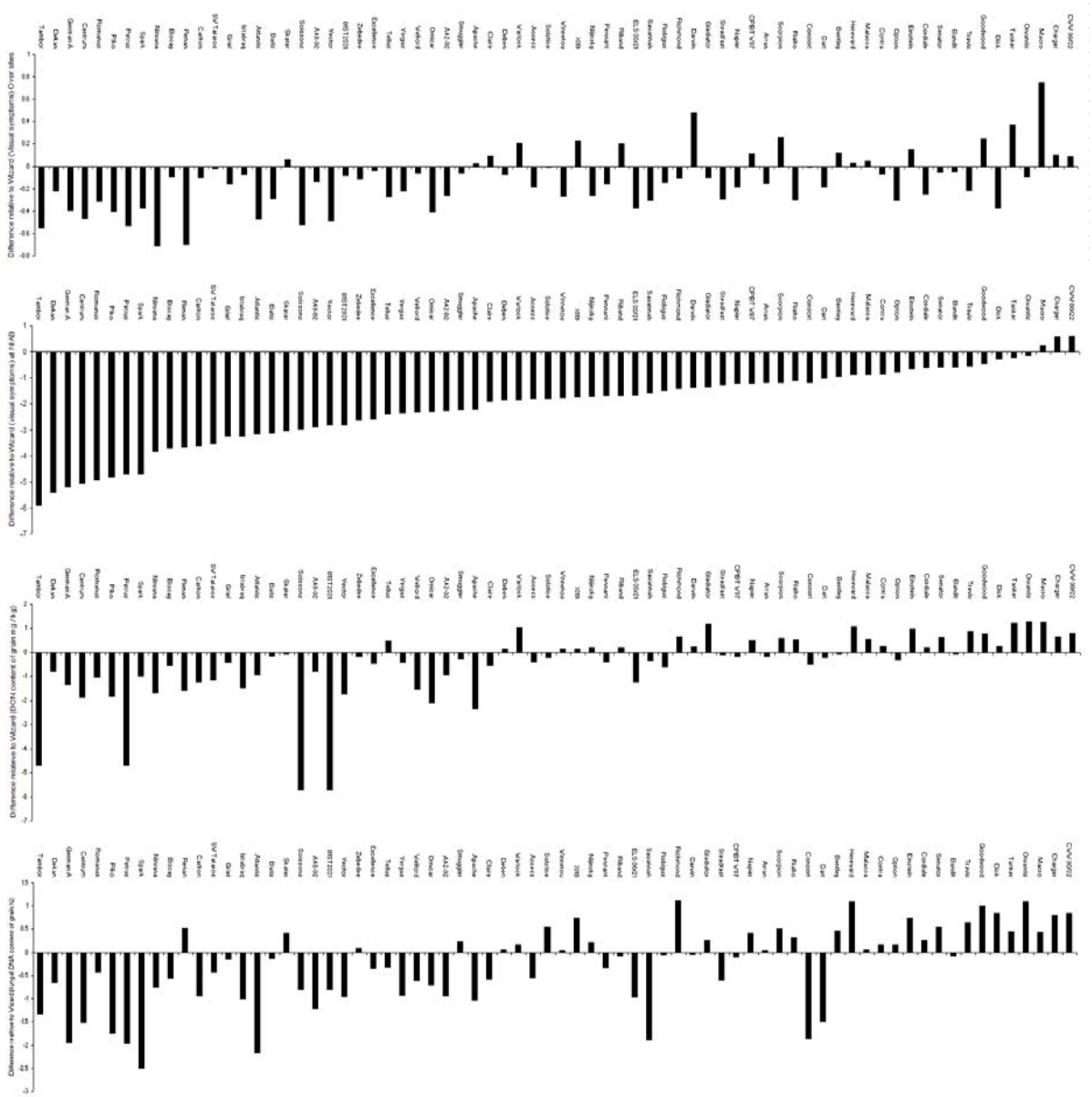

Figure 3.1 (A) Visual disease over three sites, (B) visual disease at the NIAB trials site (C) deoxynivalenol (DON) (ng / mg) and (D) fungal DNA (FDNA) (LOG10 fungal DNA as a % of total) content of grain from 72 wheat lines. Values are average deviation from the highly FHB susceptible variety Wizard. All entries are ordered (greatest relative disease reduction on the left) according to the mean deviation from Wizard at the NIAB site (which provided the samples for DON and FDNA analysis)

3.1.2 Relationship between FHB symptoms and the deoxynivalenol (DON) and fungal DNA content of grain

In addition to visual disease, grain samples from the NIAB site were assessed for the trichothecene deoxynivalenol (DON) and for fungal DNA (FDNA) content. The average DON content of samples was 1.07 mg kg^{-1} with a range of < 0.01 mg kg^{-1} to 3.75 mg kg^{-1} and the average FDNA level was 0.031%, with a range of 1.72 x 10^{-4}% to 0.38%. The entries with the lowest toxin content were French varieties Soissons and 01ST2031 and the entry with the highest was the French variety Orvantis. The entry with the lowest FDNA content was the UK variety Spark and the one with the highest was the UK variety Richmond (Figure 3.1). Analysis of traits relative to the FHB susceptible cultivar Wizard indicated that estimates of disease severity sometimes did not correspond with DON and FDNA levels in grain samples. For example, 01ST2031 and Soissons showed only moderate resistance at NIAB but both had the lowest toxin levels in the trial. Similarly, grain samples from the moderately resistant French cultivar Renan had a level of FDNA which was non-significantly ($P > 0.05$) different to entries with the highest levels of visual disease (Figure 3.1). However, coefficients of correlation were high (0.62, $P < 0.001$) for the relationship between visual disease at NIAB (FHB-NIAB) and DON and moderate between FHB-NIAB and FDNA (0.36, $P = 0.01$) (Table 3.2).

Table 3.2 Coefficients of correlation for the relationship between visual disease (% damage) over sites (FHB-OS), visual disease (percent damage) at NIAB (FHB-NIAB), deoxynivalenol (DON) and fungal DNA (FDNA) content of grain samples from the NIAB site.

	FHB-OS	FHB-NIAB	FDNA
FHB-NIAB	0.61 ***	--	--
FDNA (%)	0.53 ***	0.36 **	--
DON (mg kg^{-1})	0.45 ***	0.62 ***	0.61 ***

*** Significantly different from zero at $P < 0.001$
** Significantly different from zero at $P < 0.01$

3.1.3 Haplotyping with Known SSRs linked to FHB resistance QTL

A total of 17 microsatellite markers associated with six FHB QTL were used to genotype 83 wheat varieties (72 entries plus 11 reference cultivars) (Table 4). The polymorphic information content (PIC) values of the SSR loci ranged from 0.38 to 0.86 (mean = 0.65). Markers detected from four to 12 (mean = 7.35) alleles, giving rise to 83 unique haplotypes. Hierarchical cluster analysis was used to group lines based on marker haplotype; reference cultivars are shown in bold type (Figure 3.2).

Varieties grouped together into five main clusters the composition of which largely reflected their country of origin. The largest cluster was group 2 which consisted of mainly UK germplasm (21 entries) with a few (4 entries) varieties from France and Germany. Group 1 (6 entries) consisted of FHB resistant germplasm from France and Germany. Groups 3 and 4 (8 and 7 entries, respectively) consisted of only UK varieties, and group 5 consisted of the FHB resistant reference varieties, Wuhan 2-37E, CM82036, WEK0609 and Fundulea F201R. The Russian variety Aurora was not closely related to any of the other entries, and the most divergent entry was the German variety Petrus.

Interval length and haplotype diversity (total number of haplotypes and number of haplotypes per centi-Morgan (cM)) was assessed for each QTL interval. Relative to the other chromosome regions, 6BS and 1B had the shortest interval length (Table 4). In terms of haplotype number, *QFhs.inra-5a2* had the highest haplotype diversity; however, 6BS had the highest number of haplotypes per cM. By contrast, *Qfhs.ifa-5A* had the longest interval length and one of the lowest levels of haplotype diversity.

The allele distribution of the 17 microsatellite loci, grouped according to QTL interval, together with the FHB reaction for each variety is presented in Table 5. The most resistant (Nirvana) and susceptible (Macro) varieties in trial were used as standards in regression analysis at the 0.05% level of significance to classify entries into resistant, susceptible and moderate FHB resistance categories.

At the 1B locus the FHB resistant entry Petrus (German) and FHB resistant reference varieties Wuhan 2-37E, CM82036, Fundulea F201R and WEK0609 all carried the 1RS-1BL translocation and shared exactly the same haplotype as Aurora. However, varieties with a range of reactions to FHB carried the 1RS-1BL rye translocation and had the same allele size as Aurora at the Barc008 locus; these were A42-02, Access, Atlantis, ELS00/21, Excellence, Gladiator, Napier, Pennant, Rialto, Savannah, Senator, Steadfast, Travix, Vector, Vergas and Welford. There were no alternative haplotypes consistently associated with resistant or moderately resistant entries. The most common haplotype was (numeric values are base pairs) Xgwm018 (*196*)*, 1RS-1BL absent,* Barc008 (*257*) with a total of 15 occurrences. This haplotype was shared by a group of varieties of diverse origin with a range of FHB reactions (Table 3.3).

		1B			3BS			5A			5AL (2)			5AL (3)		6B			
Genotype	FHB reaction	Xgwm018	1BL/1RS	Barc008	Xgwm389	Xgwm533	Xgwm493	Xgwm293	Xgwm304	Xgwm156	Xgwm639	Wmc415	Barc151	Wmc524	Xgwm410	Xgwm133	Xgwm088	Wmc105	Barc198
Sumai-3	R	200	Abs	261	133	160	203	207	215	330	157	168	262	210	353	143	128	332	146
Wuhan 2-37E	R	Abs	Pres	191	133	160	203	207	215	330	173	170	252	210	353	143	128	332	146
CM82036	R	Abs	Pres	191	133	160	203	207	215	330	157	168	262	210	353	135	138	350	130
Aurora	R	Abs	Pres	191	137	123	146	203	213	295	170	176	250	172	351	133	134	350	127
F201R	R	Abs	Pres	191	nd	123	146	203	213	295	170	176	248	210	351	145	134	350	127
WEK0609	R	Abs	Pres	191	133	160	203	203	213	295	179	176	246	210	351	180	138	352	130
Renan	R	194	Abs	257	135	130	170	203	213	295	134	140	250	210	349	133	134	350	127
01ST2031	M	196	Abs	261	115	145	146	201	219	325	155	168	286	210	347	190	124	336	162
A42-02	M	198	Pres	191	137	Null	146	174	195	330	134	140	238	172	347	190	124	340	156
A48-02	M	198	Abs	261	137	128	146	174	195	310	144	140	232	172	347	189	141	360	133
Access	M	198	Pres	191	119	125	146	174	195	325	134	140	234	172	347	190	124	336	162
Apache	M	204	Abs	261	137	130	146	203	215	325	144	140	246	214	349	190	124	340	156
Arran	S	196	Abs	257	137	Null	146	197	199	310	134	140	246	172	347	190	124	336	162
Atlantis	R	198	Pres	191	137	123	146	203	205	325	155	176	246	216	351	187	124	340	156
Bandit	S	196	Abs	261	142	145	199	174	195	310	134	140	238	172	347	190	141	360	133
Batis	M	190	Abs	251	137	123	146	174	195	325	144	144	248	172	347	187	124	336	162
Bentley	S	196	Abs	261	137	145	146	174	195	310	146	140	230	172	347	187	124	336	162
Biscay	M	196	Abs	257	119	Null	146	174	195	325	144	140	234	172	347	190	124	336	162
Carlton	M	198	Abs	191	137	Null	146	174	213	310	134	140	238	214	349	180	141	360	133
Centrum	R	196	Abs	261	142	130	146	174	195	295	144	144	232	172	351	180	124	336	162
Charger	S	196	Abs	261	137	145	148	174	195	310	144	140	244	172	351	190	124	340	156
Claire	S	198	Abs	261	137	145	146	174	195	310	144	140	230	172	347	133	141	360	133
Consort	S	196	Abs	257	137	Null	146	197	199	295	134	140	246	172	347	190	124	340	156
Contra	S	196	Abs	257	137	120	146	174	195	310	144	140	238	172	347	175	132	356	165
Cordiale	S	196	Abs	261	142	145	146	174	195	310	144	140	232	172	351	190	124	336	162
CPBT W87	S	196	Abs	257	137	Null	146	197	199	330	134	140	246	172	347	190	124	336	162
CWW 00/22	S	196	Abs	261	142	145	199	174	195	310	144	140	244	172	351	190	124	340	156
Dart	S	196	Abs	259	142	145	199	197	199	295	134	140	246	172	347	190	124	340	156
Darwin	S	196	Abs	257	142	130	146	203	205	295	144	140	244	172	347	190	124	340	156
Deben	M	198	Abs	261	137	130	146	174	195	310	144	140	246	172	351	187	124	336	162
Dekan	M	196	Abs	261	142	145	146	174	195	310	157	168	246	172	351	187	124	340	156
Dick	S	196	Abs	259	119	Null	146	203	205	295	144	140	232	172	347	190	124	340	156
Einstein	S	196	Abs	259	137	Null	146	174	195	295	144	140	238	172	351	190	141	360	133
ELS 00/21	M	198	Pres	191	137	Null	146	174	195	310	144	140	232	172	349	133	134	350	127
Excellence	M	198	Pres	191	142	145	199	174	195	310	134	140	238	172	351	190	141	nd	133
GermanA	M	196	Abs	257	137	125	146	203	205	330	144	144	246	216	351	180	124	340	156
Gladiator	M	198	Pres	191	117	145	146	174	195	325	144	140	238	172	351	190	124	340	156
Goodwood	S	198	Abs	261	117	Null	146	174	195	310	144	140	232	172	351	190	141	360	133
Grief	M	198	Abs	259	117	145	146	174	195	310	144	140	238	172	347	133	124	340	156
Hereward	S	196	Abs	257	137	123	146	203	205	310	144	140	244	172	351	190	141	360	133
Istabraq	M	196	Abs	259	137	Null	146	197	199	325	144	140	246	172	347	190	124	340	156
Macro	S	196	Abs	263	142	145	146	174	195	310	134	140	252	172	347	190	124	336	162
Malacca	S	196	Abs	261	117	Null	146	174	195	310	144	140	232	172	347	190	124	336	162
Napier	M	198	Pres	191	119	120	146	174	195	325	144	140	234	172	347	190	124	336	162
Nirvana	R	196	Abs	261	119	Null	146	174	195	325	144	140	230	172	351	190	124	340	156
Nijinsky	M	198	Abs	261	137	Null	146	197	199	310	144	140	246	172	347	190	124	340	156
Option	S	196	Abs	257	137	125	199	174	195	325	144	140	230	216	347	190	124	340	156
Ornicar	M	198	Abs	261	115	145	166	205	205	310	157	176	230	172	353	190	124	340	156
Orvantis	S	196	Abs	257	137	145	146	197	199	325	144	140	230	172	347	133	124	340	156
Pennant	M	198	Pres	191	137	125	146	174	195	325	144	140	232	172	349	192	134	350	127
Petrus	R	Abs	Pres	191	135	120	146	203	205	310	144	140	244	214	347	133	132	356	165
Piko	R	198	Abs	261	137	123	146	203	201	310	157	178	232	172	347	180	124	340	156
Rialto	M	198	Pres	191	142	145	199	174	195	310	144	140	238	216	nd	180	141	360	133
Riband	S	198	Abs	257	137	Null	146	197	199	325	144	140	246	172	347	190	124	336	162
Richmond	S	198	Abs	263	137	Null	146	174	195	310	144	140	234	172	351	190	141	360	133
Robigus	S	196	Abs	257	113	Null	148	174	195	295	144	144	230	172	351	190	124	336	162
Romanus	M	196	Abs	261	137	120	146	201	201	325	144	140	246	214	347	133	124	336	162
Savannah	M	198	Pres	191	137	Null	146	197	199	330	144	140	246	172	347	190	124	336	162
Scorpion 25	S	186	Abs	255	115	125	146	174	195	310	170	170	238	216	351	190	124	336	162
Senator	S	198	Pres	191	142	145	199	174	195	310	134	140	232	172	351	190	141	360	133
Skater	M	196	Abs	257	117	123	146	203	215	310	144	168	250	216	351	187	124	336	162
Smuggler	M	196	Abs	257	119	130	146	174	195	310	144	168	246	172	347	187	124	340	156
Soissons	R	198	Abs	261	115	130	170	201	219	295	157	168	240	210	345	190	122	340	153
Solstice	M	196	Abs	257	142	130	146	174	195	310	144	140	244	216	351	190	141	360	133
Spark	M	196	Abs	261	115	160	146	174	195	310	170	170	230	172	351	190	124	336	162
Steadfast	M	198	Pres	191	142	145	199	174	195	310	144	140	234	172	351	190	141	360	133
SW Tataros	M	198	Abs	257	148	123	146	201	201	325	157	176	244	172	347	180	134	350	127
Tambor	R	198	Abs	nd	148	Null	146	201	201	325	157	176	244	172	347	190	134	350	127
Tanker	S	198	Pres	nd	142	145	146	174	195	310	144	144	238	172	347	190	124	336	162
Tellus	M	198	Abs	261	148	125	146	174	195	325	144	140	234	172	347	187	124	336	162
Travix	S	198	Pres	191	142	145	199	174	195	310	144	140	238	216	351	190	124	336	162
Vector	M	198	Pres	191	142	145	146	174	195	310	144	140	238	214	347	190	134	358	133
Vergas	M	200	Pres	191	137	130	146	201	195	295	157	168	246	216	347	187	124	nd	156
Warlock 24	S	186	Abs	255	115	125	146	199	195	325	170	170	248	216	351	190	124	336	162
Welford	M	198	Pres	191	137	Null	146	174	195	310	144	140	238	214	349	190	141	360	133
Winnetou	M	196	Pres	nd	137	130	146	174	195	330	144	140	238	216	351	135	124	360	133
Wizard	S	196	Abs	257	142	Null	146	203	205	310	144	140	246	172	347	190	124	340	156
XI19	S	186	Abs	255	115	145	146	199	195	325	170	170	248	216	351	190	124	336	162
Zebedee	M	198	Abs	259	137	145	146	174	195	310	144	140	230	172	347	189	141	360	133

Table 3.3 Allele sizes of microsatellite loci associated with FHB resistance QTL from wheat varieties on the United Kingdom (2003) National List plus reference cultivars from China, Mexico, USA and continental Europe. Within each interval, loci have been ordered (markers closest to, or on the short arm on the left and markers closest to, or on the long arm on the right) according to their map position in the consensus wheat map published by Somers et al (2003). Numeric values are amplicon sizes in base pairs for each marker, *nd* is no data. SSR alleles associated with FHB resistance QTL identified in previously published studies are colour coded: yellow identifies entries with the same size alleles as the Sumai-3 6BS resistance QTL, red identifies entries with the same size alleles as CM82036 linked to the *Qfhs.ndsu-3BS* (3BS) and *Qfhs.ifa-5A* (5A) QTL, blue identifies entries with the same size alleles as the Aurora 5A QTL, purple identifies entries with the same size alleles as Renan linked to the *QFhs.inra-5a2* (5AL (2)) and *QFhs.inra-5a3* (5AL (3)) QTL. The presence of rye chromatin associated with the 1RS-1BL translocation was identified using the duplex PCR assay of De Froidmont (1998), *Pres* 1RS-1BL present, *Abs* 1RS-1BL absent.

At *Qfhs.ndsu-3BS* there were no entry genotypes that matched any of the CM82036 alleles; however, reference varieties Sumai-3, Wuhan 2-37E and WEK0609 shared the same haplotype as CM82036. There were no alternative haplotypes consistently associated with resistant or moderately resistant entries. The most common haplotype was Xgwm389 (*138*), Xgwm533 (*Null*), Xgwm493 (*146*) with 13 occurrences. This haplotype was shared by moderately resistant and susceptible varieties of UK origin.

As previously mentioned, two QTL associated with CM82036 (marked red) and F201R (marked blue) have been identified at the *Qfhs.ifa-5A* locus. In the current study, there were no entry genotypes with a haplotype that exactly matched either the CM82036 or F201R haplotype. Among the reference cultivars, Sumai-3 and Wuhan 2-37E matched the CM82036 haplotype and WEK0609 and Renan matched that of Aurora but not that of F201R which differed from the other three varieties at the Xgwm156 locus. An additional 5A related haplotype of Xgwm293 (*174*), Xgwm304 (*195*) Xgwm156 (*325*) was, with the exception of Option, associated with resistant and moderately resistant entries from the UK and continental Europe; these were, Gladiator, Napier, Access, Biscay, Nirvana, Batis, Pennant, Option and Tellus. FHB susceptible UK varieties, Dick and Darwin matched with Aurora at the Xgwm293 and Xgwm156 loci, and the FHB resistant reference variety F201R (which has Aurora in its genealogy) matched with Aurora at the Xgwm293 and Xgwm304 loci. The most common haplotype was Xgwm293 (*174*), Xgwm304 (*195*), Xgwm156 (*310*) with 29 occurrences. This haplotype was shared by a group of varieties of diverse origin with a range of FHB reactions.

At *QFhs.inra-5a2* there were no entry genotypes or reference cultivars that matched the Renan haplotype. There were no alternative haplotypes consistently associated with resistant or moderately resistant entries. The most common haplotype was Xgwm639 (*134*), Wmc415 (*140*), Barc151 (*246*) with four occurrences. This haplotype was found among susceptible UK varieties.

At *QFhs.inra-5a3* there were no entry genotypes or reference cultivars that exactly matched the Renan haplotype. However, resistant and moderately resistant entry varieties Apache, Carlton, ELS 00/21, Pennant and Welford had the same allele as Renan at the Xgwm410 locus and resistant entry varieties Soissons and 01ST2031 and reference cultivars Sumai-3, WEK0609, CM82036, Fundulea F201R, and Wuhan 2-37E had the same allele as Renan at the Wmc524 locus. There were no alternative haplotypes consistently associated with resistant or moderately resistant entries. The most common haplotype was Wmc524 (*172*) and Xgwm410 (*347*) with 32

occurrences. This haplotype was shared by a group of varieties of diverse origin with a range of FHB reactions.

At the 6BS interval, with the exception of Wuhan 2-37E which matched exactly, none of the other varieties had the same allele size as Sumai-3 at any of the loci. An additional haplotype of *134*, *350*, *127* at the Xgwm088, Wmc105 and Barc198 loci respectively, was associated with the resistant and moderately resistant entry and reference varieties which were all, with the exception of Pennant, from continental Europe; these were ELS 00/21, Aurora, Renan, F201R, SW Tataros, Tambor, Pennant. The most common haplotype was Xgwm133 (*190*), Xgwm088 (*124*), Wmc105 (*336*), Barc198 (*162*) with 18 occurrences. This was shared by a group of susceptible and moderately resist.

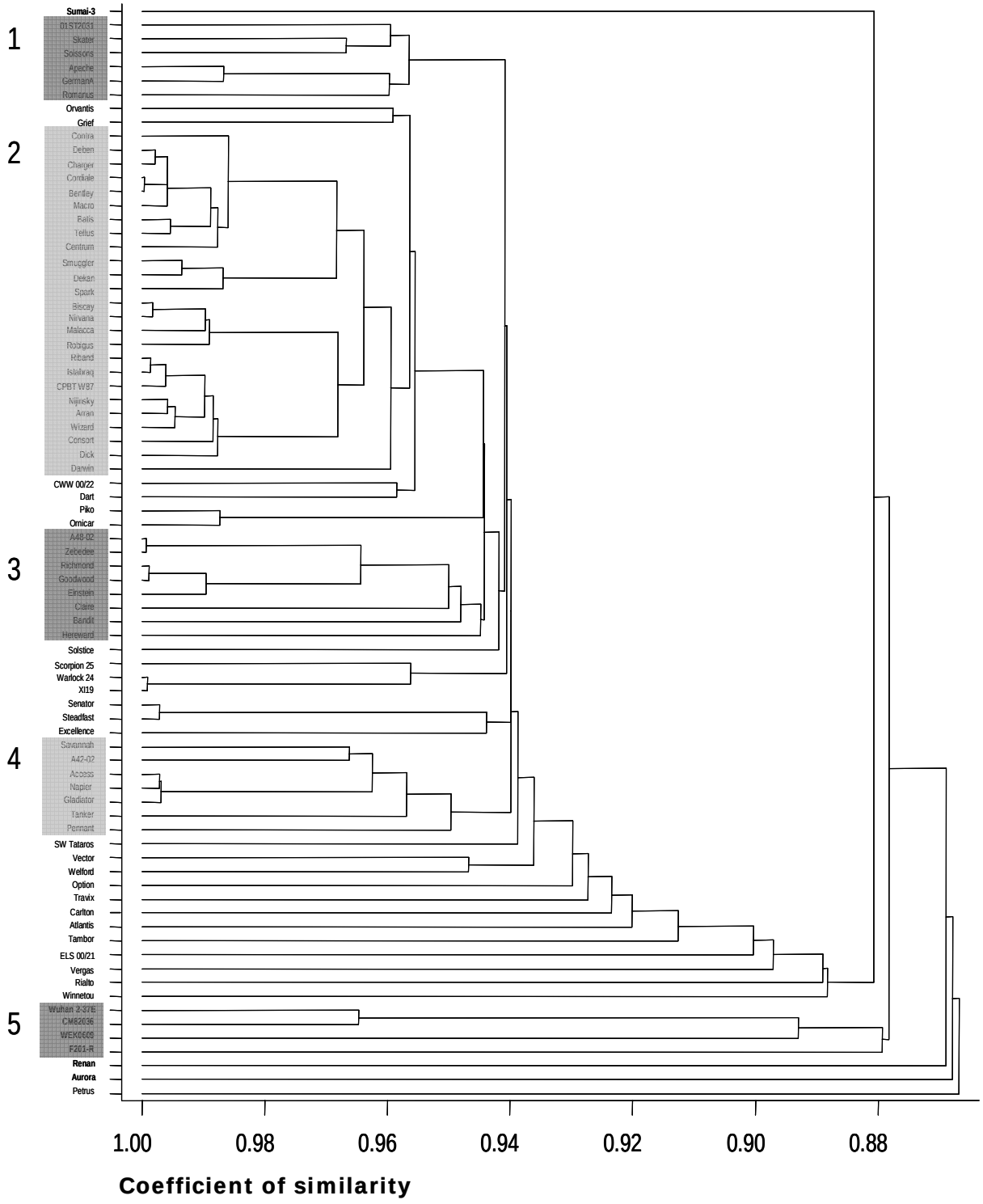

Figure 3.2 Dendrogram produced by hierarchical cluster analysis of 72 trial lines and 7 reference varieties using the Euclidian test with single link as the clustering method. The similarity matrix was calculated using allele size data from 17 microsatellite loci associated with six FHB resistance QTL. Shaded areas identify major groupings.

3.2 Discussion

3.2.1 FHB status of UK winter wheat varieties

A total of 72 winter wheat varieties were phenotyped in the UK for FHB resistance in the summer of 2003. Trial entries included 53 varieties on the UK National List (NIAB, Cambridge) plus 19 check cultivars from France and Germany in which the FHB reaction had previously been characterised by project collaborators. FHB resistance was assessed by spray inoculation with a single aggressive isolate of *F. culmorum* which is appropriate since there is no evidence that resistance to FHB is race or species specific (Van Eeuwijk et al, 1995). Three contrasting environments, two field sites at the Central Science Laboratory (CSL) and NIAB (low to moderate disease levels respectively) and one controlled environment at the John Innes Centre (JIC) (high disease level), were used to assess the stability of FHB reaction. In addition to visual disease, the trichothecene mycotoxin, deoxynivalenol (DON) and fungal DNA (FDNA) content of grain samples from the NIAB field site were assessed in order to improve phenotype reliability and further characterise resistance. Although genotypic variance was highly significant, environment and genotype by environment components were also large. Similar sized environmental effects are often reported in studies of FHB resistance in wheat mapping populations (Gervais et al, 2003; Buerstmayr et al, 2003) and inbred lines (Buestmayr et al, 2004). Much of this variance is probably due to variation in climatic conditions over years and between sites (Mesterhazy et al, 1999). However, an unpublished study by Gosman et al (2004) suggests that there can also be significant variation between winter wheat cultivars for the period of optimal susceptibility to FHB. Assessment of visual disease, DON and FDNA data from three inoculation timings indicated that some varieties had a very narrow (three days or less) period of optimal susceptibility whereas others remained highly susceptible over a six day period. These effects may, to some extent, explain significant differences in disease response between sites observed in some varieties.

Very few varieties on the UK National list had resistance levels which were comparable those of resistant check cultivars. Of the 53 National List varieties tested, only Soissons, Spark and Vector were significantly ($P < 0.05$) more resistant than the FHB susceptible variety Wizard. In addition, even under the moderate disease pressure at NIAB, a total of 24 National List varieties had DON levels which were above the proposed EU action limits of 1.25 parts per million (ppm). These results indicate that a significant effort will be required by the UK plant breeding community

to improve overall levels of FHB resistance. In general, increased resistance to symptom development was correlated with reduced DON content of grain. Indeed, certain resistant varieties (Tambor, Petrus and Soissons) and the moderately resistant 01ST2031 had particularly low toxin levels suggesting that they may have the ability to degrade DON (Miller & Arnison, 1986) or reduce DON production by the pathogen. However, some cultivars with significant levels of DON and FDNA (Dekan, Biscay, Carlton, SW Tataros, Istabraq and Grief) appeared to be resistant suggesting that visual disease may not be closely correlated to DON levels in some genotypes. A comparative analysis by Paul et al (2005) suggests that the method of visual assessment used may in part be responsible for lack of correspondence between DON levels and symptom development in some instances. Over studies, these authors found that DON content correlates better with assessments of damaged spikelets within a head than with assessments of percentage infection within a plot.

Comparison of microsatellite (SSR) allele size was used to infer the origin of FHB resistance and identify germplasm with potentially novel loci. From published mapping studies, 17 SSR loci associated with six FHB resistance QTL on chromosomes 1B, 3B, 5A and 6B were selected. In accordance with haplotype analysis of wheat FHB QTL by McCartney et al (2004) hierarchical cluster analysis of SSR allele size data tended to group cultivars according to their country of origin producing three groups of mainly UK germplasm and a small group of control varieties from France and Germany. The most divergent variety was Petrus which had highly stable FHB resistance over environments and significantly reduced both the toxin (DON) content and fungal colonisation (FDNA) of grain. Petrus, therefore, appears to be a potent source of novel FHB resistance which could be of great value in European breeding programs.

In an attempt to identify potentially novel FHB resistance, comparison was made with three loci of major effect on chromosomes 3BS, 5A and 6BS which have been reported to condition resistance in the intensively studied cultivar Sumai-3. The interval *Qfhs.ndsu-3BS* (3BS) associated with SSR loci Xgwm389, Xgwm533 and Xgwm493 (Anderson et al 2001) has been reported in several studies where Chinese or Sumai-3 derived germplasm has been the source of resistance (Buertmayr et al, 2003; Guo et al, 2003; Shen et al, 2003b). However, there was no allele size homology between Sumai-3 and any of the test varieties in the current study indicating that FHB resistance at the 3BS locus is absent among the germplasm studied. Unsurprisingly, the Sumai-3 haplotype at *Qfhs.ndsu-3BS* was shared by the Sumai-3 derivative CM82036. The Sumai-3 haplotype was also shared by the North

American wheat variety WEK0609 indicating that potent resistance identified in this interval (Gosman et al, unpublished) may be allelic. Surprisingly, the Chinese variety Wuhan 2-37E was identical to the Sumai-3 haplotype at all three QTL. This is in contrast to McCartney et al (2004) who reported that Wuhan 2-37E had alternative alleles at these loci. This result highlights the utility of selective genotyping in identifying divergences in stocks that can arise in germplasm collections.

On 6BS, there was a similar absence of homology between test varieties for SSR loci associated with major QTL for resistance in Ning 894037 (Shen et al, 2003b) and Sumai-3 (McCartney et al, 2004). In the current study there was an alternative haplotype which was, with the exception of the variety Pennant, associated with resistant and moderately resistant germplasm of Continental European origin. However, no QTL at this position was reported in studies of resistance in Renan (Gervais et al, 2003) or F201R (Shen et al, 2003a) which also matched this haplotype suggesting that this association maybe due to chance or that the putative QTL at this locus was not segregating in the mapping populations used in the published studies (Gervais et al, 2003; Shen et al, 2003a).

Two distinct haplotypes were identified at the *Qfhs.ifa-5A* (5A) locus, one identical to CM82036 reported by Buestmayr et al (2003) shared by Sumai-3 and Wuhan 2-37E, and the other identical to the Russian variety Aurora shared by WEK0609 and Renan. However, F201R, which carries a potent QTL for resistance in a similar position to *Qfhs.ifa-5A* (Shen et al, 2003a) differed at the Xgwm156 locus suggesting that WEK0609 and Renan inherited this QTL from Aurora not F201R. No other varieties matched either of the haplotypes suggesting that this locus was also absent in the study germplasm. Interestingly, although Renan had a haplotype which matched that of Aurora suggesting that it also has a QTL at this locus, no association with FHB resistance has been reported (Gervais et al, 2003). However, potent FHB resistance was associated with the Aurora haplotype in WEK0609 (Gosman et al, unpublished) suggesting that the Aurora variant may not have been segregating in the population used by Gervais et al (2003). In view of the relatively large genetic distance delineated by *Qfhs.ifa-5A* it is also possible that the QTL may have been lost in Renan through recombination without affecting the haplotype of the markers used. According to the consensus map of Somers et al (2004), the order of markers at the *Qfhs.ifa-5A* locus from the short arm is, Xgwm293, Xgwm304 and Xgwm156. The haplotype of F201R differs from that of Aurora at the Xgwm156 locus, and the FHB susceptible cultivars Dick and Darwin match Aurora at Xgwm156 and Xgwm293. This result suggests that Xgwm293 and Xgwm156 may not be closely associated with

resistance in the Aurora variant of this locus indicating that loci more closely associated with Xgwm304 might be needed for marker assisted selection (MAS). In order that further QTL of European origin were not overlooked on 5A, markers for two additional intervals (*QFhs.inra-5a2* and *QFhs.inra-5a3*) identified in Renan by Gervais et al (2003) were included in the current study. However, once again, there was no match with the Renan haplotype among test varieties.

A less widely deployed source of resistance associated with the 1RS-1BL rye translocation in F201R (Shen et al, 2003a) was also included in the current study. Use of point inoculation by Shen et al. (2003) indicated that the 1B QTL in F201R conditions significant resistance to spread (type II resistance, sensu Schoeder & Christensen, 1963). In the current study, a large group of test varieties with varying FHB reactions had the 1RS-1BL translocation and shared the same allele as F201R at the Barc008 locus. However, only the German *cv* Petrus and the reference varieties Wuhan 2-37E, CM82036, Aurora and WEK0609 had a null allele at the Xgwm018 locus suggesting that this locus may be diagnostic for this F201R QTL in combination with the rye translocation and the 191 base pair (bp) allele of Barc008. In contrast to F201R where the 1B locus is of major effect, Buerstmayr et al (2002) reported that type II resistance on chromosome 1B in CM82036, associated with the high molecular weight (HMW) glutenin locus (*Glu-B1*), was of only minor effect. This may have been because more potent loci were segregating for resistance in CM82036 compared to F201R diminishing its apparent effect, however, analysis in WEK0609 indicated that the 1B QTL is of almost equal importance to that of *Qfhs.ndsu-3BS* (Gosman et al, unpublished). Analysis of the protein banding pattern at *Glu-B1* locus indicates that CM82036 has the '*i*' allele whereas other varieties sharing the F201R haplotype have the '*c*' allele (data not shown) indicating that CM82036 may possess a less potent variant of the QTL. In the current study, the 1RS-1BL translocation is associated with an approximately 190 bp product at the Barc008 locus in every case. However, according to the consensus map of Somers et al (2004) Barc008 is on the short arm of 1B and should, therefore, be null in the presence of the rye translocation. It is possible that there is a priming site for Barc008 on 1RS in rye however; it is more likely that this locus is in fact on the long arm, rather than the short arm.

Varieties with the lowest levels of symptom development in the current study also had toxin levels in grain that were well below proposed EU action limits. Three varieties from the 2003 UK National List, Soissons, Spark and Vector had a stable reaction over environments and should, therefore, be suitable for introducing FHB resistance into UK breeding programs. Studies of combining ability in wheat suggest

that high levels of FHB resistance can be achieved from crosses between parental lines with only moderate levels of resistance (Buerstmayr et al, 1999 and Buerstamayr et al, 2004). In view of this, genetic analysis is under way to determine the genomic location and significance of FHB resistance in Soissons and Spark with a view to eventually 'pyramiding' resistance in germplasm with more favorable agronomic characteristics. SSR haplotyping indicated that these varieties do not possess any of the FHB resistance QTL found in Sumai-3, Renan or F201R. The resistance in these varieties, therefore, appears to be novel.

None of the FHB resistant varieties from the UK or mainland Europe had SSR haplotypes indicating that their resistance is derived from Sumai-3. The introduction of potent QTL for resistance to FHB from this source should also provide a means to enhance the FHB resistance of European varieties.

4 Identification and mapping of QTL for FHB resistance and other agronomic traits in different mapping populations

Following initial studies to identify FHB resistance, detailed studies were undertaken to identify FHB QTL from three resistance sources. Soissons and Spark were found to be the most resistant UK varieties and QTL analysis was undertaken of Spark x Rialto and Soissons x Orvantis doubled haploid populations. The third population was of recombinant inbred lines from RL4137 x Timgalen. Field and polytunnel disease trials were established and each line scored at several locations. Genetic maps were produced for all three crosses and QTL analysis carried out for FHB resistance traits and for selected morphological traits to determine the genetic basis of FHB resistance and associations with other traits. These the results of these studies have been discussed in this section.

4.1 Spark/Rialto DH population

4.1.1 Results

4.1.1.1 Trait analysis in the population

Spark, Rialto and the DH lines were phenotyped for FHB across three environments. The frequency distribution for AUDPC in the ADVANTA2005, NIAB2005 and JIC2006 trials was continuous with transgressive segregation and slightly skewed towards greater susceptibility, with the population mean being greater than the mid-parent mean (Table 4.1).

Table 4.1 Summary statistics for fusarium head blight (FHB) disease and plant height assessed in different experiments

Expt. site & trait	Spark	Rialto	MPV	PM	Range
FHB-AUDPC					
ADVANTA2005	246	557	401.5	529	128-1100
NIAB2005	208	339.5	273.8	294	37-695
JIC2006	144	792.5	468	515	61-1270
Plant height (cm)					
JIC2006	103.3	101.1	102.2	102	82-130

AUDPC= area under the disease progress curve, MPV = mid-parent value
PM = population mean

The analysis of variance (ANOVA) and broad sense heritability (h^2) were calculated for AUDPC in each trial and across environments and for plant height at the JIC2006 (Table 4.2). Within-trial heritability (h^2) ranged from 0.48 to 0.88 and was highest for AUDPC-dpi at JIC2006. Across environments, the heritability was 0.48.

4.1.1.2 Association between height and disease

The parental lines had very similar plant heights: Spark (103.25cm) and Rialto (101.08cm). The frequency distribution for height within the DH population was continuous, ranging from 82-130 cm, and followed a normal distribution pattern centred on the two parents, with a population mean of 101.7cm. Analysis indicated that most of the differences in height within this population have a genetic basis with a high heritability (h^2=87%), (Table 4.2). A weak/moderate negative correlation was detected between plant height at J2006 and AUDPC with correlation coefficient 0.39 (P=<0.001), indicating that plant height has some effect on resistance.

Table 4.2 Analysis of variance and heritability of different traits in individual trials and across environments

Expt. Site and year	Trait	Variance factor	DF	MS	P-value	h^2
ADVATA2005	AUDPC	Genotypes	121	56045	<.001	0.48
		Replicates	1	99487	0.07	
		Genotype x Replicates	121	30288	0.5	
		Residual	121	30288		
NIAB2005	AUDPC	Genotypes	121	39255	<.001	0.62
		Replicates	1	18136	0.22	
		Genotype x Replicate	121	11864	0.5	
		Residual	121	11864		
JIC2006	AUDPC	Genotypes	121	194638	<.001	0.88
		Replicates	1	442255	<.001	
		Genotypes x Replicates	121	13626	0.5	
		Residual	121	13626		
Across sited	AUDPC	Genotypes	121	115644	<.001	0.48
		Locations	2	4189900	<.001	
		Genotypes x Locations	237	96395	<.001	
		Residual	424	27539		
JIC2006	Plant height	Genotypes	121	231.97	<.001	0.87
		Replicates	1	25.94	0.24	
		Genotypes x Replicates	121	15.45	0.8	
		Residual	53	18.67		

DF = degrees of freedom, MS = variance expressed as mean squares, h^2 = experimental repeatability/heritability.

4.1.1.3 Identification of FHB resistance QTL

A total of ten FHB resistance QTL exceeding genome wide LOD threshold significance were detected. Five QTL were consistently detected in more than one environment (Table 4.3 and Fig. 4.1). Four major QTL, one each on 1B, 4A, 4D and 6A were detected. LG 3A detected two QTL (*Qfhs.jic-3a.1* and *Qfhs.jic-3a.2*) which are separated by about 50 cM in more than one environment. The QTL *Qfhs.jic-3a.1* closely linked to WMC11(2) and DArT markers, wPt-7992, were contributed by the alleles from Rialto. Similarly, *Qfhs.jic-3a.2* closely linked two SSR markers, GWM497

and BAC19, was from the other parent Spark. The two major QTL detected in this study are of particular interest. *Qfhs.jic-4d.2* on 4D with LOD value 6.8 to 28.2 (R^2 = 21.4 to 52.7 % except at Elsoms), is a consistent QTL identified in all the environments. This QTL was closely linked to *Rht-D1b* (Rht2), a sequence based marker and PSP3103, an SSR marker. This LG also detected a minor QTL at JIC for AUDPC-days. Surprisingly the alleles from Spark contributed for both these QTL. Another major QTL closely linked to the DArT marker, wPt-8833, was detected in more than one environment. Two minor QTL, one each on initial segment of 6A and terminal segment of 7A, are only suggestive (Table 4.3 and Fig. 4.1).

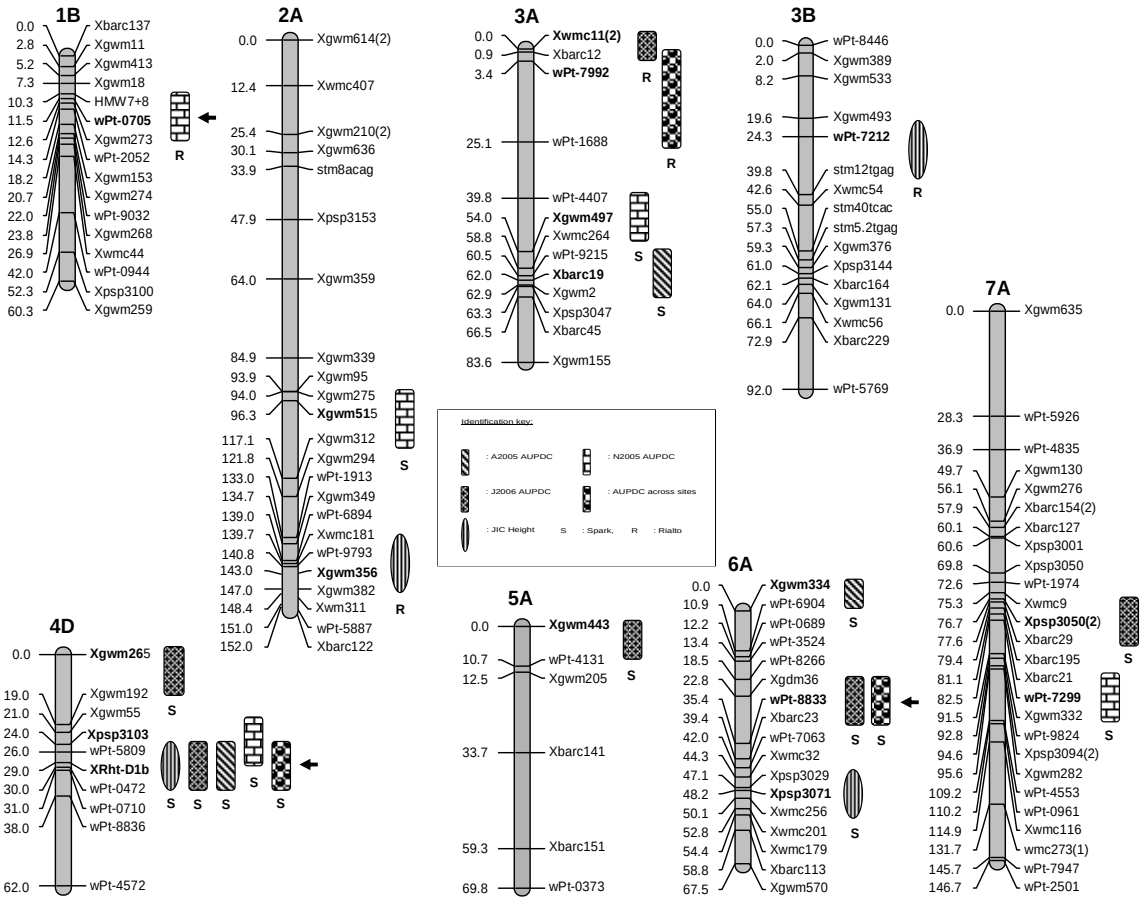

Fig. 4.1 Linkage maps of chromosome segments constructed from the Spark x Rialto doubled haploid population. Putative QTL positions for FHB resistance and plant height are shown on the right of each linkage group. Genetic distances are shown in cM to the left of each linkage group. Major QTL have been indicated with an arrow and loci in bold are closest to the peak LOD score.

4.1.1.4 Plant height QTL

The details on the genomic regions associated with plant height are shown in the Table 4.3. From both the trials, a total of five QTL which were above the LOD threshold value 2.9, were detected. They were mapped on LGs 2A, 3A, 3B, 4D and 6A. A major QTL contributed by Spark on LG 4D with closet marker *Rht-D1* (LOD value of about 29 and R^2 = 54 %), was detected in both the trials. The LG 3B detected two minor QTL (about 40 cM apart) contributed by Rialto. Although two QTL contributed by Spark were mapped on 2B, they were only 10 cM apart and therefore it could just be single QTL.

Table 4.3 Summary of QTL for FHB resistance and plant height identified in Spark/Rialto DH population in different experiments

Name	Closest marker	Origin	Position	ADVANTA2005		NIAB2005		JIC2006		Across Expt.	
				LOD	R^2	LOD	R^2	LOD	R^2	LOD	R^2
FHB resistance:											
Qfhs.jic-1b	*wPt-0705*	Rialto	11.5(1B)	_	_	5.2	13.4	_	_	_	_
Qfhs.jic-2a	*Xgwm515*	Spark	96.3(2A)	_	_	2.9	7	_	_	_	_
Qfhs.jic-3a.1	*Xwmc11(2)*	Rialto	0(3A)	_	_	_	_	2.7’	2.7	_	_
Qfhs.jic-3a.1	*wPt-7992*	Rialto	3.4(3A)	_	_	_	_	_	_	2.8	4
Qfhs.jic-3a.2	*Xgwm497*	Spark	54(3A)	_	_	3.3	8	_	_	_	_
Qfhs.jic-3a.2	*Xbarc19*	Spark	62(3A)	2.8	7.6	_	_	_	_	_	_
Qfhs.jic-4d.1	*Xpsp3103*	Spark	24(4D)	_	_	9.3	25.5	_	_	_	-
Qfhs.jic-4d.1	*XRht-D1b*	Spark	29(4D)	6.8	21.4	_	_	28.2	52.7	23.5	50.9
Qfhs.jic-4d.2	*Xgwm265*	Spark	0(4D)	_	_	_	_	3.1	3.1	_	_
Qfhs.jic-5a	*Xgwm443*	Spark	0(5A)	_	_	_	_	3.5	3.9	_	_
Qfhs.jic-6a.1	*Xgwm334*	Spark	0(6A)	2.2’	6.2	_	_	_	_	_	_
Qfhs.jic-6a.2	*wPt-8833*	Spark	35.4(6A)	_	_	_	_	8.3	10.5	5.2	7.8
Qfhs.jic-7a	*Xpsp3050(2)*	Spark	76.7(7A)	_	_	_	_	2.9	3.7	_	_
Qfhs.jic-7a	*wPt-7299*	Spark	82.5(7A)	_	_	_	_	2.2’	5.2	_	_
Plant height:	*Xgwm356*	Rialto	143(2A)	_	_	_	_	6.9	8	_	_
	wPt-7212	Rialto	24.3(3B)	_	_	_	_	3.3	3.7	_	_
	XRht-D1b	Spark	29(4D)	_	_	_	_	29.2	53.1	-	_
	Xpsp3071	Spark	48.2(6A)	_	_	_	_	2.9	3.1	_	_

R^2 = percentage phenotypic variance explained, ’= LOD below the permutation test threshold for significance, _ = no data

4.2 Soissons/Orvantis DH population

4.2.1 Results

4.2.1.1 Fusarium head blight resistance

FHB severity showed a continuous distribution both in individual environments and for mean over environments (data not shown). The distributions were slightly skewed towards resistant parent Soissons. The segregation of FHB severities in the population differed between environments. Transgressive segregation was noticed in all environments and was more towards Soissons in JIC2006 and NIAB2005 while it was more towards susceptible parent Oravantis in CSL2005. The parents displayed different FHB severities in individual environment (Table 4.4). The parent means (mid parent value) were greater than the population means except in NIAB2005 (Table 1). The plant height (PH) showed normal distribution with nearly equal number of trangressive segregants on either side of the parents (data not shown). The PH of Soissons and Orvantis were 90.3 cm and 96.1 cm, respectively (Table 4.4).

Table 4.4. Summary statistics for plant height and AUDPC in Soissons/Orvantis DH population

Expt. site	Trait	Soisson	Orvantis	MPV	Range	PM
CSL2005	AUDPC	0	519.2	259.5	743.2	238.7
JIC2006	AUDPC	208.6	1838.4	1023.5	1670	470.6
NIAB2005	Incidence	6.6	45	25.8	95	34.6
	Severity	4.1	13.9	9	58.8	13.7
	AUDPC	34	677	356	5508	659
Across sites	AUDPC	80.9	1011	556	2382	546
JIC2006	Plant height	90.3	96.1	93.2	70	92.9

MPV = mid-parent value, PM = population mean

4.2.1.2 Correlations, ANOVA and broad sense heritability

Correlations of AUDPC from all environments was found to be significant ($P < 0.001$) (data not shown) and the correlation coefficient (R^2) ranged from 0.138 to 0.839 with an average R^2 value 0.443. PH also showed weak to moderate levels of correlation with AUDPC ($R^2 = 0.04$ to 0.138). The genotypes were found to be highly significant in all the environments ($P = 0.007$ to <0.001) and moderate significance of replication

was also noticed in CSL2005 and NIAB2005 (Table 4.5). The broad sense heritability (h^2) ranged from 42 to 90 % with an average heritability of 69 %.

4.2.1.3 FHB and Plant height QTL

The map based QTL analysis revealed the association of genomic regions on 1B, 3B, 4B, 4D and 7A and three of these QTL were found to be significant (Table 4.6 and Fig. 4.2). The alleles from Soissons contributed for all these QTL except for the QTL on 1B. A major QTL *Qfhs.jic.4d* was detected in all the environments. Two minor QTL *Qfhs.jic.3b* and *Qfhs.jic.7a* were observed in N2006.

A total of five QTL on three wheat chromosomes namely, 1BL, 4BL, 4DS and 7AL were found to be associated with PH and the alleles from both parents contributed for the QTL. Of these, QTL on 4B and 4D with the closest loci *Rht-B1b* and *Rht-D1b*, respectively were found to be major PH-QTL in this population. The alleles from Soissons and Orvantis contributed for the QTL on 4DS and 4BL, respectively. Two minor QTL one each from Soissons and Orvantis were mapped on 1BS and 1BL. As majority of the makers on the map were DArTs, either consensus wheat maps published on the Triticarte website or wheat genetic maps (DArT markers anchored with SSRs) was used to identify the positions of QTL mapped in this study.

A major FHB QTL *Qfhs.jic.4d* co-localised with a major PH-QTL with the closest marker *Rht-D1b* on 4DS and the alleles from Soissons contributed for both these QTL. Similarly, a minor FHB QTL contributed by the alleles from Orvantis also co-localised with major PH-QTL with the closest maker *Rht-B1b* on 4BS. None of the other PH-QTL were co-localized with other FHB-QTL detected in this study.

4.2.1.4 Phenotyping of *Rht-B1b* and *Rht-D1b* near-isogenic lines

Experiment 1: In the Poly-tunnel, when Mercia and Maris Huntsman were spray inoculated with *F. culmorum* or *F. graminearum*, both variety ($P<0.001$, Table 4) and Rht status ($P=0.002$) were highly significant, no interaction of Rht by variety ($P=0.451$) was observed. Compared to Rht (tall), both *Rht-B1b* ($P = 0.090$) and *Rht-D1b* ($P = 0.087$) were moderately significant, however no significant differences ($P = 0.987$) existed between them. The mean AUDPC were 11209, 11784 and 6861 for *Rht-B1b*, *Rht-D1b* and Rht (tall), respectively.

When the same genotypes were sprayed with *F. culmorum*, the Rht status was found to be highly significant ($P = 0.010$, Table 4.7) and the Rht by variety interaction showed moderate effect ($P = 0.237$), variety was not significant (Table 4.7). The mean AUDPC of *Rht-D1b* (13417) was significantly higher ($P<0.001$) than Rht (tall).

No significant differences existed between *Rht-D-B1b* (P = 0.109) and Rht (tall), and *Rht-B1b* and *Rht-D1b* (P = 0.180).

Table 4.5 Variance components identified using generalised linear model for FHB-AUDPC and plant height in the Soissons/Orvantis DH population

Source of variation	Disease								Plant height	
	NIAB2005		CSL2005		JIC2006		Across sites			
	M.S.	P-value	M.S.	P-value	M.S.	P-value	M.S.	P-value	M.S.	P-value
Genotype (DH line)	3.77E+08	0.007	1171490	<.001	486578	<.001	631795	<.001	137.962	<.001
Genotype x Replication	2.85E+07	0.5	432762	0.5	27577	0.5	431372	<.001	8.384	0.5
Replication or Expt. Site	1.28E+08	0.082	3884302	0.003	2529	0.762	35521978	0.486	106.381	<.001
Residual error	2.40E+07		432762		27577		430715		8.384	
R or h^2	0.88		0.58		0.9		0.42		0.9	

M.S. refers to mean square, R refers to experimental repeatability and h^2 refers to broad sense heritability.

Table 4.6 Summary of QTL for FHB resistance identified in Soissons/Orvantis DH population in N2005, C2005 and J2006 trials

FHB trait	Name	Closest locus	Parent	Position	JIC2006		NIAB2005		CSL2005		Pooled data	
					LOD	R^2	LOD	R^2	LOD	R^2	LOD	R^2
AUDPC	*Qfhs.jic.4b*	*RhtB-1b*	Soissons	0(4BS)	2.1$^{\$}$	5.3	_	_			1.6$^{\$}$	4.2
AUDPC	*Qfhs.jic.4d.1*	wPt-7569	Soissons	0(4DS)	6.4	16.1	_	_	4	10.6	7.5	18.4
Incidence	*Qfhs.jic.1b*	wPt-1403	Orvantis	3.6(1BL)	_	_	2.3$^{\$}$	5.4	_	_	_	_
	Qfhs.jic.7a	wPt-7034	Soissons	4.4(7AL)	_	_	3.4	8.5	_	_	_	_
	Qfhs.jic.4d.2	*RhtD-1b*	Soissons	9.3(4DS)	_	_	3.2	7.4	_	_	_	_
Severity	*Qfhs.jic.3b*	wPt-2559	Soissons	3.9(3BL)	_	_	2.5	6.1	_	_	_	_
	Qfhs.jic.4d.1	wPt-0710	Soissons	0(4DS)	_	_	2.6	6.1	_	_	_	_
Disease Index	*Qfhs.jic.3b*	wPt-2559	Soissons	3.9(3BL)	_	_	2.6	6.7	_	_	_	_
	Qfhs.jic.4d.1	wPt-0710	Soissons	0(4DS)	_	_	2.8	7.1	_	_	_	_
	Qfhs.jic.7a	wPt-6447	Soissons	0(7AL)	_	_	2.2$^{\$}$	5.3	_	_	_	_
Plant height		*wPt-5562*	Soissons	0(1B)	3	5.1	_	_	_	_	_	_
		wPt-9809	Orvantis	14.7(1B)	4.1	7.4	_	_	_	_	_	_
		Rht-B1b	Orvantis	0(4B)	8.6	15.9	_	_	_	_	_	_
		Rht-D1b	Soissons	9.3(4D)	11.6	21.9	_	_	_	_	_	_
		wPt-2780	Orvantis	0(7A)	3.2	5.4	_	_	_	_	_	_

LOD = Log of adds ratio, R^2 = percentage variance explained and $^{\$}$ = below LOD threshold

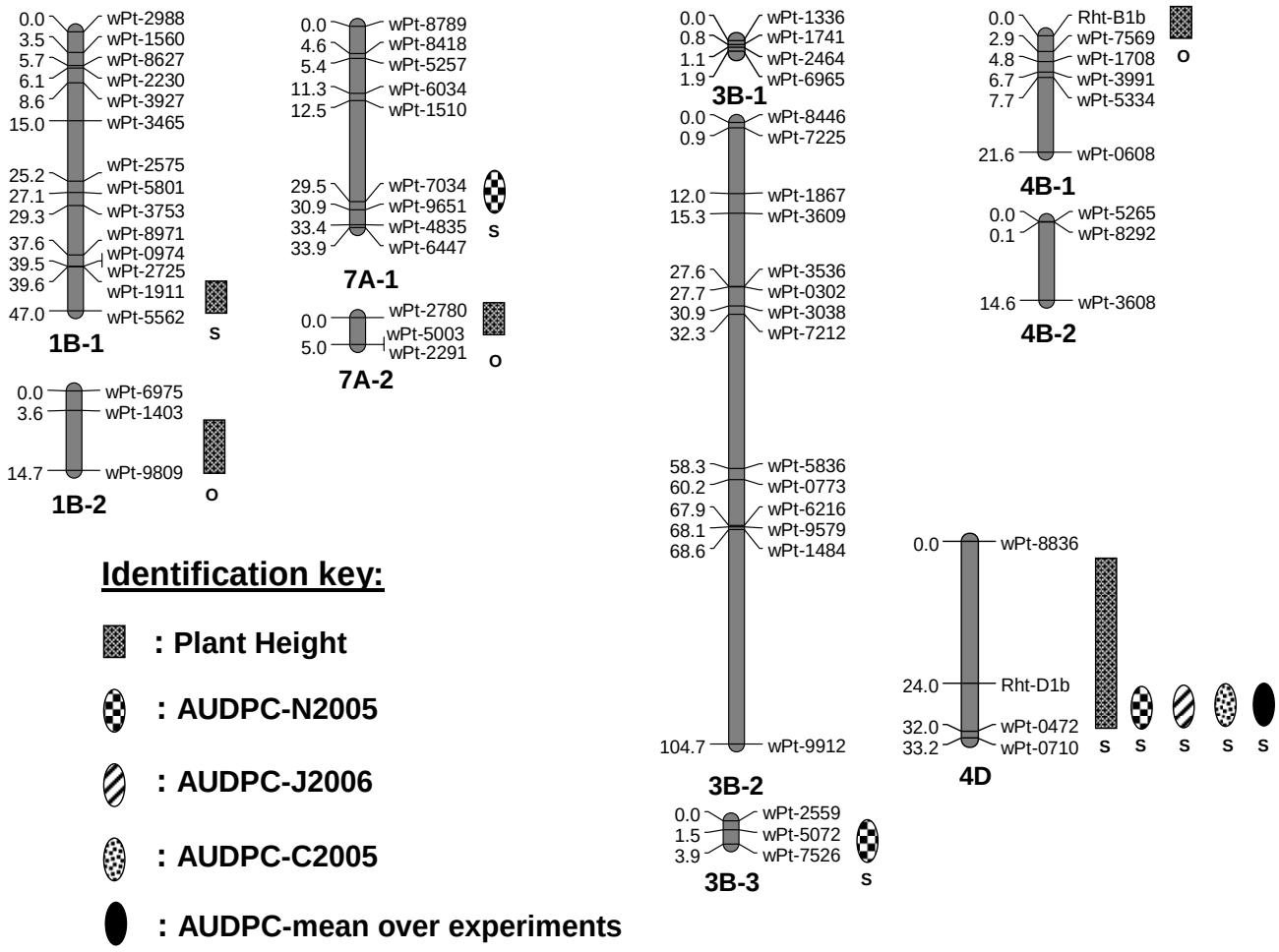

Figure 4.2 Linkage maps of Soissons/Orvantis DH population with QTL positions for FHB resistance and plant height. Genetic distances are shown in cM to the left of each linkage map. **S** and **O** refer to alleles contributed by Soissons and Orvantis, respectively. Only significant QTL have been indicated in the figure

In the point inoculation, similar to previous experiments, the Rht status was highly significant and the variety was also significant but no Rht by variety interaction was observed. Both *Rht-B1b* and Rht tall (P = 0.001, Table 4.7) and *Rht-B1b* and *Rht-D1b* (P<0.001) were found to be highly significantly different. The mean AUDPC of *Rht-B1b* (6.492679) was less than the Rht (tall) (8.194712) and *Rht-D1b* (8.784821).

Table 4.7 Analysis of variance and t-test results of FHB disease levels on *rht*, *Rht-B1b* and *Rht-D1b* near isogenic lines of winter wheat varieties Mercia and Maris Huntsman in a field trial and following spray and point inoculation in a polytunnel

Experiment	**Analysis of Variance**					**T-test**	
	Source of variation	**d.f.**	**m.s.**	**v.r.**	**F.pr.**	**Comparison**	**F.pr.**
Field trial	Variety	1	1680556	0.29	0.6	*Rht-B1b* with *rht*	0.109
Mercia and Huntsman	*Rht* status	2	4016667	6.95	0.01	*Rht-D1b* with *rht*	<0.001
	Rht x Variety	2	938889	1.62	0.237	*Between Rht-B1b* and *Rht-D1b*	0.18
	Error	12	5777778				
Spray inoculation	*Rht* status	2	108042823	4.08	0.019	*Rht-B1b* with *rht*	0.015
Mercia	Replicate	3	163413722	6.18	<0.001	*Rht-D1b* with *rht*	0.013
	Rht status x Block	6	47578640	1.8	0.104	*Between Rht-B1b* and *Rht-D1b*	0.853
	Error	134	26457077				
Spray inoculation	*Rht* status	2	575215	5.48	0.007	*Rht-B1b* with *rht*	0.005
Huntsman	Replicate	2	55077	0.52	0.595	*Rht-D1b* with *rht*	0.005
	Rht status x Block	4	97709	0.93	0.453	*Between Rht-B1b* and *Rht-D1b*	0.573
	Error	54	105030				
Point inoculation	Variety	1	49.77	7.45	0.007	*Rht-B1b* with *rht*	0.001
Mercia and Huntsman	*Rht* status	2	73.96	11.07	<0.001	*Rht-D1b* with *rht*	0.269
	Rht x Variety	2	5.17	0.77	0.463	*Between Rht-B1b* and *Rht-D1b*	0.001
	Error	154	6.68				

d.f., refers to degrees of freedom; m.s., refers to mean square, v.r., refers to variance ratio and F.pr., refers to F-probability.

4.3 RL4137/Timgalen Recombinant Inbred population

4.3.1 Results

4.3.1.1 Performance of RILs

The PH showed normal distribution and the parents RL4137 and Timgalen 97.97 and 162.33 cm, respectively and the population mean 121.1 cm (Table 4.8). A Few trangressive segregants towards RL4137 were evident. The population also showed similar trend for awns. Correlations between field and pot experiments for AUDPC was highly significant ($P<0.01$) and the correlation coefficient ranged from 0.5 (between JIC2000 and NIAB2006) to 0.9 (JIC1999 and JIC2000) (Table 4.9). The PH, awns and SW also showed a continuous distribution. The PH showed negative relationship with AUDPC and the correlation coefficient ranged -0.22 ($P = 173$ with JIC1999) to -0.47 ($P<0.001$ with NIAB2006). The awns also showed weak but non-significant negative relationship ($r = -0.13$ to -0.23) except with JIC2000 ($r = 0.12$, $P = 0.062$) (Table 4.9). Similarly, the spikelet weight showed significant negative relationship and the correlation coefficient ranged from -0.61 to -0.80 ($P = 0.001$ to <0.001). For AUDPC, the genotypes were always highly significant in all the three experiments ($P<0.001$) and blocks were not significant except in JIC2000 ($P<0.001$) (Table 4.10). However, significant block and block by genotype interactions were observed for PH, awns and WIS. The repeatability ranged from 0.68 (JIC1999) to 0.71 (JIC2000) and 0.58 (WIS) to 0.9 (PH) for AUDPC and FHB related traits, respectively (Table 4.10).

Table 4.8 Summary statistics for Fusarium head blight (FHB) and associated traits disease assessed in different trails

Experiment and trait recorded	RL4137	Timgalen	MPV	PM	Range
JIC1999-AUDPC	545.64	1161.08	853.36	835.9	50-1796.7
JIC2000-AUDPC	357.38	983.85	670.62	477.4	0-1540
NIAB2006-AUDPC	75.31	435.88	255.59	276.7	12-835
JIC2000-Spkilet weight	0.08	0.04	0.06	0.59	0.04-1.49
JIC2000-Awns	1	5	2.5	4.336	1-5
JIC2000-Plant height	162.3	97.7	130.0	121.1	73-170

AUDPC = area under disease progress curve, MPV = mid-parent value, PM = population mean
Awns were measured on 1 to 5 scale.

Table 4.9 Correlation coefficients of AUDPC and other FHB related traits in RL4137/Timgalen RIL population

Trait	J1999 AUDPC	J2000 AUDPC	N2006 AUDPC	Pooled AUDPC	J2000 PH	J2000 A	J2000 WIS
J1999-AUDPC	1						
J2000-AUDPC	0.70	1					
N2006-AUDPC	0.56	0.51	1				
Pooled-AUDPC	0.90	0.88	0.77	1			
J2000-PH	-0.22	-0.42	-0.47	-0.42	1		
J2000-A	-0.22	0.116	-0.27	-0.13	-0.46	1	
J2000-WIS	-0.68	-0.75	-0.61	-0.80	0.54	-0.06	1

J = JIC; N = NIAB; PH = plant height; A = awns; WIS = weight of infected spikelet

Table 4.10 Components of variation for AUDPC and other FHB associated traits in RL4137/Timgalen RIL population

Source of	FHB-AUDPC				FHB associated traits			
variation	m.s.	v.r.	F pr.		m.s.	v.r.	F pr.	
JIC1999					*Plant height-JIC2000*			
Blocks	239953	2.66	0.035		1402.96	36.28	<.001	
Genotypes	430371	4.77	<.001		3347.97	86.57	<.001	
Residual	90194				38.67			
h^2				0.7				0.94
JIC2000					*Awns-JIC2000*			
Blocks	819205	10.08	<.001		0.2808	1.53	0.218	
Genotype	458747	5.65	<.001		7.2132	39.25	<.001	
Residual	81256				0.1838			
h^2				0.71				0.92
NIAB2006					*Spikelet weight-J2000*			
Blocks	143	0.01	0.934		0.00224	4.48	0.012	
Genotypes	54885	2.69	0.007		0.00457	9.14	<.001	
Residual	20396				0.0005			
h^2				0.68				0.58

m.s. = mean square, v.r. = variance ratio, Fpr. = F probability, h^2 = repeatability, J = JIC, N = NIAB

4.3.1.2 Mapping of QTL for FHB resistance and associated traits

The genetic map based QTL analysis was performed in single experiment and across experiments on AUDPC values. The pooled average AUDPC from the three experiments was treated as another environment. The analysis identified a total of six

putative QTL for AUDPC on the chromosomes 1B, 2A, 2B, 3A, 6B and 7A (Table 4.11 and Fig. 4.3). The alleles from RL4137 contributed for all the QTL except for the QTL on 6B. Among these, three major QTL detected one each on chromosomes 2B, 3A and 6B were detected. Three QTL namely *Qfhs.jic-1b*, *Qfhs.jic-2b* and *Qfhs.jic-6b* were detected in more than one environment. The alleles for the QTL on 1B and 3B were contributed by RL4137 and that of QTL on 6B was contributed by the alleles from Timgalen. Both PH and awns detected a QTL on 2B with the closet AFLP marker S13/M23G. The alleles from RL4137 and Timgalen, respectively contributed for PH and awns QTL. SW detected one QTL each on 2B and 6A and the alleles from RL4137 contributed for two SW QTL one each on 2B and 6A. The QTL for PH, WIS and awns co-localized with a major FHB resistance QTL *Qfhs.jic-2b*. The alleles from RL4137 contributed for the FHB, PH and WIS QTL and the QTL for awns was contributed by the alleles from Timgalen.

Table 4.11 Summary of QTL identified for FHB resistance and plant height in RL4137/Timgalen RILs in different experiments

Trait	QTL	Closest marker	Position	Origin	JIC1999		JIC2000		NIAB2006		Over expt.	
					LOD	R^2	LOD	R^2	LOD	R^2	LOD	R^2
AUDPC	*Qfhs.jic-1b*	wPt-6425	51.3(1B)	RL4137	_	_	_	_	2.6'	9.9	2.4'	9.1
	Qfhs.jic-2b	wPt-5292	4.6(2B)	RL4137	_	_	_	_	4.8	21.5	_	_
	Qfhs.jic-2b	S24/M16I	6.2(2B)	RL4137	_	_	5.2	19.8	_	_	2.1'	8.4
	Qfhs.jic-3a	S22/M14F	48.1(3A)	RL4137	_	_	_	_	4.6	20.8	_	_
	Qfhs.jic-6a	wPt-9132	12.3(6A)	RL4137	3.4	25.8	_	_	_	_	_	_
	Qfhs.jic-6b	wPt-3376	16.6(6B)	Timgalen	_	_	_	_	_	_	2.4'	9.2
	Qfhs.jic-6b	wPt-4930	17.3(6B)	Timgalen	_	_	3.8	14	_	_	_	_
	Qfhs.jic-7a	wPt-3836	3.1(7A)	RL4137	_	_	2.1'	7.4	_	_	_	_
WIS	*Qfhs.jic-2b*	S13/M23G	5.5(2B)	RL4137	_	_	_	_	3.5	33.9	_	_
	Qfhs.jic-6a	wPt-9132	12.3(6A)	RL4137	_	_	_	_	4.2	40.4	_	_
DON-AUG	*Qfhs.jic-2b*	S24/M16I	6.2(2B)	RL4137	2.1'	23.3	_	_	_	_	_	_
	Qfhs.jic-7A	wPt-6273	3.3(7A)	RL4137	2.3'	20.3	_	_	_	_	_	_
Plant height		S13/M23G	5.5(2B)	RL4137	_	_	15.9	47.9	_	_	_	_
		wPt-7524	3.4(4A)	Timgalen	_	_	2.9	5.4	_	_	_	_
		wPt-9454	57.8(5B)	RL4137	_	_	2.7'	5.9	_	_	_	_
Awns		S13/M23G	5.5(2B)	Timgalen	_	_	3.4	14.6	_	_	_	_

R^2 = Percentage phenotypic variance explained, ' = LOD below the permutation test threshold for significance
WIS = weight of infected spikelet, AUDPC = area under disease progress curve, DON = deoxynivalenol, AUG = area under growth

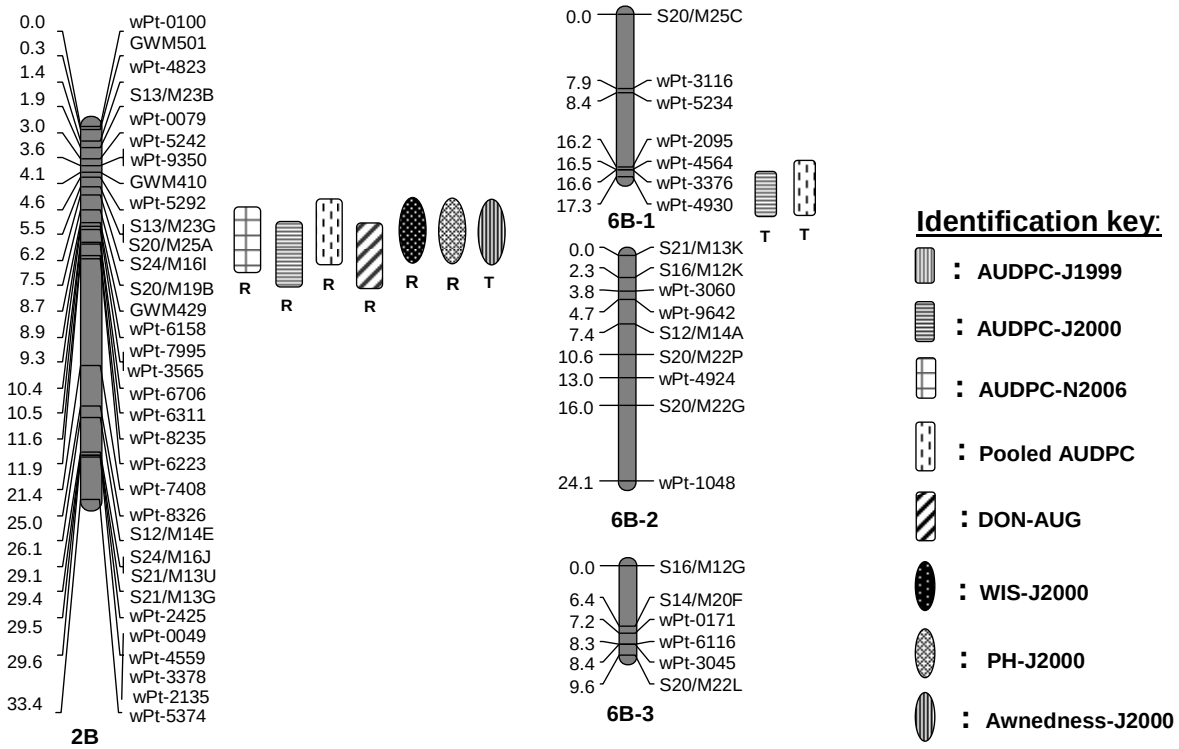

Figure 4.3 Putative QTL positions identified in RL4137/Timgalen for the major consistent FHB resistance along with the co-localized QTL for the DON-tolerance (effect of DON on seed germination and growth, DON-AUG), plant height (PH), weight of infected spikelet (WIS) and awns are shown on the right of each linkage map. J and N refer to JIC and NIAB trail sites, respectively. Genetic distances are shown in cM to the left of each linkage map. **R** and **T** refer to alleles contributed by RL4137 and Timgalen, respectively.

4.4 Discussion

4.4.1 FHB resistance QTL in Spark and the role of *Rht-D1b* semi-dwarfing allele in FHB susceptibility

These studies have clearly demonstrated that the *Rht-D1b* semi-dwarfing allele is associated with increased susceptibility to FHB. Furthermore, we have shown that the effect relates to a decrease in Type I resistance with no apparent effect on Type II resistance. Numerous authors have reported an association between plant height and resistance to FHB (Mesterhazy, 1995; Somers et al, 2003; Klahr et al, 2007). It has been proposed that in natural FHB epidemics, the smaller distance between the soil and the first leaf and between the flag leaf and the head in short genotypes may

result in greater exposure to primary inoculum where it is splash dispersed from the soil or stem-base as suggested by (Jenkinson & Parry, 1994). Even with direct spray and the application of mist irrigation the influence of plant height on susceptibility has been observed (Buerstmayr, et al, 2000; Gervais et al, 2003) supporting the hypothesis that the microclimate about the head is more conducive to infection in shorter varieties (Klahr et al, 2007). The importance of the negative correlation between FHB and plant height in field trials has been found to vary in magnitude, depending upon the population under study. Somers et al. (2003) observed a very significant negative correlation (r=-0.65) in progeny from a cross between Wuhan-1 and Maringa whereas Klahr et al (2007) observed an effect in only two of four environments (r=-0.27 and -0.32).

Coincident QTL for FHB resistance traits and plant height have been reported in several studies (Gervais et al, 2003; Somers et al, 2003; Paillard et al, 2004; Steiner et al, 2004). It has been suggested that taller lines may escape from infection by having heads further from the soil and that the microclimate about the ear may differ in short and tall varieties (Somers et al, 2003). However, several studies indicate that the relationship between FHB resistance and plant height may be more complex. Somers et al (2003) determined that a QTL on 2DS influencing the accumulation of DON in grain was coincident with a QTL for plant height although this QTL had no apparent effect on disease symptoms. However, a second QTL for DON accumulation (5A) and a QTL for FHB symptoms (4B) were not associated with PH. In a cross between Renan and Recital QTL for FHB and PH were coincident on 5A whereas the PH QTL on 4A was not associated with differences in resistance to FHB (Gervais et al, 2003). Steiner et al (2004) found coincident QTL for FHB and PH in a similar position on 5A derived from Frontana. Paillard and coworkers also found that while some PH QTL overlapped with those for FHB (e.g. 5B) the main PH QTL (2AL and 5AL) in their population from a cross between Arina and Forno were not coincident with FHB QTL (Paillard et al, 2004). A FHB and PH QTL on 6A was found to be coincident in a cross between Dream and Lynx, although other FHB and PH QTL were independent in this cross (Schmolke et al, 2005). These authors concluded that the coincidence of FHB and PH QTL following spray inoculation suggested that it has a genetic basis- either linkage or pleiotropy rather than being due to escape. While PH may be correlated with resistance to FHB, the finding that some QTL for PH coincide with those for FHB, while others do not, supports this view.

The negative effect of the semi-dwarfing alleles *Rht-B1b* and *Rht-D1b* (formerly termed *Rht1* and *Rht2*, respectively) on resistance to FHB has been observed or

inferred in previous studies. Hilton et al (1999) observed that, in crosses between varieties carrying these alleles (semi-dwarf) with those carrying the wild-type alleles (tall) there was a clear tendency for tall strawed lines to appear more resistant than short strawed lines. These authors also spray-inoculated near-isogenic lines differing in *Rht-B1* and *Rht-D1* alleles with a mixture of *Fusarium* species and *M. nivale.* In most cases lines carrying either the *Rht-B1b* or *Rht-D1b* alleles were more susceptible than lines carrying the wild-type alleles (Hilton et al, 1999). Relative humidity at ear height was not found to differ between tall and short isogenic lines and it was concluded that the effect on FHB susceptibility was not due to higher humidity at ear height in the shorter genotypes.

The negative effect associated with the *Rht-D1b* allele carried by Rialto has also been observed in Riband, another UK variety (Draeger et al, 2007). As in the present study Draeger and colleagues (2007) showed that the main FHB QTL segregating among progeny from a cross between Arina and Riband co-localised with the *Rht-D1* locus. These authors concluded that the effect is not due to plant height per se but rather to plieotropy or linkage. It was also noted that the great majority of UK wheat varieties carry the *Rht-D1b* allele and that they are generally highly susceptible to FHB (Gosman et al, 2007). In combination with the present work these studies indicate that *Rht-D1b* (*Rht2*) is associated with reduced resistance to FHB but less is known of the effect of *Rht-B1b* (*Rht1*) on resistance. Hilton et al (1999) found no evidence for differences in the effect of the *Rht-B1b* and *Rht-D1b* alleles. However, in a separate study of a population derived from a cross between Frontana and Remus a FHB QTL identified on 4B coincident with a PH QTL thought to correspond to *Rht-B1b* accounted for only 7.4% of phenotypic variance (Steiner et al, 2004). Further study is required to determine whether the *Rht-B1b* and *Rht-D1b* alleles differ in their influence on susceptibility to FHB. It is perhaps significant that the semi-dwarfing alleles of the *Rht-B1* and *Rht-D1* alleles carried on separate chromosomes (4B and 4D, respectively) are both associated with increased susceptibility to FHB, suggesting that the effect may be due to pleiotropy rather than linkage to deleterious genes.

The results from different types of inoculation procedure in the present study clearly demonstrate that the negative effect of the *Rht-D1b* allele on FHB resistance acts on resistance to initial infection (Type 1 resistance) (Schroeder & Christensen, 1963) with little, if any effect on spread within the head (Type II resistance). Other studies also found that the relationship between resistance to FHB and PH was evident in field trials measuring Type I resistance (incidence) or a combination of Type I and Type II resistance (severity) but not where point inoculation was used or spread

within the head was measured (Somers et al, 2003; Steiner et al, 2004). It remains to be determined how the *Rht-B1b* and *Rht-D1b* alleles might alter susceptibility to infection (Type I resistance) and this is the subject of ongoing research.

In addition to the *Qfhs.jic-4d.1* coincident QTL for FHB resistance and PH, two other chromosomes carried QTL for both FHB resistance and PH. On 2A, however, the allele from Spark contributed the FHB resistance while that for PH was contributed by Rialto. Additionally, although, QTL for FHB and PH were located on 6A, they did not overlap.

Two major QTL conferring resistance to FHB were detected on 4D and 6A insinuating regions for candidate genes/QTL for novel sources for FHB. Our study also provides molecular markers for MAS and gives access to a previously uncharacterized source of FHB resistance. Hitherto, the successful application of MAS needs QTL validation in other genetic backgrounds and germplasms.

In the present study, ten putative FHB QTL were observed to segregate within the Spark x Rialto population. These were on chromosomes 1B, 2A, 3A, 4D, 5A, 6A and 7A. The total variance explained for AUDPC by all the QTL across environments varied being greatest at JIC (76.6%). A total of three major ($R^2 \geq 10\%$) and seven minor ($R^2 < 10\%$) QTL were mapped. Of these, the alleles from Spark and Rialto contributed resistance for eight and two QTL, respectively. The contribution to FHB resistance of alleles from both the parents is not uncommon and previous studies of FHB resistance in winter wheat have indicated that resistance is controlled by several loci on different chromosomes, each with only a weak or moderate effect (Paillard et al, 2004; Schmolke et al, 2005; Draeger et al, 2007). This is in contrast to spring wheat where several FHB resistance genes of major effect have been identified (Cuthbert et al, 2006; Cuthbert et al, 2007). An exception appears to be *Qfhs.jic4.1* associated with the *Rht-D1* locus in winter wheat which explained up to 53% of phenotypic variance in the Spark x Rialto population used here and up to 24% in the Arina x Riband population (Draeger et al, 2007).

Other than *Qfhs.jic-4d.1,* two relatively stable QTL of moderate effect were also observed to segregate in the Spark x Rialto population. *Qfhs.jic-6a.2* on 6A (R^2 = 7.8 to 10.5%) and *Qfhs.jic-3a.2* on 3A (R^2 = 7.6 to 8%), both originating from Spark. *Qfhs.jic-6a.2* appears to be in a similar location to a QTL identified in the FHB resistant winter wheat variety Dream (Schmolke et al, 2005). These authors reported that the FHB QTL from Dream partially overlapped with a PH QTL and, interestingly, Spark also possessed a PH QTL near *Qfhs.jic-6a.2* but distinct from this locus. The *Qfhs.jic-3a.2* from Spark is located in a similar position on chromosome 3A to FHB

QTL reported from the spring wheat variety Frontana (Steiner et al, 2004) and Fundulea 201R, a winter wheat line (Shen et al, 2003). Further work is required to establish the relationship between these three FHB QTL.

Rialto carries the 1BL/1RS wheat-rye translocation (Snape et al, 2007). Several studies have found that this translocation is associated with Type II resistance to FHB (Buerstmayr et al, 2002; Shen et al, 2003; Schmolke et al, 2005). In the present study a QTL of major effect associated with chromosome 1B from Rialto was observed at a single location (NIAB2005). Whereas the QTL in the CM-82036-derived population was also only detected in some trials (Buerstmayr et al, 2002) that from Fundulea 201R was consistently expressed across environments (Shen et al, 2003). It may be that expression of the FHB QTL associated with the 1BL/1RS wheat-rye translocation is influenced by the genetic background in which it is present.

Spark has previously been shown to be one of the most resistant UK winter wheat varieties (Gosman et al, 2007). The present work has demonstrated that most of this effect may be due to the presence of the wild-type (tall) allele at the *Rht-D1* locus in this variety in contrast to the semi-dwarfing allele (*Rht-D1b*) that is present on most UK varieties. The *Rht-D1b* allele appears to confer enhanced susceptibility to FHB by reducing the level of Type I resistance. This allele however, has little or no effect on Type II resistance. Additional FHB QTL of lesser effect are also present in Spark (3A and 6A) but additional work is required to establish their value in breeding programmes.

4.4.2 FHB resistance QTL identified in Soissons and the role of *Rht-B1b* and *Rht-D1b* semi-dwarfing alleles in resistance

The DH population of Soissons (*Rht-D1b*)/Orvantis (*Rht-B1b*) was phenotyped for FHB resistance in the field at NIAB2005, CSL2005 and JIC2006. The genotypes were always significant both in individual environments and mean over environments. FHB traits showed moderate to high levels of broad sense heritability. A total of five QTL regions on 1BL, 3BL, 4BS, 4DS and 7AL appear to be involved in increased resistance to FHB. The alleles from Soissons contributed for all the QTL except for a minor QTL on 3BL. Despite variation in the levels of correlation for AUDPC between environments, a major QTL on 4DS was consistently detected in all the environments with an explained variance of 6.1 to 18.4 %. In addition to this, two minor QTL one each on 3BL and 7AL were also detected. Similar to FHB trait, the PH also detected four QTL and the loci *Rht-B1b* on 4B and *Rht-D1b* on 4D were the major height affecting QTL in this study.

In wheat-Fusarium interaction, because of passive resistance, often plants either completely escape from infection or show fewer FHB symptoms. Thus resistance breeding may be hindered by the association between FHB resistance with other important agronomic traits such as the presence or absence of awns, flowering time and plant height. It is therefore, important to consider pleiotropic effects of such traits on FHB infection. Generally taller and early heading genotypes show fewer FHB symptoms (Miedaner, 1997; Hilton et al, 1999; Steiner et al, 2004) and significant negative association between PH and FHB resistance and/associated traits such as accumulation of DON in grains has also been reported (Mesterhazy, 1995; Somers et al, 2003; Draeger et al, 2007; Klahr et al, 2007 and Srinivasachary et al, 2008). These studies are often supported by the co-localization of QTL for these traits (Gervais et al, 2003; Paillard et al, 2004; Steiner et al, 2004; Schmolke et al, 2005; Draeger et al, 2007; McCartney et al, 2007 and Srinivasachary et al, 2008). Similar to the findings in Arina by Draeger et al (2007) in Arina/Riband cross and Srinivasachary et al (2008) in Rialto in Spark/Rialto cross containing *Rht-D1b*, in the current study also a major PH-QTL co-localized with a major FHB-QTL on 4DS. In addition to this, *Rht-B1b* also co-localized with a minor effect FHB QTL on 4B. The long arm of 7A comprised of a major and a minor LG. A minor PH QTL contributed by the alleles from Soissons and a PH-QTL contributed by Orvantis was mapped on to two independent LGs.

Rht-D1b (Rht2), as in previous studies by Hilton et al (1999), Draeger et al (2007) and Srinivasachary et al (2008) using the isogenic lines of Rht-D1 have showed the detrimental effect on FHB resistance and but this effect is due to more than just height, shown by comparison with second semi-dwarfing allele. When a series of experiments on isogenic lines of Rht-B1 and Rht-D1 by spray (Type I and Type II) and point inoculations (Type II), it was clear that both alleles compromise for Type I resistance but *Rht-B1b* differs from *Rht-D1b* by showing positive effect for Type II resistance.

In a cross between 98B69-L47 (carries *Rht-B1b*) and HC374, McCartney et al (2007) observed a strong association of a major FHB QTL with plant height as FHB QTL was in the region of Rht-B1 locus. Wuhan-1, which as responsible for 4B resistance was associated with increase in plant height. However they could not study further if this is due is linkage of Rht-B1 and 4B FHB resistance QTL or pleiotrophic effect of Rht1-B1 on FHB resistance. Similar to our studies, Jia et al (2006) along with Lin et al (2006) and Lie et al (2007) have also reported a major effect QTL on 4B from different spring wheats.

Some of the QTL detected in this population have also been reported by several workers in different populations. The studies involving either Sumai 3 or its derivatives have reported a major QTL on 3BS (Anderson et al, 2001; Bai et al, 1999; Zhou et al, 2002; Buerstmayr et al, 2002; Somers et al, 2003; Jia et al, 2005; Mardi et al, 2005; Shen et al, 2002; Ma et al, 2006). Lie et al (2007) have also reported a FHB-QTL in the centromeric region on 3B. In this study, a minor FHB-QTL was mapped to the long arm of 3B. Paillard et al (2004) have also mapped a minor QTL on 3BL in Arina (resistant) / Forno (susceptible). Similarly, Gervais et al (2003) also reported a minor FHB QTL on 3BL. More recently, Klahr and Zimmermann (2007) have also reported QTL on 3B in winter wheat Cansas, moderately resistant winter wheat. The findings from this study, together the studies of Gervais et al (2003) and Paillard et al (2004), insinuate the involvement of different genomic regions for FHB resistance in winter wheat compared to spring wheat cultivar Sumai 3 and its derivatives. Our study also detected a minor FHB QTL on 7AL at NIAB2005. Similar to our studies, Jia et al (2005), Mardi et al (2006), Semagn et al (2007) and Zhang and Mergoum (2007) also reported a minor QTL on 7AL in Spring wheats Wangshuibai, Frontana, NK93604 and Stoa, respectively. In the current study, when FHB resistance was measured in terms of incidence (Type I- resistance to initial infection), severity (Type II –resistance to the spread of fungus within the spikelet) and disease index (Type I and Type II) in C2005 and used in QTL analysis, it was clear that incidence and severity may be useful to identify the QTL specifically expressed for Type I and Type II resistance, respectively while disease index could be useful to detect QTL for field resistance.

In summary, five FHB resistant QTL were identified in this study and two of these QTL co-localized with semi-dwarfing alleles, *Rht-D1b* and *Rht-B1b*. A series of experiments on the isogenic lines showed that compared to *Rht-D1b, Rht-B1b* has no negative effect on resistance under field conditions (low disease pressure) w. Further, *Rht-B1b* has a positive effect with regards to Type II resistance compared to *Rht-D1b* allele. It is therefore, *Rht-B1b* is the better dwarfing allele to use in breeding programmes as it has no detrimental effects on FHB resistance under field conditions.

4.4.3 FHB resistance QTL detected in RL4137/Timgalen cross

4.4.3.1 Disease assessment and QTL mapping for FHB resistance

RL4137 is Canadian awnless, red-grained spring wheat which is highly resistant to FHB. It is known to have derived from a Brazilian cultivar Frontana, a widely used FHB resistant source in breeding programmes after Sumai 3. The classical genetic studies

have shown the presence of a minimum of two or three additive genes in Frontana (Singh et al, 1995; VanGinkel et al, 1996). Timgalen is white-grained Australian spring wheat and is moderately resistant to FHB. It carries an introgressed region on 2B from *Triticum timopheevi* (AAGG) (Devos et al, 1993). RILs of a cross between RL4137 and Timgalen were screened for FHB resistance in two polytunnel and a field experiment using Fu42, a DON-producing isolate of *F. grameniarum*. Generally, distribution of AUDPC was continuous and was marginally skewed towards RL4137. Few transgressive segregants towards RL4137 were observed. The correlation for AUDPC between experiments was highly significant. Similar to AUDPC, FHB related traits PH, awns and SW also showed continuous distributions. PH and SW showed a strong significant negative relationship with AUDPC. The linkage map was constructed using 341 loci majority of which were DArT (236) and AFLP (90) which organized into 20 major and 24 minor groups. The map based QTL analysis detected consistent QTL on 1B (*Qfhs.jic-1b*), 2B (*Qfhs.jic-2b*) and 6B (*Qfhs.jic-6b*). The study also indentified three QTL for FHB resistance which were detected only once either in the polytunnel or field experiment. The alleles from RL4137 contributed for most of the QTL identified in this study. The study also identified a positive effect QTL on 6B coming from Timgalen which is moderately resistant to FHB. It is not uncommon that a moderately resistant or susceptible parent contributing alleles for resistance when used as one of the parent with a resistant line. The transgressive segregation of FHB resistance occurs when wheat with varying levels resistance to FHB are crossed. For example, Waldron et al (1999) reported a QTL for FHB resistance from a moderately susceptible parent Stoa, similarly Alondra, a FHB susceptible parent contributed the favourable allele (Shen et al, 2003).

Poor representation of the D genome in the map and non-representation of 5A chromosomes in the map may have had some effect on our ability to identify QTL for FHB resistance and associated traits. Further, majority of the makers used in the study were DArTs and AFLPs and thus, we were only able to assign the LG to wheat chromosomes and further extrapolation of QTL positions on the chromosome arms was not possible.

4.4.3.2 QTL detection for other traits and their role in FHB resistance

Studying the relationship between FHB resistance and other important agronomic traits is crucial for the development of resistant cultivars. The breeding programmes can be seriously affected if FHB resistance is linked to undesirable traits. However, some of the morphological traits associated with FHB resistance potentially limit the

number of spores reaching the infection sites on heads and/minimise the chances of spores gaining entry into head tissues (Steiner et al, 2004). Often association of traits such as PH, awns, spikelet number, flowering time and other agronomic traits with FHB resistance have been studied by several workers (Ban and Suenaga, 2000; Buerstmayr et al, 2000; Draeger et al, 2007; Hilton et al, 1999; Jiang et al, 2006; Liu et al, 2007; Mesterhazy, 1995; Srinivasachary et al, 2008). In the current study, the possible role of PH, awns and SW with FHB resistance were investigated.

The PH has been considered as an escape mechanism. Generally, it has been shown that taller plants alter the micro-climate by having heads further from the soil creating less favourable conditions for FHB infection and symptom development (Klahr et al, 2007; Somers et al, 2003; Hilton et al, 1999; Miedaner, 1997; Steiner et al, 2004). In the current study, PH showed significant negative correlation with AUDPC which could be due to more favourable microclimate for Fusarium infection in short genotypes. QTL mapping detected a major PH QTL which co-localized with a major FHB resistant QTL on 2B and the alleles from RL4137 contributed a positive effect for both. This is in agreement with co-incident QTL for FHB resistance and PH reported in several studies (Gervais et al, 2003; Paillard et al, 2004; Somers et al, 2003; Steiner et al, 2004). However, some of the studies have also revealed a more complex relationship between FHB resistance and PH (Draeger et al, 2007; Gervais et al, 2003; Paillard et al, 2004; Schmolke et al, 2005; Somers et al, 2003; Srinivasachary et al, 2008). These studies suggested that co-incidence of QTL for FHB resistance and PH has a genetic basis, linkage or pleiotropy, rather than being due to escape. The study also identified two minor PH QTL which did not co-localize with any of the FHB resistance QTL identified in this population. While PH may be correlated with resistance to FHB, the finding that some QTL for PH coincide with those for FHB, while others do not, supports this view.

The precise role of awns with FHB resistance in not clear and the literature is often contradictory. Tamburyic-llincic et al (2007) showed that awned genotypes showed lower FHB index than awnless genotypes and similarly Ban and Suenaga (2000) reported that fully awned genotypes were more resistant than tip-awned genotypes. Further, Snijiders (1990) reported that the association of awns with FHB are genetically linked and suggested that awns could be used as a marker to select the resistant lines in the progenies if the resistant parent involved carry awns. However, Buerstmayr et al (2002) mapped a QTL for FHB resistance on 5A in a Sumai 3 derived line CM-82036 that was not associated with the presence or absence of awns. Similarly, the studies on Ernie by Liu et al (2007) showed that the presence of

awns was not associated with FHB resistance. In the current study, a major QTL for awns contributed by the alleles from Timgalen on 2B co-localized with a major FHB resistant QTL contributed by the alleles from RL4137. Most of the variation in the population for SW was explained by two QTL (2B and 6A) contributed by the alleles from RL4137 and one of which on 2B co-localized with a major FHB QTL. Co-localization of QTL for FHB resistance and spike architecture have been reported eg. in wheat (Draeger et al, 2007) and barley (Ma et al, 2000; Zhu et al, 1999).

As a measure of FHB resistance/tolerance to DON toxin, a trichothecene produced by Fusarium species was tested on the germination and the growth of wheat seeds as described in Lemmarks et al (1994) and Gosman et al (2005). The degree of germination retardation was expressed as the area under disease progress curve (AUGRC) calculated from seven measurements over ten days. The AUGRC of seed exposed to DON was expressed as a percentage of the AUGRC of controls. QTL analysis identified two minor insignificant QTL one each on 2B and 7A with the closest markers S24/M16I and wPt-6273, respectively which co-localized with the FHB QTL and both the QTL were contributed by the alleles from RL4137. Moderate correlation between visual disease scores, DON accumulation in the kernels and *in vitro* DON tolerance was reported by Lemmens et al (1994; 1997). Further, the studies by Wang and Miller (1988) reported that FHB resistant cultivars were more tolerant to DON than those of FHB susceptible cultivars, however, the experiments by Bruins et al (1993) and Snijders (1990b) provided an evidence for the lack of link between FHB resistance and DON tolerance.

The current study identified two major consistent QTL one each on 2B and 6A, contributed by the alleles from RL4137 and Timgalen, respectively. The QTL for the FHB related traits PH, SW and awns co-localized with the FHB resistant QTL on 2B. Co-localization of QTL may be due to linkage or pleiotropy and it has been hypothesized that the developmental architecture traits determine QTL for FHB resistance (Zhu et al, 1999). At the level of resolution afforded by this mapping population, whether the co-localization is due to linkage or pleiotropy could not be distinguished. Therefore, more studies are necessary to determine whether it is due to linkage or pleiotropy before attempting to introgress resistance alleles. However, 2B chromosome of Timgalen carries a large DNA segment from *T. timopheevi* (Devos et al, 1993) which makes it less amenable for recombination in that region for precise mapping of QTL in this region. Hitherto, another mapping population involving RL4137 as one of the parent might be ideal to define the precise position of FHB QTL on 2B.

The study provides a starting point for manipulating RL4137 derived resistance in wheat.

5 Identification of new sources of resistance from CIMMYT wheat lines

5.1 Background

Fusarium head blight (FHB) is a destructive disease of wheat worldwide (Parry et al, 1995; Waldron et al, 1999). The predominant causal agents *Fusarium graminearum* and *F. culmorum,* both reduce yield and can contaminate grain with mycotoxins that render it unsuitable for human and livestock consumption (Gilbert and Tekauz, 2000). Thus, FHB can be major threat for producers, processors and consumers of wheat. Agronomic practices and fungicides are not fully effective in controlling the disease and thus breeding of resistant cultivars has been the strategy adopted to minimize yield and grain quality losses (Shen et al, 2004).

Resistance of wheat to FHB appears to be horizontal and non-species specific with no clear evidence for host by pathogen species interaction (van Eeuwijk et al, 1995). Several components of resistance to FHB have been proposed, of which two have been commonly accepted, Type 1 and Type 2 (Schroeder and Christensen 1963). Resistance to initial infection (Type 1) is assessed as disease incidence following natural infection or inoculation by spraying heads at mid-anthesis with conidia (Miedaner et al, 2003). Resistance to spread within the head (Type 2) is assessed by injection of inoculum into single florets within the head.

Use of point and spray inoculation in conjunction with molecular mapping has identified several major quantitative trait loci (QTL) conditioning predominantly Type II resistance (Anderson et al, 2001; Buestmayr et al, 2002; Shen et al, 2003a, 2003b) but only a few studies have identified QTL for Type I resistance (Buerstmayr et al, 2002; Steiner et al, 2004; Steed et al, 2005). This may reflect a paucity of Type I resistance in the germplasm under study, but it is also probable that the need to infer Type I resistance is hampering the identification of this form of resistance. If species, or isolates, that produce little or no toxin can infect but not spread within the spike they might be used as tools to identify Type I resistance. The aim of the current study was to 1) indentify a method for reliable and easy identification of Type 1 resistance 2) identify and characterise potential sources of FHB resistance among a collection of winter wheat lines obtained from the CIMMYT, Mexico.

5.2 Results

5.2.1 *Experiment 1*: Characterisation of FHB resistance in wheat varieties inoculated with toxin producing and non-producing species in the glasshouse

Thirty Europen winter wheat varieties were inoculated in the glasshouse with either a single DON-producing isolate of *F. culmorum* or an isolate of the toxin non-producing species *M. majus* and assessed for resistance to FHB. Spray inoculation was used to estimate the level of combined Type I plus Type II resistance and point inoculation was used to detect Type II resistance alone. Point inoculation experiment was repeated in 2005.

5.2.1.1 Spray inoculation

Although the average disease severity caused by *F. culmorum* (51.5%) was significantly ($P < 0.001$) higher than that caused by *M. majus* (14.8%) the coefficient of correlation for the relationship between pathogens was moderate (0.52, $P = 0.003$). For inoculations with *F. culmorum* the most resistant variety was Spark (31.79% (The low score for Orvantis (25.67%) was due to late inoculation of this variety), and the most susceptible was Riband (79.4%), however for inoculations with *M. majus*, the most resistant variety was Grief (4.39%) and the most susceptible was Riband (30.88%) (Table 5.1).

5.2.1.2 Point inoculation

Average disease severity (number of damaged spikelets per head) caused by *F. culmorum* in 2004 ranged from 12.17 (Goodwood) to 0.70 (Dekan) with an average of 6.74. *M. majus* was, however, generally unable to spread beyond the spikelet into which it was inoculated (Table 5.1). *F. culmorum* produced similar levels of disease when the point experiment was repeated in 2005, ranging from 11.22 (Riband) to 1.54 (Claire) with an average of 5.80, but *M. majus* was, once again, unable to spread beyond the inoculated spikelet (Table 3). Coefficients of correlation for the relationship between spray and point inoculation with *F. culmorum* in 2004 were moderate (0.48, $P = 0.006$), but low between point inoculations over years (0.37, $P = 0.041$). However, for *M. majus*, the relationship between point and spray inoculation in 2004 and between point inoculations over years was non-significant ($P > 0.05$).

Table 5.1 Average visual disease scores under controlled environment conditions (glasshouse) for a collection of winter wheat varieties after spray (21 days post inoculation (dpi) and point inoculation (14 dpi) with a deoxynivalenol (DON) producing isolate of *F. culmorum* and the toxin non-producing species *M. majus* in (2004) and (2005)

Variety	*F. culmorum*			*M. majus*		
	Spray 2004	Point 2004	Point 2005	Spray 2004	Point 2004	Point 2005
A43-02	56.36	8	5.6	11.51	0.91	0.25
Batis	38.15	4.04	4.96	13.36	1.06	0.35
Bentley	51.12	5.3	10.58	16.98	0.89	0.32
Biscay	54.41	7.54	4.42	15.53	0.84	0.46
Centrum	48.04	5.09	5.36	11.69	0.86	0.13
Charger	63.97	5.75	8.38	20.49	1.06	0.87
Claire	40.07	7.75	1.54	11.5	0.86	1.17
Consort	76.51	7.96	2.46	26.02	0.96	1.18
Dekan	50.22	0.7	5.34	5.28	0.8	0.5
Einstein	59.87	9.15	9.41	14.62	1.03	0.48
Goodwoo	45.73	12.17	8.49	15.28	0.95	0.41
Grief	45.23	4.67	6.58	4.39	0.74	0.07
Istabraq	41.8	5.88	3.32	10.27	0.96	0.36
Napier	46.32	4.86	3.83	11.11	0.8	0.84
Nirvana	46.45	3.52	4.2	4.61	1.17	0.49
Nijinsky	42.79	5.67	1.89	12.01	1.01	1.01
Orvantis	25.67	8.39	7.42	11.27	1.26	0.68
Quest	38.08	4.1	7.16	9.65	0.93	0.67
Renan	48.04	7.1	2.42	6.4	1.05	0.28
Riband	79.4	9.83	11.22	30.88	0.86	1.32
Richmond	43.43	3.98	4.5	11	1.05	0.53
Robigus	55.43	9.67	4.25	16.72	1.03	0.83
Savannah	59.9	7.81	9.16	24.55	0.96	0.32
Scorpion	78.74	10.19	9.45	26.75	1.13	0.4
Smuggler	39.39	2.03	5.08	12.08	0.86	0.26
Spark	31.79	2.9	1.75	4.56	1.11	0.67
Tanker	64.76	6.2	9.26	16.73	0.95	0.86
Warlock	74.29	11.08	7.37	19.76	1	0.4
Winnetou	40.79	6.23	5.42	7.22	0.99	0.23
Wizard	46.59	10.01	8.76	21.97	1.01	0.93

5.2.2 Characterisation of resistance

The contrasting spreading capabilities of *F. culmorum* (able to spread) and *M. majus* (unable to spread) and method of inoculation were used to infer the presence of Type 1 and/or Type II resistance in the wheat varieties according to their reaction to each pathogen, e.g. *M. majus* spray (Type I only), *F. culmorum* point (Type II only) and *F. culmorum* spray (Type I + II) (Table 5.1). For example, Spark was shown to possess both Type I and Type II resistance as revealed by its response to *M. majus* spray (4.56) and *F. culmorum* point (2.3 (mean over 2004 and 2005) inoculation. It had a high level of overall resistance (Type I+II), being resistant (31.79) to *F. culmorum* spray. Similarly, Dekan was resistant to *M. majus* spray (5.28) and *F. culmorum* point

(3.1) inoculation indicating that it possessed both Type I and Type II resistance. However, its overall resistance was only moderate as shown by its response to *F. culmorum* spray inoculation (50.22). Grief (5.6) and Renan (4.8) appeared less resistant to the *F. culmorum* point inoculation than to the *M. majus* spray inoculation (4.39 and 6.4) and were assumed to possess predominantly Type I resistance. Varieties like Batis (4.5), Napier (4.3) and Istabraq (4.6) were relatively more resistant to *F. culmorum* point inoculation than to *M. majus* spray (13.36, 11.11 and 10.27) inoculation and were assumed to possess predominantly Type II resistance. The combined resistance of these varieties was however, only moderate (*F. culmorum* spray) with the exception of Batis (38.15).

5.2.3. Spray and point inoculation of Mercia winter wheat with DON and NIV producing isolates of *F. graminearum*

In 2005, Mercia variety was spray or point inoculated with either a DON (UK1) or NIV (F86) producing isolate of *F. graminearum* in two separate experiments with different spore titres. Over all, both in low (5 ml per spike of 1×10^4 ml^{-1} for spray and 10 µl of 1×10^5 ml^{-1} for point) and high titre (5 ml per spike of 1×10^5 ml^{-1} for spray and 10 µl of 1×10^6 ml^{-1} for point) experiments, it was evident that both DON and NIV-producing isolates produced similar disease levels in point and spray experiments (Fig. 5.1). In the spray inoculation experiment, the DON and NIV isolates were able to infect and cause similar disease levels. In the point inoculation experiment, the DON-producing isolate was able to infect and spread rapidly to other spikelets within the ear, contrastingly, the NIV-producing isolate was largely restricted to the inoculated spikelet until 18dpi in both low and high titre experiments indicating the inability of NIV-producing isolate to spread beyond the point of inoculation.

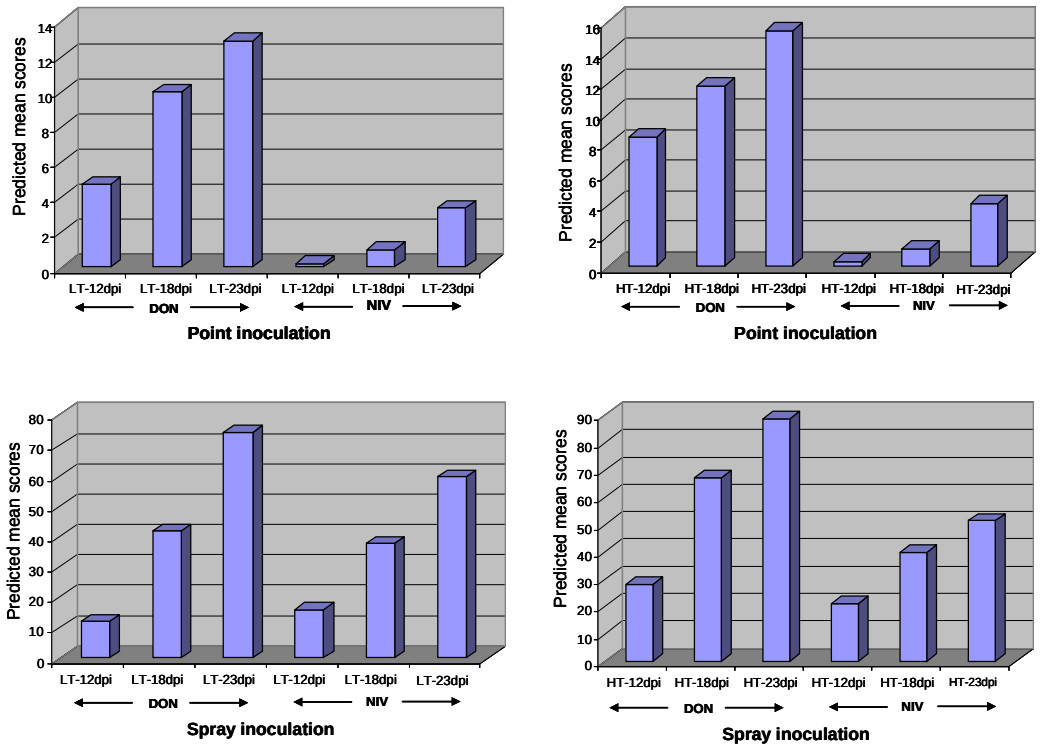

Fig. 5.1 Predicted mean scores on Mercia following spray or point inoculation with *F. graminearum* of different chemotypes UK1 (DON) and F86 (NIV). LT and HT refer to low and high titres used for inoculation

5.2.4 Spray and point inoculation of CIMMYT wheat lines with NIV and DON-producing isolates of *F. graminearum*

A total of 300 genotypes developed in the FHB breeding programme at International Maize and Wheat Improvement Center (CIMMYT), Mexico were screened both in field and polytunel for to identify Type 1 and Type II resistance and characterise them. These genotypes were developed at CIMMYT, Mexico and were kindly provided by Dr. Maarten van Ginkel. Initially all the 300 genotypes were screened for field resistance by spraying DON-producing isolate of *F. graminearum* and then 90 best lines were further characterised. The range of AUDPC scores across the 300 lines assessed in the field trial of 2005 was very high, ranging from 41 to 2110 with a mean of 412. In 2006, the disease levels (AUDPC) induced by the two chemotypes of *F. graminearum* were ranged from 11 to 579 for the DON-producing isolate and 21-469 for the NIV producing isolate and disease levels were significantly higher for the DON-producing isolate at all three score dates (Table 5.2). The lines also differed significantly in resistance at 21, 28 and 32 dpi (Table 5.2). The interaction due to DON and NIV producing isolates was not significant at 21 dpi (P=0.065) but was highly significant

by 28 dpi (P=0.002) became non-significant by 32 dpi (P=0.179) reflecting on the relative impact of different resistance components at each score date.

Table 5.2 ANOVA for disease scores on CIMMYT lines following spray inoculation with a DON (UK1) or NIV (F86) producing isolate for *F. graminearum* in the 2006 field trial.

Disease scores		21DPI			28DPI			32DPI			AUDPC		
Source of variation	DF	MS	VR	F pr.	MS.	VR	F pr.	MS	VR	F pr.	MS	VR.	F pr.
Pathogen	1	679.5	51.7	<.001	2422.1	67.3	<.001	3073.8	31.66	<.001	355234	46.87	<.001
Line	59	86.8	6.6	<.001	461.1	12.8	<.001	839.7	8.65	<.001	88088	11.62	<.001
Pathogen*Line	59	17.6	1.3	0.065	62.5	1.7	0.002	116.1	1.2	0.179	9554	1.26	0.118
Residual	230	13.1			36			97.1			7579		
Total	349	28.2			119.2			234.4			22519		
Repeatability				0.83			0.88			0.88			0.9

DF = degrees of freedom, MS = mean square, VR = variance ratio, F pr. = F probability, DPI = days post inoculation.

In 2006 field experiment with spary inoculation with a DON producing isolate, genotype 54, 91, 105, 112 and 146 expressed high level of FHB resistance (Table 5.3). The genotypes 54, 105 and 146 also showed high level of resistance to the NIV isolate indicating that they also possess a high level of Type I resistance. Other lines, most strikingly 128 exhibited a high level of resistance to the NIV producer while appearing less resistant to the DON producer indicating that they their FHB resistance is predominantly of Type I. Many lines exhibited a high level of Type II resistance following point inoculation. Of these, only 105 and 112 had also expressed high overall resistance (product of Type I and Type II) following spray inoculation with the DON isolate in the field trials. The study identified that line 54, 210 and 232 seem to possess high levels of Type I resistance.

Table 5.3 List of potential CIMMYT genotypes for FHB resistance identified using DON or NIV producing isolates of *F. graminearum* or *F. culmorum*

Wheat Line/variety	*F. c.* DON (Fu42) Spray	*F.g.* DON (UK1) Spray		*F. g.* NIV (F86) Spray		*F. g.* DON (UK1) Point	
	J05-AUDPC	J-06 AUDPC	Type I+II	AUDPC	Type I	AUDPC	Type II
7	133.5	579.2	S	271.7	S	62.9	S
14	247	530	S	229	MR	62.3	S
18	222	249.7	MR	111.3	MR	41	MR
24	192	424.2	S	112.7	MR	-	-
33	151	190.8	MR	74.7	MR	33.1	MR
54	119	24.3	R	14.3	R	50	S
55	109	94.3	MR	23	R	48.3	MR
61	158	187	MR	23.3	R	31.2	MR
62	172	202.3	MR	44	MR	90.8	S
66	61	75.2	R	21	R	38.1	MR
74	174.5	386	MR	67	MR	44.9	MR
80	216	172.8	MR	68	MR	44	MR
83	109	58.7	R	41	MR	36.4	MR
87	133.5	313.2	MR	81.7	MR	88.1	S
88	192	265.8	MR	65.5	MR	-	-
90	133.5	57.7	R	22	R	40.5	MR
91	130	48	R	28.7	R	42	MR
95	161.5	558.8	S	181.7	MR	51.6	S
103	81	71.5	R	29	R	11.9	R
105	116.5	22.5	R	12.3	R	21.3	R
108	85	92.5	R	20.3	R	13.8	R
110	65	114.5	R	20.3	R	13.9	R
112	120.5	38.8	R	22	R	19.3	R
116	155	259.8	MR	81.7	MR	67.5	S
122	192.5	53	R	15.3	R	43.5	MR
128	102	118.3	R	12.7	R	39.2	MR
132	126	132.7	MR	54	MR	66	S
146	186.5	10.8	R	12.3	R	48.5	MR
147	132.5	203.7	R	43.3	MR	22.2	R
161	198.5	146.7	MR	77.7	MR	43.5	MR
168	89	160.8	MR	60	MR	71.4	S
171	212	521.7	S	159	MR	92.2	S
172	219	307.8	MR	158.3	MR	26.9	MR
173	105.5	62.7	R	41.7	MR	20.6	R
174	218	278.3	MR	86.7	MR	33	MR

]

Table 5.3 continued List of potential CIMMYT genotypes for FHB resistance identified using DON or NIV producing isolates of *F. graminearum* or *F. culmorum*

Wheat Line/variety	*F. c.* DON (Fu42) Spray	*F.g.* DON (UK1) Spray		*F. g.* NIV (F86) Spray		*F. g.* DON (UK1) Point	
	J05-AUDPC	J-06 AUDPC	Type I+II	AUDPC	Type I	AUDPC	Type II
176	124	340	MR	50	MR	30.9	MR
178	133	194	MR	59.7	MR	88.6	S
182	123	383.3	MR	130.7	MR	65.5	S
183	187	179.2	MR	81.7	MR	56.1	S
186	71.5	55.7	R	14.3	R	29.1	MR
187	170.5	57.5	R	18.3	R	19	R
189	193	418.4	S	63.3	MR	44.9	MR
193	109	191.5	R	37	R	36.4	MR
194	196	79.5	R	25	R	23.4	R
195	138	67.2	R	18.3	R	40.9	MR
200	237	372.8	MR	185.7	MR	38.3	MR
205	143	71.8	R	28.3	R	23.4	R
206	105	400	S	144	MR	30.1	MR
209	109	227.3	MR	44.3	MR	28.4	MR
210	103	96.5	R	29.7	R	83.8	S
224	165	233.2	MR	82	MR	90.4	S
232	55	59.5	R	34	R	62.4	S
233	122	177.7	R	29	R	40.9	MR
234	259	296.6	MR	58	MR	39.5	MR
240	161.5	147.2	R	30	R	39.8	MR
241	162	425	S	144	MR	68.7	S
244	274	152.5	MR	82.7	MR	38.8	MR
251	95.5	53	R	13.3	R	19.4	R
257	200	298.8	MR	53	MR	61.3	S
264	202	485	S	96.5	MR	-	-
268	194.5	337.5	MR	78.7	MR	-	-
Renan	-	75.6	R	17.8	R	-	-
Spark	-	93.2	R	39.4	R	-	-
Riband	-	632.2	S	265.4	S	-	-
Sumai 3	-	-	-	-	-	7.7	R
Timgalen	-	-	-	-	-	43.3	MR
Remus	-	-	-	-	-	70	S

F. c. = Fusarium culmorum, *F. g*. = *F. graminearum,* J05 = JIC2005, J06 = JIC2006, JIC = JIC2007,
AUDPC = area under disease progress curve; R, resistant; I, moderately resistant; S, susceptible; -, no data
Type I and Type II refers to resistance to initial infection and resistance to spread with in the spike (sensu Schroeder & Christensen (1963).

5.3 Discussion

The resistance of wheat to FHB is of two types: resistance to initial infection (now termed type I) and resistance to spread within the plant (now termed type II) (Schroeder and Christensen 1966) and the varieties may contain either or both types of resistance. However, much research has concentrated upon identifying and mapping type II resistance (see Lin et al, 2006). Generally type I resistance evaluation is more tricky and is carried out in field trials using spray or natural inoculation and measuring disease as the percentage of infected heads in plots or as disease severity (Steiner et al, 2004; Lin et al, 2006). The DON myco-toxin acts as a virulence factor required to enable spread of *F. graminearum* between spikelets in earheads (Jansen et al, 2005). The isolates of *F. grameniarum* with disrupted in *Tri5*, a gene essential for trichothecene biosynthesis (non-DON producers), are unable to spread from inoculated floret (Proctor et al, 1995). The current study species, *M. majus* or a NIV-producing isolate of *F. graminearum* could be used to screen for resistance to initial infection (type I resistance) minimising the confounding effects caused by spread within the wheat head.

Following spray inoculation, both *F. culmorum* and *M. majus* produced disease although *M. majus* was much less aggressive under the conditions used herein. Numerous reports suggest that *M. majus* is a weak pathogen of wheat heads (Brennan et al, 2005). In contrast, following point inoculation, *M. majus* was unable to spread beyond the infected spikelet whereas the DON-producing *F. culmorum* spread into the rachis and throughout the head. The few studies that have compared the resistance of wheat varieties to different FHB species concluded that resistance acts similarly against all species (Mesterhazy et al, 2005; Toth et al, 2008). However, no comprehensive comparison has been made for *M. majus* and *Fusarium* species. It has been reported that the infection processes and colonisation of wheat florets by *M. majus* is similar to that of *F. graminearum* and *F. culmorum* but spread into adjacent spikelets was not noted (Kang et al, 2004). Symptoms produced by *M. majus*, a non toxin-producing species (Jennings, 2005) are almost identical to those produced by *Tri5*$^{-}$transformants (Cuzick et al, 2008), being restricted to single spikelets and unable to spread throughout the head. Our suggest that spray inoculation with *M. majus* may be used to assess Type I resistance directly in the absence of any confounding effects caused by differing levels of Type II resistance. This technique would complement point inoculation with a DON-producing isolate to evaluate Type II resistance. Results following spray inoculation with the DON-producing

isolate of *F. culmorum* were deduced to represent the product of Type I and Type II resistance in each variety.

A number of the varieties examined appeared to lack appreciable levels of either Type I or Type II resistance. These included Riband, Charger, Scorpion 25, Tanker, Consort and Goodwood. In many cases, combination of moderate and resistant Type I and Type II components appeared to be additive and resulted in a higher level of overall resistance. The variety Spark exhibited the best level of overall resistance and appeared to possess both Type I and Type II resistance. The variety Grief had the most contrasting response to point inoculation with *F. culmorum* and spray inoculation with *M. majus* with the relatively high level of resistance against the latter indicating that it possesses predominantly Type I resistance with very little Type II. The varieties Nirvana, Centrum and Renan also appeared to possess greater Type I than Type II resistance but the effect was less marked than that of Greif. However, there were also many cases where combinations of moderate or resistant components did not appear to be additive. Combining ability analysis in wheat has identified both general (GCA) and specific (SCA) combining effects associated with FHB resistance (Buerstmayr et al, 1999; Mardi et al, 2004). In addition, genetic analysis of combinations of quantitative trait loci (QTL) by Miedaner et al (2006) indicated that certain combinations of QTL conditioning Type I and Type II components produced higher levels of resistance than others hinting at the presence of additive by additive epistatic effects.

Although the non toxin-producing *M. majus* did infect wheat heads, disease levels were consistently low under the conditions used herein. Therefore, a more virulent alternative pathogen was sought to assess Type I resistance. NIV has been shown to be much less phytotoxic than DON on both wheat and Arabidopsis (Eudes et al, 2000; Desjardins et al, 2007). While NIV-producing isolates of *F. graminearum* can spread within wheat heads, disease progress is often slow (Maier et al, 2006), although this may be due to a reduced level of toxin biosynthesis rather than to the type of trichothecene (Goswami & Kistler, 2005). Disease severity on Mercia 12 dpi following spray inoculation with a NIV-producing isolate (F86) was similar to that induced by a DON producing strain (UK1) indicating that both strains were equally aggressive during initial infection. In contrast, following point inoculation the DON producer rapidly spread through the spike, whilst the NIV isolate remained largely confined to the inoculated spikelet for at least the first 18 days and had only significantly moved beyond the infection site by 23 dpi. Taken together, the spray and point data suggest that both isolates are equally virulent, but the

DON producer is able to move beyond the point(s) of infection to cause greater over-all disease severity. These data are in accordance with field studies of disease severity in wheat and rye which reported that NIV producing isolates of *F. culmorum* caused less disease and accumulated less toxin in grain samples than DON producing isolates (Miedaner and Reinbrecht, 2001). While the isolates UK1 and F86 are DON (15ADON) and NIV chemotypes, respectively and no attempt was made to determine the amounts of toxin made by them. It is possible that F86 produced only small amounts of NIV *in planta* that may account for it not spreading within the spike until very late in infection relative to the DON producer (UK1). However, as NIV is intrinsically less phytotoxic than other trichothecenes such as DON (Eudes et al, 1997) and this may explain the reduced ability of the NIV chemotype to spread within the spike. Whatever the underlying cause, we conclude that virulent NIV chemotype isolates of *F. graminearum*, such as F86, might be used in spray inoculation trials to determine relative levels of Type I resistance in wheat. We used a combination of point and spray trials with appropriate NIV and DON producing isolates of *F. graminearum* to characterise the FHB resistance within 60 lines of wheat obtained from the wheat breeding of the International Maize and Wheat Improvement Center (CIMMYT), Mexico. The field trials confirmed the differential rate of spread of the NIV and DON producing isolates and showed those with high overall FHB resistance and those with predominantly Type I resistance. Several lines exhibiting high levels of FHB resistance in field trials were found to also possess high levels of Type II resistance following point inoculation. More significantly, a few lines (54, 210 and 232), while exhibiting high levels of FHB resistance in field trials, were highly susceptible in point inoculation studies indicating that their resistance is predominantly of Type I. While a relatively large number of Type II resistance genes and QTL have been reported (Shen et al, 2003; Ma et al, 2006; Cuthbert et al, 2007; 2008), few sources of Type I resistance have been identified to date (Lin et al, 2006; Steed et al, 2005). This, in large part, is due to the greater technical challenges associated with the unequivocal identification of Type I resistance.

Point inoculation with a virulent DON chemotype of *F. graminearum* or *F. culmorum* is recognised as an appropriate method to screen wheat varieties for relative levels of Type II FHB resistance. On the basis of our results from disease trials, we propose that spray inoculation with a virulent non DON-producing FHB pathogen may be used to directly assess relative levels of Type I resistance in the absence of the confounding effects due to differences in Type II resistance. *M. majus* may be suitable but this species

is generally not highly virulent in our experience. The use of a virulent NIV chemotype isolate of *F. graminearum* or *F. culmorum* would be more appropriate. Some NIV chemotypes within the *F. graminearum* species complex have been shown to be able to spread within wheat spikes (Goswami & Kistler, 2005). Therefore, prior to use in assessments of Type I resistance, the virulence of NIV chemotype isolates should be evaluated by both spray and point inoculation to select those (like F86) that are virulent but unable to spread within the wheat spike. We propose that the use of appropriate non DON-producing FHB species or isolates in spray inoculation trials combined with point inoculation using DON-producing isolates will assist researchers seeking to identify and characterise resistance to FHB in wheat.

6. Evaluation of FHB resistance within UK spring barley varieties

6.1 Background

Relatively little is known about the genetics of FHB resistance in barley, although it is believed that barley generally has good Type 2 resistance (resistance to spread of infection within the ear). The implication is that differences in Type 1 resistance (resistance to initial infection) are likely to be of paramount importance in barley varieties. There is currently no information available on the resistance of UK barley varieties to FHB.

The aim of the work described here was to carry out an initial screen of spring barley varieties on the current UK Recommended List to look for evidence of varietal differences in resistance to FHB.

6.2 Materials and Methods

Field experiments investigating the resistance of UK spring barley varieties to FHB were carried out in two years, 2005 and 2006, at NIAB, Cambridge.

2005

27 spring barley varieties, comprising those in current UK Recommended List trials, were grown in two experiments, each being a randomised block design with 4 replicates. Plots were approximately 1m x 1m. The first experiment ('early') was inoculated with spores of *F. culmorum* on 7 June, to coincide with mid anthesis of the earlier flowering varieties. The second experiment ('late') was inoculated three days later on 10 June 2005, to

coincide with mid anthesis of the later flowering varieties. A single very late flowering variety in the late trial was individually inoculated on 14 June. A spore suspension of a DON-producing isolate of *F.culmorum* (Fu42, from JIC), at a concentration of 1 x 10^6 spores / ml, was applied at a rate of 100ml per plot, using a hand held pump sprayer.

Assessment of FHB infection was carried out in the late experiment only, as disease levels were extremely low in the early experiment. Symptoms were assessed visually on 4 July, at around GS 80, using an infection index method (% ears infected x mean severity of infection of infected ears). At harvest, grain samples were taken for analysis of DON content.

2006

22 spring barley varieties from amongst the 27 tested in 2005 were grown in a single experiment in 2006. Experimental design was a randomised block with 4 replicates. Plots were approximately 0.5m x 1m. All plots were inoculated twice, the earlier inoculation, on 9 June 2006, being when the earliest flowering varieties started to flower and the later inoculation, on 12 June, being when the majority of other varieties reached anthesis. A few late flowering varieties required an additional inoculation on 15 June. Inoculation methods were similar to those in 2005, except that 50ml of inoculum was applied per plot, to reflect their smaller size.

Symptoms of FHB were assessed on three dates, 22 June (GS 71), 29 June (GS 83) and 7 July (GS 85), using the same method as in 2005.

6.3 Results

FHB infection was considerably more severe in the 2006 trial than the 2005 trial. However, in both years there were highly significant differences in infection level between varieties, with a significant correlation between years for the 22 common varieties ($r = 0.857$, $P<0.001$).

Table 6.1 summarises assessments of FHB symptoms in the two years' trials. Varieties were ranked in order of severity of infection in 2006. The data for 2005 are from a single assessment, whereas the 2006 data are means of three assessments made over a period of 15 days.

Table 6.1. FHB severity (0-100) in two years' trials.

Variety	FHB severity in 2005 trial (0-100)	FHB severity in 2006 trial (0-100)
Cocktail	11.29	51.67
Cellar	8.23	34.08
Doyen	7.08	33.17
NFC Tipple	6.28	27.83
Static	10.52	27.75
Power	3.05	24.83
Hydra	6.84	23.75
Wicket	4.69	22.08
Kirsty	2.68	21.08
Tocada	0.72	20.58
Decanter	2.49	19.83
Poker	1.95	19.33
Chalice	4.30	17.83
Riviera	2.70	17.50
Rebecca	0.75	17.17
Waggon	4.49	15.84
Oxbridge	2.12	14.51
Optic	0.71	14.51
Appaloosa	1.46	13.34
Spire	0.55	11.68
Troon	0.08	10.68
Westminster	0.92	9.51
Putney	6.32	-
Carafe	2.70	-
Cribbage	2.16	-
Centurion	2.05	-
Beatrix	2.03	-
Anovar		
Variety effect		
P value	<0.001	<0.001
SE difference	1.906	3.468
lsd P<0 05	3.775	6.868

Table 6.2 compares the results of DON analyses of grain samples taken from the 2005 trial (bulked over replicates) with FHB severity. There was a significant correlation between the two measures ($r = 0.747$, $P<0.001$).

Table 6.2. FHB severity and DON content of grain in 2005

Variety	FHB severity (0-100)	DON (ppm)
Cocktail	10.72	1.50
Static	10.07	1.70
Cellar	7.95	1.52
Doyen	7.16	3.05
Hydra	6.61	2.08
Chalice	4.20	1.10
Wicket	4.00	0.70
Waggon	3.75	1.00
Power	2.63	1.90
Kirsty	2.47	0.30
Decanter	2.31	0.81
Riviera	2.11	0.65
Oxbridge	1.85	0.20
Poker	1.68	0.29
Aquila	1.39	0.31
Westminster	0.88	0.35
Rebecca	0.73	0.26
Tocada	0.73	0.13
Optic	0.72	0.37
Spire	0.54	0.08
Troon	0.05	0.35

6.4 Discussion

The results obtained from the two trials provide clear indications of significant and consistent differences between barley varieties in resistance to FHB. More detailed work is needed to dissect the components of resistance and determine whether the apparent resistance may be influenced by the flowering characteristics of a variety, in particular its tendency to flower while the ear is still in boot.

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